frontiers | Research Topics

The side effects of insecticides on insects and the adaptation mechanisms of insects to insecticides

Edited by
Youhui Gong, Ting Li, Qingli Shang, Xiaoming Xia,
Adil Hussain and Asad Ali

Published in
Frontiers in Physiology

ISSN 1664-8714
ISBN 978-2-8325-3644-5
DOI 10.3389/978-2-8325-3644-5

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

The side effects of insecticides on insects and the adaptation mechanisms of insects to insecticides

Topic editors

Youhui Gong — State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection (CAAS), China

Ting Li — Alabama State University, United States

Qingli Shang — Jilin University, China

Xiaoming Xia — Shandong Agricultural University, China

Adil Hussain — Abdul Wali Khan University, Pakistan

Asad Ali — Abdul Wali Khan University, Pakistan

Citation

Gong, Y., Li, T., Shang, Q., Xia, X., Hussain, A., Ali, A., eds. (2023). *The side effects of insecticides on insects and the adaptation mechanisms of insects to insecticides*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-3644-5

Table of contents

frontiers | Frontiers in Physiology

TYPE Editorial
PUBLISHED 22 September 2023
DOI 10.3389/fphys.2023.1287219

OPEN ACCESS

EDITED AND REVIEWED BY
Sylvia Anton,
Institut National de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE), France

*CORRESPONDENCE
Youhui Gong,
gongyh922@126.com,
gongyouhui01@caas.cn

†These authors have contributed equally to this work

RECEIVED 01 September 2023
ACCEPTED 18 September 2023
PUBLISHED 22 September 2023

CITATION
Gong Y, Li T, Hussain A, Xia X, Shang Q and Ali A (2023), Editorial: The side effects of insecticides on insects and the adaptation mechanisms of insects to insecticides.
Front. Physiol. 14:1287219.
doi: 10.3389/fphys.2023.1287219

Editorial: The side effects of insecticides on insects and the adaptation mechanisms of insects to insecticides

Youhui Gong[1*†], Ting Li[2†], Adil Hussain[3], Xiaoming Xia[4], Qiangli Shang[5] and Asad Ali[3]

[1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China, [2]Department of Biological Sciences, Alabama State University, Montgomery, AL, United States, [3]Department of Entomology, Abdul Wali Khan University Mardan, Mardan, Pakistan, [4]The College of Plant Protection, Shangdong Agricultural University, Taian, China, [5]College of Plant Science, Jilin University, Changchun, China

KEYWORDS

sublethal effects, beneficial insects, insecticide resistance, insecticide resistance management, insecticide resistance mechanisms

Editorial on the Research Topic
The side effects of insecticides on insects and the adaptation mechanisms of insects to insecticides

Since their initial implementation, insecticides have played a significant role in controlling agricultural and medical insect pests. However, it is essential to recognize that while these chemicals effectively target insect pests, they can also harm beneficial insects, such as pollinators and natural predators, and have unintended impacts on non-target insects. Additionally, the use of insecticides raises concerns about their lasting impact on the environment and the delicate balance of ecosystems, affecting various organisms within these intricate systems (Ansari et al., 2014). Moreover, the global challenge of insecticide resistance and pest resurgence poses a significant constraint on agricultural output (Zuo et al., 2021; Serrão et al., 2022). Considering this, "The Side Effects of Insecticides on Insects and the Adaptation Mechanisms of Insects to Insecticides" aims to explore new perspectives regarding the potential consequences of insecticide for both pest and beneficial insects, focusing on Research Topic such as hormesis effects related to aspects like reproduction, tolerance, and insect behavior. This subject also covers the biochemical and molecular mechanisms that regulate insecticide resistance and its corresponding fitness costs in insects.

Following their initial application in the field, the potency of insecticides in terms of lethal concentrations diminishes gradually, leading to exposure to low lethal and/or sublethal concentrations (Desneux et al., 2005; Desneux et al., 2007). This exposure can give rise to various sublethal effects within the affected insects, as indicated by Desneux et al. (2007). These effects encompass a range of negative impacts on crucial life history traits of beneficial insects. Afza et al. delve into the realm of sublethal and transgenerational repercussions stemming from the application of six distinct synthetic insecticides on the seven spotted lady beetle, *Coccinella septempunctata*—a valuable predatory insect. Their findings reveal that even at a low concentration (LC_{30}), all tested insecticides substantially hinder the emergence

of adults, adult weight, fertility, and fecundity in the parental generation when compared to the control treatment. Additionally, exposure to sublethal concentrations of these insecticides resulted in a reduction of predation rates among the F_1 generation adults. This serves as a compelling example, highlighting the detrimental consequences of insecticides on beneficial arthropods within agroecosystems. Understanding the unintended impacts of insecticides on non-target organisms, particularly biological control agents, is imperative for the successful integration of insecticides into pest management strategies (Stark et al., 2007; Liu et al., 2019). Furthermore, this Research Topic presents yet another illustration of the consequences of insecticides on pollinators. Han et al. study uncovered that exposure to field-realistic concentrations of acetamiprid and/or difenoconazole triggered alterations in the honeybee gut microbiome and gene expression, ultimately impacting the health of these vital pollinators.

It is widely recognized that subjecting insects to mild stress from insecticides can elicit hormetic (stimulatory) responses, which holds significant implications for insect management, as well as the ecological balance and dynamics within agroecosystems (Cutler et al., 2022). Shedding light on this phenomenon, Li et al. have presented their research outcomes concerning the influence of hormesis induced by imidacloprid on the developmental and reproductive aspects of the rose-grain aphid, *Metopolophium dirhodum*. Conducted by Liu et al., another investigation delved into the sublethal impacts of emamectin benzoate on adult diamondback moths (DBM), *Plutella xylostella*, along with their subsequent generations. The most intriguing revelation from this study lies in the observation of reproductive hormesis among DBM adults when exposed to LC_{20} concentrations. The discovery of hormetic effects on reproduction holds profound significance in the realm of insect pest management, as it could potentially underlie the mechanisms driving pest resurgence following applications of insecticides (Wu et al., 2019; Cutler et al., 2022). In a recent study, Gong et al. (2023) examined the transgenerational hormetic effects of nitenpyram on fitness, as well as the development of insecticide tolerance and resistance, in *Nilaparvata lugens*. The researchers put forth the hypothesis that the population outbreaks of *N. lugens*, following exposure over multiple generations to low concentrations of nitenpyram in field crops, might arise due to increased reproduction and the subsequent development of resistance.

The emergence of insecticide resistance among insects poses a significant challenge to the long-term effectiveness of insecticides, which remain a primary means of controlling both agricultural and medical pests (Gould et al., 2018; Van Leeuwen et al., 2020). Initially, conducting thorough risk assessments of insecticide resistance is paramount as part of insecticide resistance management (IRM) strategies, particularly before the widespread adoption of new insecticides (Zhang et al., 2020). Triflumezopyrim (TFM) represents a new type of mesoionic insecticide developed by Corteva Agriscience, which showed high biological activity in controlling piercing-sucking insect pests such as planthopper (Zhang et al., 2020; Wen et al., 2021). Wen et al. reported that after 21 generations of continuous selection with TFM, the *Laodelphax striatellua* developed a 26.29-fold resistance to TFM with no cross-resistance to five other insecticides. This research indicated that there is a risk of insecticide resistance development during the continuous selection of TFM in fields, which is consistent with the results obtained by Zhang et al. (2022). Furthermore, insecticide resistance monitoring within field populations across different regions holds paramount importance in IRM (Huang et al., 2021). This practice aids in making informed choices regarding insecticides and the rotation of insecticide Modes of Action (MoA) groups (Huang et al., 2021). Meng et al. meticulously assessed the susceptibility of forty-six field populations of *Chilo suppressalis* to three distinct insecticides in three provinces of Central China from 2010 to 2021. Their findings indicated that *C. suppressalis* populations developed varying degrees of resistance over time. While demonstrating moderate to high levels of resistance to triazophos, these populations still exhibited susceptibility, and low to moderate levels of resistance to chlorpyrifos and abamectin. As a result, the application of triazophos should be suspended in efforts to control *C. suppressalis*, while the frequent use of chlorpyrifos and abamectin should be reduced across Central China. Chen et al. conducted a meticulous analysis of insecticide resistance in 11 field-collected populations of fall armyworm, *Spodoptera frugiperda*, originating from Sichuan Province. The findings revealed that the resistance levels of the *S. frugiperda* field populations within Sichuan remained within a sensitive range concerning emamectin benzoate and chlorpyrifos. Notably, these populations demonstrated low to moderate resistance to chlorantraniliprole.

The development of insect resistance to insecticides can be attributed to two primary mechanisms: One mechanism involves target insensitivity resulting from gene mutations, while the other involves metabolic resistance stemming from alterations in the quantity and quality of detoxification enzyme genes (Bass and Nauen, 2023). The extensive use of diamide insecticides has notably led to the emergence of resistance among lepidopteran pests (Boaventura et al., 2020). Specifically, the G4946E and I4790M/K mutations within the ryanodine receptor (RyR) have been closely associated with resistance to diamide insecticides in various field populations of *P. xylostella*, as well as several other pest insects (Roditakis et al., 2017; Wei et al., 2019). Ren et al. identified two potential mutation loci, namely Gly4911Glu and Ile4754Met, that contribute to the development of insecticide resistance in the leaf beetle, *Galeruca daurica*. This beetle has emerged as a significant grassland pest, marked by sudden outbreaks in the Inner Mongolia grasslands since 2009. Remarkably, the G4911E mutant model exhibited reduced affinity and an altered mode of action towards two diamide insecticides. Consequently, their study strongly suggests that the G4911E mutation in GdRyR could potentially serve as a key mechanism driving resistance to diamide insecticides in *G. daurica*. Additional contributions within this Research Topic center on the pivotal role of detoxification enzymes and genes in the development of insecticide resistance. Wen et al. have proposed that the development of Triflumezopyrim resistance in *L. striatellus* is closely linked to several P450 genes. In the 12th paper, Chen et al. have illuminated the upregulation of five specific P450 genes (*CYP6AE43*, *CYP321A8*, *CYP305A1*, *CYP49A1*, and *CYP306A1*) as potential contributors to the moderate-level resistance observed towards chlorantraniliprole. Furthermore, the eighth paper conducted by Chen et al. have confirmed the critical role of a cytochrome P450 gene, MusiDN2722 in the resistance of *Megalurothrips usitatus* Bagnall to acetamiprid through RNA interference targeting the P450 gene.

The phenomenon of insecticide resistance often carries a fitness cost, forming the foundation for resistance management strategies that involve altering insecticide usage (ffrench-Constant and Bass, 2017). However, this principle does not hold in every scenario. Hasnain et al. have presented findings indicating that the chlorantraniliprole-reduced susceptible strain of *S. frugiperda* demonstrates higher performance in fecundity and other life table traits compared to the chlorantraniliprole-susceptible strain. To counteract the emergence of insecticide resistance, it is imperative to implement a multifaceted approach encompassing biological control, crop rotation, transgenic plants, and cultural practices, in conjunction with refined insecticide application strategies. These refined insecticide application strategies include alternating insecticide usage, adjusting mixtures, and reducing application frequencies. Feng et al. introduced an innovative approach wherein combining pymetrozine with the fungicide zhongshengmycin amplifies the insecticidal effects of pymetrozine while concurrently carrying fitness costs in *Nilaparvata lugens* (Stål), which has developed elevated resistance to pymetrozine. This research unveils a fresh avenue to stave off resistance development and enhance the efficacy of pest management. In another exploration, Idrees et al. evaluated the insecticidal potency of the entomopathogenic fungus *Metarhizium anisopliae* MA against *S. frugiperda*. Their study demonstrated the efficacy of this fungus in inducing mortality among second instars, eggs, and neonate larvae under controlled laboratory conditions.

In summary, this Research Topic delved into the multifaceted aspects of insecticide's impact on insects and their adaptive responses. The outcomes illuminated various dimensions, encompassing effects on pollinators and predators, hormesis effects influencing pest insect populations, risk assessment for insecticide resistance, and the intricate mechanisms underlying the development of insecticide resistance. Furthermore, valuable instances of utilizing biological control and combining pesticides with fungicides for pest management and resistance mitigation were highlighted within this Research Topic. However, despite these significant advancements, numerous questions remain unanswered. For instance, while many studies have identified an array of detoxification genes exhibiting overexpression in resistant insects, the exact roles of these genes require further investigation and validation in the future.

We extend our gratitude to all authors for their insightful contributions, as well as to the reviewers and the dedicated editorial team for their invaluable input, comments, and suggestions. With its diverse insights, we anticipate that this Research Topic will prove engaging and enlightening for the broad readership of Frontiers in Physiology.

Author contributions

YG: Writing–original draft. TL: Writing–review and editing. AH: Writing–review and editing. XX: Writing–review and editing. QS: Writing–review and editing. AA: Writing–review and editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The authors declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

Author disclaimer

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

References

Ansari, M. S., Moraiet, M. A., and Ahmad, S. (2014). "Insecticides: impact on the environment and human health," in *Environmental deterioration and human health: Natural and anthropogenic determinants*. Editors A. Malik, E. Grohmann, and R. Akhtar (Dordrecht: Springer Netherlands), 99–123.

Bass, C., and Nauen, R. (2023). The molecular mechanisms of insecticide resistance in aphid crop pests. *Insect Biochem. Mol. Biol.* 156, 103937. doi:10.1016/j.ibmb.2023.103937

Boaventura, D., Bolzan, A., Padovez, F. E., Okuma, D. M., Omoto, C., and Nauen, R. (2020). Detection of a ryanodine receptor target-site mutation in diamide insecticide resistant fall armyworm, *Spodoptera frugiperda*. *Pest. Manag. Sci.* 76, 47–54. doi:10.1002/ps.5505

Cutler, G. C., Amichot, M., Benelli, G., Guedes, R. N. C., Qu, Y., Rix, R. R., et al. (2022). Hormesis and insects: effects and interactions in agroecosystems. *Sci. Total Environ.* 825, 153899. doi:10.1016/j.scitotenv.2022.153899

Desneux, N., Decourtye, A., and Delpuech, J. M. (2007). The sublethal effects of pesticides on beneficial arthropods. *Annu. Rev. Entomol.* 52 (1), 81–106. doi:10.1146/annurev.ento.52.110405.091440

Desneux, N., Fauvergue, X., Dechaume-Moncharmont, F. X., Kerhoas, L., Ballanger, Y., and Kaiser, L. (2005). Diaeretiella rapae limits *Myzus persicae* populations after applications of deltamethrin in oilseed rape. *J. Econ. Entomol.* 98 (1), 9–17. doi:10.1093/jee/98.1.9

ffrench-Constant, R. H., and Bass, C. (2017). Does resistance really carry a fitness cost? *Curr. Opin. Insect Sci.* 21, 39–46. doi:10.1016/j.cois.2017.04.011

Gong, Y. H., Cheng, S. Y., Desneux, N., Gao, X. W., Xiu, X. J., Wang, F. L., et al. (2023). Transgenerational hormesis effects of nitenpyram on fitness and insecticide tolerance/resistance of *Nilaparvata lugens*. *J. Pest Sci.* 96, 161–180. doi:10.1007/s10340-022-01494-4

Gould, F., Brown, Z. S., and Kuzma, J. (2018). Wicked evolution: can we address the sociobiological dilemma of pesticide resistance? *Science* 360, 728–732. doi:10.1126/science.aar3780

Huang, J. M., Zhao, Y. X., Sun, H., Ni, H., Liu, C., Wang, X., et al. (2021). Monitoring and mechanisms of insecticide resistance in *Spodoptera exigua* (Lepidoptera: noctuidae), with special reference to diamides. *Pestic. Biochem. Physiol.* 174, 104831. doi:10.1016/j.pestbp.2021.104831

Liu, T. F., Yao, W., Zhang, L. X., Xu, Y. Y., Zhang, Z. Q., and Mu, W. (2019). Sublethal effects of four insecticides on the seven-spotted lady beetle (Coleoptera: coccinellidae). *J. Econ. Entomol.* 112, 2177–2185. doi:10.1093/jee/toz146

Roditakis, E., Steinbach, D., Moritz, G., Vasakis, E., Stavrakaki, M., Ilias, A., et al. (2017). Ryanodine receptor point mutations confer diamide insecticide resistance in tomato leafminer, Tuta absoluta (Lepidoptera: Gelechiidae). *Insect Biochem. Mol. Biol.* 80, 11–20. doi:10.1016/j.ibmb.2016.11.003

Serrão, J. E., Plata-Rueda, A., Martínez, L. C., and Zanuncio, J. C. (2022). Side-effects of pesticides on non-target insects in agriculture: a mini-review. *Sci. Nat.* 109, 17. doi:10.1007/s00114-022-01788-8

Stark, J. D., Vargas, R., and Banks, J. E. (2007). Incorporating ecologically relevant measures of pesticide effect for estimating the compatibility of pesticides and biocontrol agents. *J. Econ. Entomol.* 100, 1027–1032. doi:10.1603/0022-0493(2007)100[1027:iermop]2.0.co;2

Van Leeuwen, T., Dermauw, W., Mavridis, K., and Vontas, J. (2020). Significance and interpretation of molecular diagnostics for insecticide resistance management of agricultural pests. *Curr. Opin. Insect Sci.* 39, 69–76. doi:10.1016/j.cois.2020.03.006

Wei, Y., Yan, R., Zhou, Q., Qiao, L., Zhu, G., and Chen, M. (2019). Monitoring and mechanisms of chlorantraniliprole resistance in Chilo suppressalis (Lepidoptera: crambidae) in China. *J. Econ. Entomol.* 112, 1348–1353. doi:10.1093/jee/toz001

Wen, S., Liu, C., Wang, Y., Xue, Y., Wang, X., Wang, J., et al. (2021). Oxidative stress and DNA damage in earthworm (*Eisenia fetida*) induced by triflumezopyrim exposure. *Chemosphere* 264, 128499. doi:10.1016/j.chemosphere.2020.128499

Wu, J., Ge, L., Liu, F., Song, Q., and Stanley, D. (2019). Pesticide-induced planthopper population resurgence in rice cropping systems. *Annu. Rev. Entomol.* 65, 409–429. doi:10.1146/annurev-ento-011019-025215

Zhang, S., Gu, F., Du, Y., Li, X., Gong, C., Pu, J., et al. (2022). Risk assessment and resistance inheritance of triflumezopyrim resistance in *Laodelphax striatellus*. *Pest Manag. Sci.* 78 (7), 2851–2859. doi:10.1002/ps.6909

Zhang, Y. C., Feng, Z. R., Zhang, S., Pei, X. G., Zeng, B., Zheng, C., et al. (2020). Baseline determination, susceptibility monitoring and risk assessment to triflumezopyrim in *Nilaparvata lugens* (Stål). *Pestic. Biochem. Physiol.* 167, 104608. doi:10.1016/j.pestbp.2020.104608

Zuo, Y., Shi, Y., Zhang, F., Guan, F., Zhang, J., Feyereisen, R., et al. (2021). Genome mapping coupled with CRISPR gene editing reveals a P450 gene confers avermectin resistance in the beet armyworm. *PLoS Genet.* 17 (7), e1009680. doi:10.1371/journal.pgen.1009680

frontiers | Frontiers in Physiology

TYPE Original Research
PUBLISHED 19 October 2022
DOI 10.3389/fphys.2022.1025959

OPEN ACCESS

EDITED BY
Youhui Gong,
Institute of Plant Protection (CAAS), China

REVIEWED BY
Lei Guo,
Qingdao Agricultural University, China
Jianhong Li,
Huazhong Agricultural University, China
Farman Ullah,
China Agricultural University, China

*CORRESPONDENCE
Chao-Bin Xue,
cbxue@sdau.edu.cn

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Invertebrate Physiology, a section of the journal Frontiers in Physiology

RECEIVED 23 August 2022
ACCEPTED 10 October 2022
PUBLISHED 19 October 2022

CITATION
Liu K-X, Guo Y, Zhang C-X and Xue C-B (2022), Sublethal effects and reproductive hormesis of emamectin benzoate on Plutella xylostella. *Front. Physiol.* 13:1025959. doi: 10.3389/fphys.2022.1025959

Sublethal effects and reproductive hormesis of emamectin benzoate on *Plutella xylostella*

Kong-Xing Liu†, Yong Guo†, Can-Xin Zhang and Chao-Bin Xue*

College of Plant Protection, Shandong Agricultural University, Key Laboratory of Pesticide Toxicology and Application Technique, Taian, China

The diamondback moth (DBM), *Plutella xylostella* L., is an important pest of cruciferous vegetables, and population control mainly depends on chemical pesticides. Emamectin benzoate is a highly effective insecticide used for controlling DBM. However, it is unknown how the sublethal effects of low concentration residues of emamectin benzoate on DBM. So the population development sublethal effects of emamectin benzoate, at LC_5, LC_{10}, and LC_{20} with concentrations of 0.014 mg/L, 0.024 mg/L and 0.047 mg/L, respectively, on adult DBM and their progeny were investigated in this study. The pupal weight, pupal period, female fecundity, and vitellin content of the F_0 DBM generation increased significantly compared to the control. And the single female oviposition number of DBM was increased by 20.21% with LC_{20} treatment. The pupation rate, adult longevity and ovariole length of the treatment groups decreased significantly. The fecundity of DBM in the treatment groups increased, and this increased the population by a presumptive 13.84%. Treatment also led to the shortening of ovarioles and the reduction of egg hatching, and increased pupal weight in the F_1 generation. We concluded that the effects of sublethal/low concentration emamectin benzoate on the different life stages of DBM were variable, and the reproductive hormesis on DBM adults were attractive findings.

KEYWORDS

fecundity, sublethal concentration, emamectin benzoate, hormesis, development

Introduction

The diamondback moth (DBM), *Plutella xylostella* L., is a widely distributed lepidopteran pest that causes serious damage to cruciferous vegetables. It has strong adaptability of host, long-distance migration, and overlapping generations (Furlong et al., 2013). DBM control relies on chemical insecticides. However, excessive pesticide use has selected for DBM resistance to more than 90 pesticides (Whalon et al., 2019).

Studies have found that pesticide applications usually induce strong lethal effects in most of arthropods. It primarily through direct mortality of exposed arthropods and a variety of sublethal physiological biological, and/or behavioral effects on arthropod

individuals (Desneux et al., 2007; Shan et al., 2020). Arthropods may experience exposure to sublethal doses because of suboptimal spray coverage during applications and owing to a decrease in residue concentrations after the initial application (Guedes et al., 2016). Pesticide applications can significantly impact the effectiveness of biocontrol agents in most agroecosystems (Lu et al., 2012; Huang et al., 2020). It may also influence habitat shifts, induces hormesis in insect pests, resistance development, and direct and indirect interactions between species within food webs. Some insecticides may also disrupt biological control of secondary pests, leading to a secondary pest outbreak (Wang et al., 2017; Liang et al., 2021; Zhang et al., 2021a). Pesticide exposure can stimulate reproductive hormesis of *Nilaparvata lugens* and lead to *N. lugens* population outbreaks (Wu et al., 2019). All of the cases bring great challenges to the rational use of pesticides and pest control, which require extensive attention from entomologist and agrochemical scientists all of world.

Emamectin benzoate (4″-epi-methylamino-4″-deoxyavermectin B1) is a highly efficient, broad-spectrum, semi-synthetic insecticide used for control of agricultural and forestry insect pests. It is especially useful for controlling lepidoptera including *Spodoptera exigua*, *Helicoverpa zea*, *P. xylostella*, and *Spodoptera littoralis* (López et al., 2010; El-Sheikh, 2015). Field residues of emamectin benzoate gradually decrease to sublethal concentrations due to chemical, biological and/or natural degradation in the environment (Biondi et al., 2012; Khan et al., 2018). The half-life of emamectin benzoate on cabbage was determined to be 3.81 days (Wang et al., 2009). The LC_5 and LC_{15} of emamectin benzoate prolonged the development time and longevity of *Spodoptera littoralis* and reduced the population (Mokbel and Huesien, 2020). The LC_{30} of emamectin benzoate had a significant negative impact on egg laying, ovarian development, mating rate, and survival of *Conopomorpha sinensis* (Yao et al., 2018).

It was found that the third-instar larvae of DBM were treated by LC_{10} and LC_{25} of chlorantraniliprole. Which of the insecticide could reduce pupation, pupal weight, adult emergence rates, increase the duration of female preoviposition period, decrease fecundity and egg hatch, and decrease survival rates of the offspring. And, the mean values of the net reproductive rate (R_0), intrinsic rate of increase (r_m), and finite rate of increase (λ) were also significantly decreased in LC_{10} and LC_{25} treatment (Han et al., 2012). The negative effects by indoxacarb, metaflumizone, methylthio-diafenthiuron, spinetoram, broflanilide and fluxametamide with sublethal dose/concentration were also studied (Wang et al., 2011; Zhang et al., 2012; Su and Xia, 2020; Tamilselvan et al., 2021; Gope et al., 2022; Sun et al., 2022). However, a few of insecticides, e.g. fenvalerate, chlorpyrifos, chlorfenapyr (LC_1) and abamectin with sublethal dose/concentration (Fujiwara et al., 2002; Deng et al., 2016; Rodríguez-Rodríguez et al., 2021; Jia et al., 2022) could stimulate or lead to hormesis effect on DBM. The LC_1 (0.274 mg/L) of chlorfenapyr significantly increased female pupa weight of F_0 and F_1 generations, and F_0 fecundity as well as F_1 gross reproduction rate of DBM. And, the LC_1-elicited rise in emergency rate and fecundity was significantly greater in F_0 than in F_1 (Jia et al., 2022). How does the sublethal effects of emamectin benzoate on DBM remains unknown until now. In this paper, we studied the effects of sublethal/low concentrations of emamectin benzoate, at LC_5, LC_{10}, and LC_{20} with concentrations of 0.014 mg/L, 0.024 mg/L and 0.047 mg/L, respectively, on the population development of DBM, and hope carry out a science assessment of its application on DBM, avoid or delay resurgence of DBM in the field.

Materials and methods

Insect

P. xylostella was collected from the experimental station of the South campus of Shandong Agricultural University in 2006 and cultured indoors on radish seedlings and cabbage leaves without pesticide exposure. The insect rearing room was maintained at 25 ± 2°C, relative humidity 60%–70%, and a photoperiod of 14:10 h (L:D). The adults were fed on 10% honey: water.

Insecticides and reagents

Emamectin benzoate (95.0%) was provided by Qingdao Dingfeng Biotechnology Co., Ltd. Shandong Province, China. Acetone, ether, and other solvents were analytical grade and were purchased from Tianjin Damao Chemical Reagent Factory, Tianjin, China.

Bioassay of acute toxicity

Acute toxicity was determined by the leaf dipping method (Liang et al., 2003). Emamectin benzoate was dissolved in acetone to prepare a concentrate of 200 mg/L. The concentrate was serially diluted with 1% Tween 80 aqueous solution to obtain emamectin benzoate solution concentrations of 0.05, 0.10, 0.20, 0.40 and 0.80 mg/L. Fresh cabbage leaves were cut into 5.0 ± 0.5 cm disks, dipped in the solution for 10 s and held vertically to allow excess solution to drip off, and placed on a rack to dry. Twenty third-instar DBM larvae were added to each culture dish containing a treated leaf disk. An aqueous solution without emamectin benzoate used as the negative control. After 72 h exposure on the cabbage leaves treated with the different concentration of emamectin benzoate, counted the surviving larvae that moved when touched slightly, and transferred to fresh leaves for subsequent experiments.

TABLE 1 Toxicity of emamectin benzoate to third-instar larvae of *P. xylostella* (72 h).

Insecticides	Regression equation	LC_5 LC_{10} LC_{20} (mg/L)	LC_{50} (mg/L)	95% CL (mg/L)	Correlation coefficient *r*	χ^2	*df*
Emamectin benzoate	y = 1.134 + 1.489x	0.014 0.024 0.047	0.173	0.125–0.223	0.986	44.39	16

Bioassay of sublethal effects on DBM

Thirty third-instar DBM larvae were treated with emamectin benzoate for 72 h use the same leaf dip method as described above at sublethal/low concentrations of LC_5, LC_{10}, and LC_{20}, respectively. The effects of the sublethal/low concentrations of emamectin benzoate on DBM development were determined. These effects included pupation rate, pupal weight, pupal period, adult emergence rate, adult survival number, single female oviposition number, adult longevity, eggs hatching rate, larval survival rate, and larval development duration. Both F_0 and F_1 generations were studied. An aqueous solution without emamectin benzoate was used as the control.

After the emergence of the treated insects, select one couple of male and female adults randomly eclosing on the same day, and put the couple into a can bottle with fresh cabbage leaves, providing with 10% honey solution. When the female adult begins to lay, count the number of eggs laid and the number of eggs hatched every 24 h until the adult died. Each treatment was 5 couple of adults and repeated 3 times independently.

Ovary anatomy and vitellin content determination

Third-instar DBM larvae were exposed to emamectin benzoate for 72 h use the leaf dip method mentioned above at sublethal/low concentrations of LC_5, LC_{10}, and LC_{20} and surviving female adults were collected. The female adults were anesthetized with CO_2 and the thorax/abdomen was removed with ophthalmic surgical scissors. The abdomen was placed on a glass slide coated with physiological saline. Then, the end of the abdomen was squeezed gently with an insect pin and the ovaries were removed. The fat particles adhering to the ovarioles were removed with dissecting forceps and the ovarioles were stained with safranine dye solution for 5 min. Excess dye solution was then washed off the ovarioles. The ovarioles were observed and photographed with a continuously variable magnification stereomicroscope (SZX 10). The length or width diameter of mature eggs and ovarioles lengths were measured with ImageJ image processing software.

The vitellin content at 0–96 h female emergence was determined using an insect vitellin linked immunoassay (ELISA) kit (Shanghai Meilian Biotechnology Co., Ltd., Shanghai China) according to the directions of the kit.

Data analysis

Three independent replicates were used for each test. Probit analysis was used to determine the value of lethal concentration. All biological traits data were processed using SPSS V16.0 (SPSS, Inc., Chicago, IL, USA), and the results are shown as the mean ± standard deviation (SD, $n = 3$). All biological traits data were subjected to the analysis of variance (ANOVA) test, and mean differences were evaluated by Tukey's multiple comparison test ($p = 0.05$). Significance was indicated at $p < 0.05$.

Results

Toxicity

The toxicity of emamectin benzoate to the third-instar DBM larvae was determined by the leaf dip method. After 72 h exposure the LC_{50} was 0.173 mg/L, and the LC_5, LC_{10}, and LC_{10} values were also obtained (Table 1).

Sublethal effects of emamectin benzoate on the F_0 generation of DBM

Third-instar DBM larvae were treated with emamectin benzoate at the sublethal/low concentrations of LC_5, LC_{10}, and LC_{20}. The corrected larvae survival rate was 93.3%, 88.7% and 81.3%, respectively. The pupation rate of DBM larvae decreased with increased treatment concentration. The pupation rate of the LC_{10} and LC_{20} treatments was significantly ($F = 7.47$, $df = 11$, $p = 0.010$) lower than the control group (Figure 1A). The pupa weight of LC_{10} and LC_{20} treatment groups increased significantly ($F = 16.49$, $df = 79$, $p = 0.0001$) by 10.53% and 14.63%, respectively (Figure 1B), compared to the control. The pupal period of DBM in the LC_{10} treatment was significantly ($F = 3.70$, $df = 79$, $p = 0.015$) longer than the control group (Figure 1C). However, the three treatment groups had no significant ($F = 2.60$, $df = 11$, $p = 0.125$) effect on the adult emergence rate (Figure 1D). After

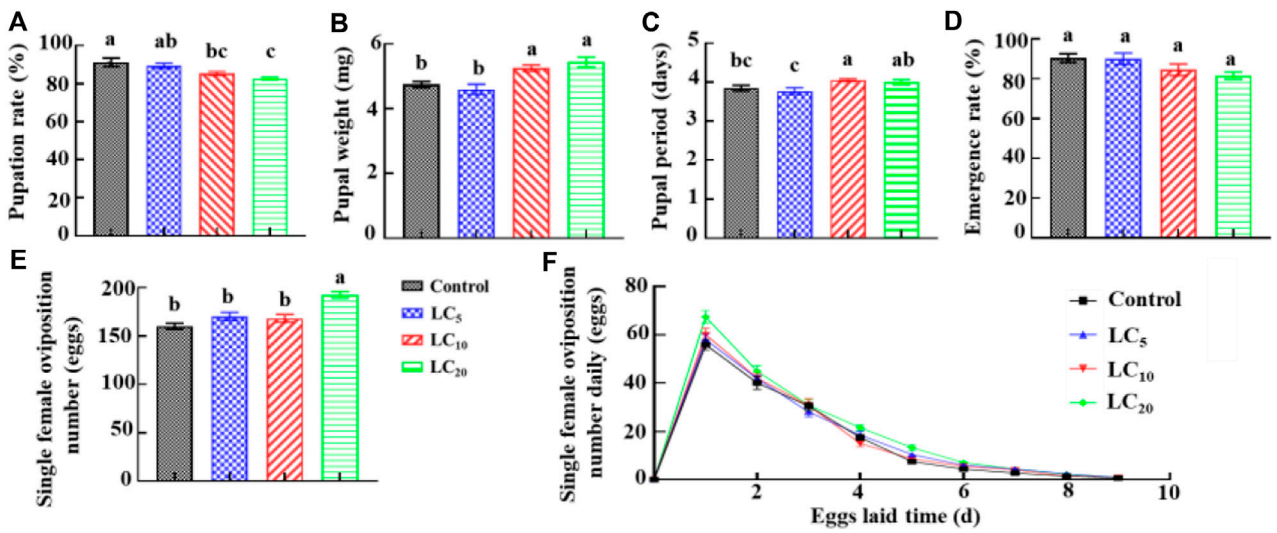


FIGURE 1
Effects of emamectin benzoate sublethal/low concentrations on pupation rate **(A)**, pupal weight **(B)**, pupal period **(C)**, emergence rate **(D)**, single female oviposition number **(E)**, and single female oviposition number daily **(F)** of *P. xylostella* F_0 generation.

pupation and adult emergence, single female oviposition number in the LC_{20} treatment group was 192.7 ± 3.37. It was increased 20.21% compared with the control (Figure 1E). The eggs laying peak in the LC_{20} group was the same as that of the control, but the daily oviposition number increased and the egg laying hours was prolonged by 7.31% (Figure 1F). When compared to the control, the egg hatching rate decreased by 4.08%; however, the average number of larvae in the F_1 generation increased by 22.19, which ultimately led to DBM quantity presumptive increase of 13.84%.

Third-instar DBM larvae were exposed to deposits of emamectin benzoate applied at LC_5, LC_{10}, and LC_{20}. After survivors developed into adults, the longevity of female adults in the LC_{20} group was determined to be significantly ($F = 7.05$, $df = 39$, $p = 0.0008$) shorter than control longevity. The male adult longevity in the three treatments was shortened to various degrees (Figure 2A). The vitellin content in adult females was determined by ELISA. With extension of the time after eclosion, the vitellin content in the LC_{20} treatment group was significantly (72 h: $F = 24.17$, $df = 11$, $p = 0.0002$) higher than in the control, and the maximum increase was 19.00%. The vitellin content of the other groups were significantly (LC_{10} 24 h: $F = 47.59$, $df = 11$, $p = 0.0001$) changed with the eclosion time (Figure 2B).

Sublethal effects of emamectin benzoate on the F_1 generation of DBM

Emamectin benzoate treatment affected the length or width diameter of adult mature eggs. Compared to the control, the

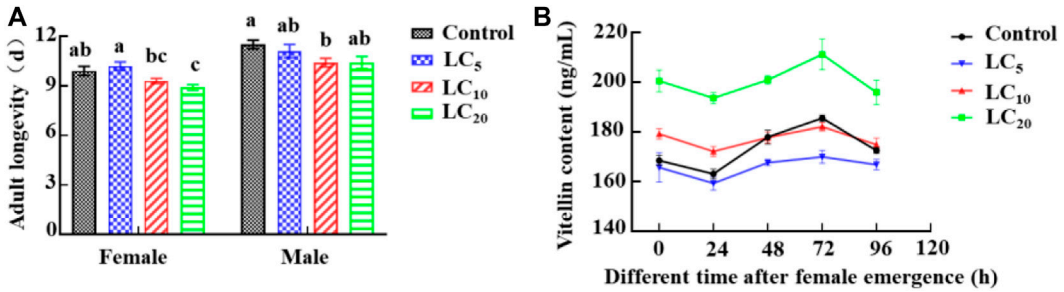


FIGURE 2
Effects of sublethal/low concentrations of emamectin benzoate on adult longevity **(A)** and vitellin content **(B)** in females of the *P. xylostella* F_0 generation.

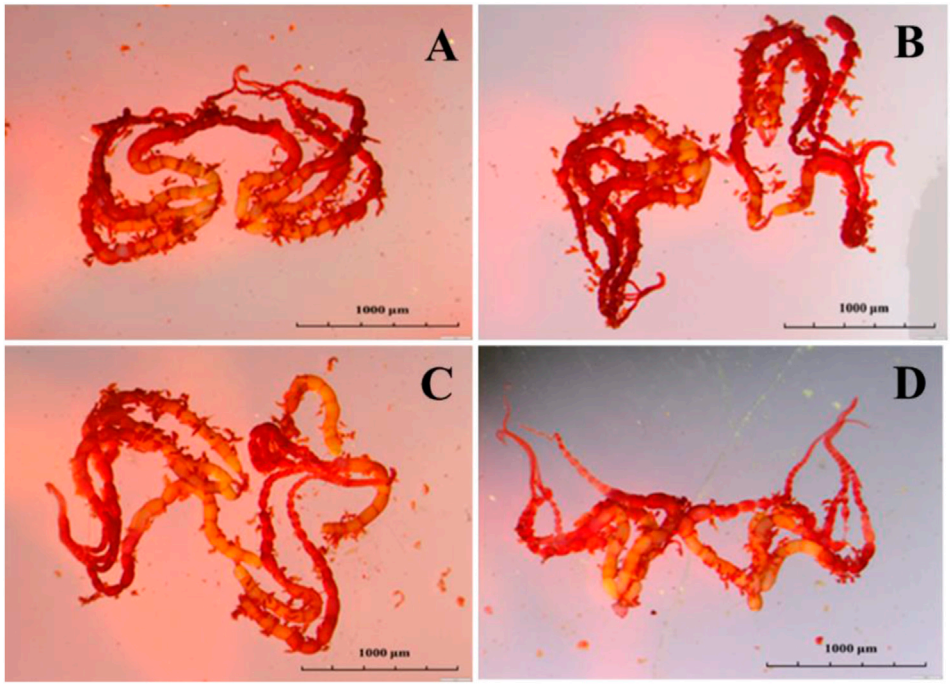


FIGURE 3
Effect of sublethal/low concentrations of emamectin benzoate on the ovary of adult female *P. xylostella*. **(A–D)** are the Control, LC_5, LC_{10}, and LC_{20} treatment group.

TABLE 2 Effects of sublethal/low concentrations of emamectin benzoate on mature eggs, ovarian canal, and larvae of F_1 generation of *P. xylostella*.

Treatment	Mature egg length diameter (μm)	Mature egg width diameter (μm)	Ovariole length (μm)	Mature egg ratio (%)	Egg hatchability (%)	Larval survival (%)
Control	141.38 ± 7.34 a	90.38 ± 4.11 a	2406.41 ± 108.0 a	63.75 ± 2.90 a	88.80 ± 0.67 a	54.44 ± 1.01 a
LC_5	141.75 ± 7.17 a	95.97 ± 3.72 a	2454.08 ± 70.27 a	58.87 ± 3.01 a	85.98 ± 1.15 b	55.22 ± 3.07 a
LC_{10}	133.80 ± 2.76 a	89.30 ± 2.04 a	2489.03 ± 74.44 a	62.63 ± 3.13 a	87.00 ± 0.67 b	52.41 ± 1.27 a
LC_{20}	133.42 ± 4.65 a	86.48 ± 2.33 a	2030.87 ± 62.04 b	58.25 ± 2.95 a	84.72 ± 0.67 b	53.66 ± 2.06 a

Different letters on right side of the same column indicate significance ($p< 0.05$).

TABLE 3 Effects of sublethal/low concentrations of emamectin benzoate on pupae and adults of F_1 generation of *P. xylostella*.

Treatment	Pupation rate (%)	Pupae weight (mg)	Pupal period (d)	Emergence rate (%)	Single female oviposition number (eggs)	Female oviposition time (d)	Adult longevity (d)	
							Female	Male
CK	90.00 ± 1.15 a	6.01 ± 0.18 b	4.47 ± 0.10 a	92.22 ± 1.12 a	180.70 ± 9.98 a	8.00 ± 0.21 a	10.70 ± 0.37 a	11.20 ± 0.25 a
LC_5	88.11 ± 1.23 a	5.98 ± 0.17 b	4.55 ± 0.09 a	93.15 ± 1.84 a	185.20 ± 11.21 a	7.70 ± 0.15 a	10.40 ± 0.40 a	10.90 ± 0.23 a
LC_{10}	89.18 ± 1.26 a	6.47 ± 0.18 ab	4.70 ± 0.10 a	92.49 ± 1.33 a	183.70 ± 9.63 a	8.10 ± 0.18 a	10.20 ± 0.39 a	10.80 ± 0.33 a
LC_{20}	87.74 ± 1.24 a	6.57 ± 0.15 a	4.60 ± 0.09 a	91.27 ± 1.65 a	190.10 ± 8.80 a	7.80 ± 0.29 a	10.20 ± 0.25 a	10.70 ± 0.30 a

Different letters on right side of the same column indicate significance ($p< 0.05$).

length of mature ovarioles in the LC_{20} group was shortened by 15.60% (Figure 3). Emamectin benzoate treatment did not significantly (F = 0.16, *df* = 39, *p* = 0.925) affect the mature egg ratio of the F_1 generation, but it significantly (F = 8.611, *df* = 11, *p* = 0.006) decreased egg hatchability. The treatments had no significant (F = 0.353, *df* = 11, *p* = 0.789) effects on larval survival (Table 2).

The pupa weight of the F_1 offspring in LC_{20} group was significantly (F = 3.187, *df* = 79, *p* = 0.028) increased. However, there was no significant effect on the pupation rate, pupal period, pupal emergence rate, single female oviposition number, female oviposition period, and adult longevity of the F_1 offspring (Table 3).

Discussion

Only a small proportion of chemical pesticide applied directly kills target pests. Most pesticide residue remains in the environment and it may exert sublethal effect on surviving insects. A sublethal/low dose of residual insecticide can effect insect development, morphology, pupa weight, longevity, and fecundity (Zhang et al., 2022). The sublethal effects of pesticides influence the biological characteristics and population development of insects and can provide insight into optimal pesticide use.

Treatment of second-instar *Spodoptera litura* larvae with sublethal doses of chlorantraniliprole or indoxacarb increased the pupal period and increased pupal weight (Moustafa et al., 2021). The fecundity of *Laodelphax striatellus* was significantly decreased by imidacloprid LC_{30} treatment. However, the fecundity was significantly increased when the test insects were treated with an LC_{10} dose of imidacloprid (Zhang et al., 2021b). When the third-instar DBM larvae were treated with a LC_{20} dose of emamectin benzoate in this study the development time of larvae was prolonged by 17.0 ± 3.0 h, the pupation rate of F_0 larvae was decreased by 9.40%, the pupa weight was increased by 14.63%, the average single female oviposition number increased by 30.9 eggs, and the longevity of female adults was shortened by 10.10%. The egg hatch rate decreased by 4.08%, but the number of larvae in the F_1 generation increased. The survival rate, pupation rate and pupae emergence rate of the F_1 generation were similar to the control, and this could ultimately lead to a presumptive population increase of 13.84%. Sublethal concentration (LC_{10} and LC_{30}) exposures of emamectin benzoate had a significant negative impact on the larval, protonymph, and deutonymph developmental periods on *Panonychus citri* (Khan et al., 2021). Female fecundity of *P. citri* was decreased, and the adult pre-oviposition period and total pre-oviposition periodwere increased in the sublethal treatments. The age-stage specific survival rates (S_{xj}), age-specific fecundity (M_x), net reproductive rate (R_0), age-stage specific life expectancy (E_{xj}), and age-stage reproductive value (V_{xj}) was reduced by LC_{10} and LC_{30} exposure (Khan et al., 2021), that of the results were different from this study. The reason need to be further study.

A low concentration nitenpyram induced transgenerational hormesis effects in terms of fitness-related traits and insecticide tolerance in *Nilaparvata lugens* after exposure to the LC_{20} concentrations for six generations (Gong et al., 2022), but, we did not find transgenerational hormesis in DBM by emamectin benzoate treated. It was found that insecticide-induced hormesis in life history traits may augment the development of insecticide tolerance or resistance in pest insects, allowing fitter individuals to survive and reproduce, with significant management and environmental implications (Guedes et al., 2016). The reproduction hormesis of parental generation maybe one of reasons of DBM resurgence. Based on this case, a few of control policies conform to IPM strategies could be used, e.g., combination use of compatible insecticides and biological control agents, 'attract-and-kill' control strategies, development of new safer, environmentally friendly and target-specific insecticides or cultivation safety transgenic crops.

Insect fecundity is mainly regulated by the synthesis of vitellogenin (Vg) and vitellin (Vn) (Jing et al., 2021). Vitellogenin is synthesized in the fat body of adult females and released into the hemolymph. It is then absorbed by oocytes through special channels to synthesize vitellin to provide essential nutrients for egg development (Tufail et al., 2014). The expression of vitellogenin and vitellogenin receptor genes were significantly increased in a flubendiamide resistant DBM strain compared to a susceptible strain, and vitellin content also increased in the resistant strain (Sun et al., 2020). The LC_{30} of emamectin benzoate can reduce the transcription of *CsVg* and *CsVgR* at 24-h, 48-h, and 72-h exposure and decrease the egg production of *C. sinensis* (Yao et al., 2018). After injecting 20-hydroxyecdysone into silkworm larvae, the Vg content in the hemolymph increased, and the Vn content in the ovary also increased. This led to a significant increase in the total egg weight (Shen et al., 2014), this is similar to the results of the present study. When DBM larvae were treated with the LC_{20} of emamectin benzoate, the number of eggs laid by single adults was significantly increased compared to the control. The Vn content of the LC_{20} DBM treatment after adult eclosion was significantly higher than that of the control. This indicated a direct correlation between the Vn content and egg production. Similar results have been elaborated in many other insecticide-exposed insects (Zhou et al., 2020).

In conclusion, the effects of sublethal/low concentration emamectin benzoate on the different life stages of DBM were variable, and the reproductive hormesis on DBM adults were attractive. However, the reason of hormesis deserve further detailed study on insect biology and genetics in combination with DBM resistance. This case makes it necessary for us to re-understand the population development of *P. xylostella*. In addition, combination use of compatible insecticides and

biological control agents, 'attract-and-kill' control strategies, development of new safer, environmentally friendly and target-specific insecticides, one or more these policies can be selected for the control of DBM in fields.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

Conceptualization, C-BX; methodology, C-BX and K-XL; validation, K-XL and YG; formal analysis, C-BX, C-XZ, and K-XL; writing—original draft preparation, YG, C-XZ, and K-XL; writing—review and editing, C-BX, YG, C-XZ, and K-XL; supervision, C-BX. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by the National Natural Science Foundation of China (31972295).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Biondi, A., Desneux, N., Siscaro, G., and Zappalà, L. (2012). Using organic-certified rather than synthetic pesticides may not be safer for biological control agents: Selectivity and side effects of 14 pesticides on the predator *Orius laevigatus*. *Chemosphere* 87 (7), 803–812. doi:10.1016/j.chemosphere.2011.12.082

Deng, Z., Zhang, F., Wu, Z., Yu, Z., and Wu, G. (2016). Chlorpyrifos-induced hormesis in insecticide-resistant and -susceptible *Plutella xylostella* under normal and high temperatures. *Bull. Entomol. Res.* 106 (3), 378–386. doi:10.1017/S000748531600002X

Desneux, N., Decourtye, A., and Delpuech, J. M. (2007). The sublethal effects of pesticides on beneficial arthropods. *Annu. Rev. Entomol.* 52, 81–106. doi:10.1146/annurev.ento.52.110405.091440

El-Sheikh, E. A. (2015). Comparative toxicity and sublethal effects of emamectin benzoate, lufenuron and spinosad on *Spodoptera littoralis* Boisd. (Lepidoptera: Noctuidae). *Crop Prot.* 67, 228–234. doi:10.1016/j.cropro.2014.10.022

Fujiwara, Y., Takahashi, T., Yoshioka, T., and Nakasuji, F. (2002). Changes in egg size of the diamondback moth *Plutella xylostella* (Lepidoptera: Yponomeutidae) treated with fenvalerate at sublethal doses and viability of the eggs. *Appl. Entomol. Zool.* 37, 103–109. doi:10.1303/aez.2002.103

Furlong, M. J., Wright, D. J., and Dosdall, L. M. (2013). Diamondback moth ecology and management: Problems, progress, and prospects. *Annu. Rev. Entomol.* 58 (1), 517–541. doi:10.1146/annurev-ento-120811-153605

Gong, Y., Cheng, S., Desneux, N., Gao, X., Xiu, X., Wang, F., et al. (2022). Transgenerational hormesis effects of nitenpyram on fitness and insecticide tolerance/resistance of *Nilaparvata lugens*. *J. Pest Sci*. doi:10.1007/s10340-022-01494-4

Gope, A., Chakraborty, G., Ghosh, S. M., Sau, S., Mondal, K., Biswas, A., et al. (2022). Toxicity and sublethal effects of fluxametamide on the key biological parameters and life history traits of diamondback moth *Plutella xylostella* (L.). *Agronomy* 12, 1656. doi:10.3390/agronomy12071656

Guedes, R. N. C., Smagghe, G., Stark, J. D., and Desneux, N. (2016). Pesticide-induced stress in arthropod pests for optimized integrated pest management programs. *Annu. Rev. Entomol.* 61, 43–62. doi:10.1146/annurev-ento-010715-023646

Han, W., Zhang, S., Shen, F., Liu, M., Ren, C., and Gao, X.-W. (2012). Residual toxicity and sublethal effects of chlorantraniliprole on *Plutella xylostella* (Lepidoptera: Plutellidae). *Pest Manag. Sci.* 68, 1184–1190. doi:10.1002/ps.3282

Huang, N. X., Jaworski, C. C., Desneux, N., Zhang, F., Yang, P. Y., and Wang, S. (2020). Long-term and large-scale releases of *Trichogramma* promote pesticide decrease in maize in northeastern China. *Entomol. Gen.* 40, 331–335. doi:10.1127/entomologia/2020/0994

Jia, B., Zhang, J., Hong, S., Chang, X., and Li, X. (2022). Sublethal effects of chlorfenapyr on *Plutella xylostella* (Lepidoptera: Plutellidae). *Pest Manag. Sci.* doi:10.1002/ps.7175

Jing, Y.-P., Wen, X.-P., Li, L.-J., Zhang, S.-J., Zhang, C., and Zhou, S.-T. (2021). The vitellogenin receptor functionality of the migratory locust depends on its phosphorylation by juvenile hormone. *Proc. Natl. Acad. Sci. U. S. A.* 118 (37), e2106908118. doi:10.1073/PNAS.2106908118

Khan, M. M., Ali, M. W., Hafeez, M., Fan, Z.-Y., Ali, S., and Qiu, B.-L. (2021). Lethal and sublethal effects of emamectin benzoate on life-table and physiological parameters of citrus red mite, *Panonychus citri*. *Exp. Appl. Acarol.* 85, 173–190. doi:10.1007/s10493-021-00667-7

Khan, M. M., Nawaz, M., Hua, H., Cai, W., and Zhao, J. (2018). Lethal and sublethal effects of emamectin benzoate on the rove beetle, *Paederus fuscipes*, a non-target predator of rice Brown planthopper. *Ecotoxicol. Environ. Saf.* 165, 19–24. doi:10.1016/j.ecoenv.2018.08.047

Liang, H.-Y., Yang, X.-M., Sun, L.-J., Zhao, C.-D., Chi, H., and Zheng, C.-Y. (2021). Sublethal effect of spirotetramat on the life table and population growth of *Frankliniella occidentalis* (Thysanoptera: Thripidae). *Entomol. Gen.* 41 (3), 219–231. doi:10.1127/entomologia/2020/0902

Liang, P., Gao, X. W., and Zheng, B. Z. (2003). Genetic basis of resistance and studies on cross-resistance in a population of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). *Pest Manag. Sci.* 59 (11), 1232–1236. doi:10.1002/ps.760

López, J. D., Latheef, M. A., and Hoffmann, W. C. (2010). Effect of emamectin benzoate on mortality, proboscis extension, gustation and reproduction of the corn earworm, *Helicoverpa zea*. *J. Insect Sci.* 10 (89), 89–16. doi:10.1673/031.010.8901

Lu, Y., Wu, K., Jiang, Y., Guo, Y., and Desneux, N. (2012). Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services. *Nature* 487, 362–365. doi:10.1038/nature11153

Mokbel, E., and Huesien, A. (2020). Sublethal effects of emamectin benzoate on life table parameters of the cotton leafworm, *Spodoptera littoralis* (Boisd). *Bull. Natl. Res. Cent.* 44 (1), 155–158. doi:10.1186/s42269-020-00412-x

Moustafa, M. A. M., Fouad, E. A., Yasmin, A. M., Hamow, K. Á. A., Mikó, Z., Molnár, B. P., et al. (2021). Toxicity and sublethal effects of chlorantraniliprole and indoxacarb on *Spodoptera littoralis* (Lepidoptera:Noctuidae). *Appl. Entomol. Zool.* 56, 115–124. doi:10.1007/S13355-020-00721-7

Rodríguez-Rodríguez, J. F., Cerna-Chávez, E., Ochoa-Fuentes, Y. M., Landeros-Flores, J., Guevara-Acevedo, L. P., and Cisneros-López, H. C. (2021). Sublethal effects and costs of resistance to abamectin in diamondback moth (*Plutella xylostella*) (Lepidoptera: Plutellidae). *Rev. Colomb. Entomol.* 47 (2), e10657. doi:10.25100/socolen.v47i2.10657

Shan, Y.-X., Zhu, Y., Li, J.-J., Wang, N.-M., Yu, Q.-T., and Xue, C.-B. (2020). Acute lethal and sublethal effects of four insecticides on the lacewing (*Chrysoperla sinica* Tjeder). *Chemosphere* 250, 126321. doi:10.1016/j.chemosphere.2020.126321

Shen, G. W., Lin, Y., Lv, Y. H., Wang, J. Y., Xing, R. M., and Xia, Q. Y. (2014). Regulation of ecdysone on vitellogenin gene expression in silkworm eggs. *Chin. J. Biochem. Mol. Biol.* 30 (11), 1106–1112. doi:10.13865/j.cnki.cjbmb.2014.11.08

Su, C.-Y., and Xia, X.-M. (2020). Sublethal effects of methylthio-diafenthiuron on the life table parameters and enzymatic properties of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). *Pestic. Biochem. Physiol.* 162, 43–51. doi:10.1016/j.pestbp.2019.08.011

Sun, S.-Q., Wang, N.-M., Li, J.-J., Jin, M.-H., and Xue, C.-B. (2020). Reduced fecundity and regulation of reproductive factors in flubendiamide-resistant strains of *Plutella xylostella*. *Pestic. Biochem. Physiol.* 169, 104668. doi:10.1016/j.pestbp.2020.104668

Sun, X., Wei, R., Li, L., Zhu, B., Liang, P., and Gao, X.-W. (2022). Resistance and fitness costs in diamondback moths after selection using broflanilide, a novel meta-diamide insecticide. *Insect Sci.* 29, 188–198. doi:10.1111/1744-7917.12917

Tamilselvan, R., Kennedy, J. S., and Suganthi, A. (2021). Sublethal and transgenerational effects of spinetoram on the biological traits of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). *Ecotoxicology* 30, 667–677. doi:10.1007/s10646-021-02385-7

Tufail, M., Nagaba, Y., Elgendy, A. M., and Takeda, M. (2014). Regulation of vitellogenin genes in insects. *Entomol. Sci.* 17 (3), 269–282. doi:10.1111/ens.12086

Wang, G., Huang, X., Wei, H., and Fadamiro, H. Y. (2011). Sublethal effects of larval exposure to indoxacarb on reproductive activities of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). *Pestic. Biochem. Physiol.* 101, 227–231. doi:10.1016/j.pestbp.2011.09.010

Wang, L., Ma, X., Hua, R. M., Tang, F., Wu, X. W., Li, X. D., et al. (2009). Degradation dynamics and compound effect of chlorpyrifos and emamectin benzoate on cabbage and glass slides. *J. Anhui. Agric. Univ.* 36 (2), 309–314. doi:10.13610/j.cnki.1672-352x.2009.02.009

Wang, S., Qi, Y., Desneux, N., Shi, X., Biondi, A., and Gao, X.-W. (2017). Sublethal and transgenerational effects of short-term and chronic exposures to the neonicotinoid nitenpyram on the cotton aphid *Aphis gossypii*. *J. Pest Sci.* 90, 389–396. doi:10.1007/s10340-016-0770-7

Whalon, M. E., Mota-Sanchez, D., and Hollingworth, R. M. (2019). *The arthropod pesticide resistance database [DB/OL]* East Lansing, MI: Michigan State University. Available at: http://www.pesticideresistance.org (Accessed 25 June 2019).

Wu, J., Ge, L., Liu, F., Song, Q., and Stanley, D. (2019). Pesticide-induced planthopper population resurgence in rice cropping systems. *Annu. Rev. Entomol.* 65 (1), 409–429. doi:10.1146/annurev-ento-011019-025215

Yao, Q., Xu, S., Dong, Y., Que, Y., Quan, L., and Chen, B. (2018). Characterization of vitellogenin and vitellogenin receptor of *Conopomorpha sinensis* Bradley and their responses to sublethal concentrations of insecticide. *Front. Physiol.* 9, 1250. doi:10.3389/fphys.2018.01250

Zhang, C.-X., Wang, Z.-J., Li, J.-J., Wang, N.-M., and Xue, C.-B. (2022). Sublethal effects of tolfenpyrad on the development, reproduction, and predatory ability of *Chrysoperla sinica*. *Ecotoxicol. Environ. Saf.* 236, 113482. doi:10.1016/J.ECOENV.2022.113482

Zhang, Q., Liu, Y., Wyckhuys, K. A., Liang, H., Desneux, N., and Lu, Y. (2021a). Lethal and sublethal effects of chlorantraniliprole on *Helicoverpa armigera* adults enhance the potential for use in 'attract-and-kill' control strategies. *Entomol. Gen.* 41 (1), 111–120. doi:10.1127/entomologia/2020/1104

Zhang, Y. Y., Xu, G., Jiang, Y., Ma, C., and Yang, G. (2021b). Sublethal effects of imidacloprid on fecundity, apoptosis and virus transmission in the small Brown planthopper *Laodelphax striatellus*. *Insects* 12 (12), 1131. doi:10.3390/insects12121131

Zhang, Z., Li, J. H., and Gao, X.-W. (2012). Sublethal effects of metaflumizone on *Plutella xylostella* (Lepidoptera: Plutellidae). *J. Integr. Agric.* 11, 1145–1150. doi:10.1016/S2095-3119(12)60108-7

Zhou, C., Yang, X. B., Yang, H., Long, G. Y., and Jin, D. C. (2020). Effects of sublethal concentrations of insecticides on the fecundity of *Sogatella furcifera* (Hemiptera: Delphacidae) via the regulation of vitellogenin and its receptor. *J. Insect Sci.* 20 (5), 14. doi:10.1093/jisesa/ieaa099

frontiers | Frontiers in Physiology

TYPE Original Research
PUBLISHED 01 November 2022
DOI 10.3389/fphys.2022.1029319

OPEN ACCESS

EDITED BY
Qingli Shang,
Jilin University, China

REVIEWED BY
J. Joe Hull,
Agricultural Research Service (USDA), United States
Chunqing Zhao,
Nanjing Agricultural University, China

*CORRESPONDENCE
Xiaolei Zhang,
zhangxiaolei@yangtzeu.edu.cn

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Invertebrate Physiology, a section of the journal Frontiers in Physiology

RECEIVED 27 August 2022
ACCEPTED 18 October 2022
PUBLISHED 01 November 2022

CITATION
Meng H, Huang R, Wan H, Li J, Li J and Zhang X (2022), Insecticide resistance monitoring in field populations of *Chilo suppressalis* Walker (Lepidoptera: Crambidae) from central China. *Front. Physiol.* 13:1029319. doi: 10.3389/fphys.2022.1029319

Insecticide resistance monitoring in field populations of *Chilo suppressalis* Walker (Lepidoptera: Crambidae) from central China

Haoran Meng[1†], Rong Huang[1†], Hu Wan[2], Jianhong Li[2], Junkai Li[1] and Xiaolei Zhang[1*]

[1]Hubei Engineering Technology Center of Forewarning and Management of Agricultural and Forestry Pests, College of Agriculture, Yangtze University, Jingzhou, China, [2]Hubei Insect Resources Utilization and Sustainable Pest Management Key Laboratory, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, China

Chilo suppressalis Walker (Lepidoptera: Crambidae) is a devastating rice crop pest in China. Chemical insecticides have been used to effectively managing *C. suppressalis* field populations in most of China's agricultural regions. However, due to the intensive and extensive application of these insecticides, *C. suppressalis* has developed widespread resistance to many active ingredients. Thus, insecticide resistance development is a genuine concern for all crop specialists and growers. In this study, using the topical application method, we assessed the susceptibility of forty-six field populations of *C. suppressalis* to three insecticides in three Central Chinese provinces from 2010 to 2021. Our findings revealed that field populations of *C. suppressalis* built moderate to high levels of resistance to triazophos (Resistance Ratio (RR) = 41.9–250.0 folds), low to moderate levels of resistance to chlorpyrifos (RR = 9.5–95.2 folds), with the exception of the Zhijiang population in 2013 and the Xinyang population in 2015 at 4.8 folds and 3.4 folds resistance rates, respectively, despite showing susceptibility, and low and moderate levels of resistance to abamectin (RR = 4.1–53.5 folds). There were significant correlations between the activity of the detoxification enzymes (CarE) and the log LD_{50} values of triazophos. These results should help effective insecticide resistance management strategies reduce the risk of rapid build-ups of resistance to insecticides and slow down the process of selection for insecticide resistance.

KEYWORDS

***Chilo suppressalis* Walker, triazophos, chlorpyrifos, abamectin, insecticide resistance monitoring**

Introduction

The striped rice borer, *Chilo suppressalis* Walker (Lepidoptera: Crambidae), is a devastating rice pest in Asia, Oceania and south Europe (Meng et al., 2019). The larvae of *C. suppressails* bore into the leaf sheaths and stems of rice, then feed on the tissues of leaf sheath and stem of rice, forming dead sheaths, dead hearts and white heads (Lu et al., 2017). Since the 1990s, *C. suppressalis* populations have spread out and caused severe damages to rice-growing areas in China due to the use of hybrid varieties and changes in the rice cultivation system (Zhu and Cheng, 2013; Xu et al., 2015). *C. suppressalis* outbreaks have led to reduced yields of rice and related economic losses (Li et al., 2020), with statistics demonstrating that they have damaged more than 14 million hectares of rice fields in China every year for the last 10 years by the National Agro-Tech Extension and Service Center (NATESC) (NATESC, 2022).

To fight the devastation caused by these insects, farmers have adopted the widespread application of insecticides to manage *C. suppressalis* over the last several decades (Zhao, 2019). Unfortunately, this has resulted in the evolution of insecticide resistance by field populations of *C. suppressalis*. *C. suppressalis* currently has different levels of resistance to organochlorines, organophosphates, carbamates, phenylpyrazoles, avermectins, nereistoxin analogues and diamide groups in China (Huang et al., 2017; Lu et al., 2017; Yao et al., 2017; Xu et al., 2018; Mao et al., 2019; Wei et al., 2019; Zhao, 2019; Huang et al., 2020; Sun et al., 2021; Zhao et al., 2021). The evolution of *C. suppressalis'* resistance to insecticides is well documented in the Arthropod Pesticide Resistance Database (APRD) (Mota-Sanchez and Wise, 2022). The development of insecticide resistance by *C. suppressalis* has diminished the ability to control field populations of this insecticide. Nonetheless, this resistance has only served to increase the use of insecticides to control *C. suppressalis*.

Chlorpyrifos, triazophos, and abamectin are the major insecticides to have been predominantly used to control *C. suppressalis* for several decades (Zhao, 2019). In China, triazophos was used to control *C. suppressalis* in the early 1990s, chlorpyrifos was adopted as an alternative agent in rice fields after the field population of *C. suppressalis* developed high resistance levels to triazophos, and abamectin has been in use against field populations of *C. suppressalis* since the end of the 20th century (Yao, 2015). Presently, only a limited number of insecticides can contain *C. suppressalis* in China. Hence, monitoring and understanding the status of insecticide resistance is fundamental to successful resistance management (Sun et al., 2021; Xu et al., 2021). In addition, insecticide resistance levels and the speed of development of resistance by field populations of *C. suppressalis* differ between regions depending on the closeness of the association with the cultivation system, the history of insecticide use, and the degree of insecticide use in a country or region. Therefore, monitoring insecticide resistance by *C. suppressalis* field populations in different areas is of great significance to the rational choice of insecticides, the rotation of insecticide MoA (modes of action) groups, insecticide mixtures, reducing the risk of insecticide resistance, preventing further insecticide resistance development, and improving insecticide control efficiency.

Managing insecticide resistance requires knowing the mechanism of insecticide resistance (Zhang et al., 2017a). Previous studies have demonstrated how understanding the mechanism behind the resistance to insecticides is critical to pest management (He et al., 2012; Zhang et al., 2017b). Insecticide resistance often stems from gene regulatory changes that culminate in increased detoxification activities, such as carboxylesterase (CarE), glutathione *S*-transferase (GST), and cytochrome P450 monooxygenases (P450) (Heckel, 2012; Mao et al., 2019; Bhatt et al., 2021; Meng et al., 2022; Nauen et al., 2022). The increase in these detoxifying enzymes is the most common resistance mechanism (Heckel, 2012; Enders et al., 2020). Furthermore, insecticides with cross-resistance share the same resistance mechanisms, such as alternate target-sites, enhanced enzyme systems, or reduced penetration (Qian et al., 2008). Thus, applying only one of them could still result in the resistance to all of them, and these insecticides cannot be used rotationally in resistance management.

The present study monitored insecticide resistance by field populations of *C. suppressalis* to organophosphate insecticides (chlorpyrifos and triazophos) and an avermectin insecticide (abamectin) from 2010 to 2021 to evaluate the levels of resistance to insecticides by these field populations. The outcome of this investigation could provide a scientific basis for the rational selection of insecticides and delay the development of resistance by *C. suppressalis* field populations.

Materials and methods

Insects

Forty-six field populations of *C. suppressalis* were gathered from the rice paddy fields of Gongan, Jianli, Wuxue, Zaoyang, Zhijiang, Ezhou, Xiaogan, Chibi, Xiantao, Qianjiang, Songzi, Changde, Changsha, and Xinyang in Central China (Table 1) from 2010 to 2021. More than 100–2000 larvae were collected from each site. The fourth instar larvae were used for bioassays. The fourth-instar larvae of the first (F1) and second (F2) generations were used for the susceptibility bioassay.

Chemicals

Technical grade chlorpyrifos (98%) (CAS#: 2921-88-2) and triazophos (80%) (CAS#: 24017-47-8) were acquired from

10.3389/fphys.2022.1029319

TABLE 1 Sampling sites, dates, and developmental stages of *C. suppressalis* collected from fields.

Population	Location	Collection date (year-month-day)	Site	Insect stage
WX-2010	Wuxue, Hubei	2010-06-23	30.11° N, 115.59° E	larva
WX-2011	Wuxue, Hubei	2011-06-30	30.11° N, 115.59° E	larva
WX-2012	Wuxue, Hubei	2012-07-01	30.11° N, 115.59° E	larva
WX-2013	Wuxue, Hubei	2013-07-02	30.11° N, 115.59° E	larva
WX-2014	Wuxue, Hubei	2014-07-02	30.11° N, 115.59° E	larva
WX-2015	Wuxue, Hubei	2015-06-02	30.11° N, 115.59° E	larva
ZJ-2010	Zhijiang, Hubei	2010-07-05	30.26° N, 111.55° E	larva
ZJ-2011	Zhijiang, Hubei	2011-06-24	30.26° N, 111.55° E	larva
ZJ-2012	Zhijiang, Hubei	2012-06-23	30.26° N, 111.55° E	larva
ZJ-2013	Zhijiang, Hubei	2013-06-29	30.26° N, 111.55° E	larva
ZJ-2014	Zhijiang, Hubei	2014-07-04	30.26° N, 111.55° E	larva
ZJ-2015	Zhijiang, Hubei	2015-07-07	30.26° N, 111.55° E	larva
JL-2010	Jianli, Hubei	2020-07-28	29.91° N, 112.77° E	larva
JL-2011	Jianli, Hubei	2011-06-25	29.91° N, 112.77° E	larva
JL-2012	Jianli, Hubei	2012-06-30	29.91° N, 112.77° E	larva
JL-2013	Jianli, Hubei	2013-06-29	29.91° N, 112.77° E	larva
JL-2014	Jianli, Hubei	2014-06-25	29.91° N, 112.77° E	larva
ZY-2010	Zaoyang, Hubei	2010-07-30	31.98° N, 112.76° E	larva
ZY-2011	Zaoyang, Hubei	2011-08-01	31.98° N, 112.76° E	larva
ZY-2012	Zaoyang, Hubei	2012-08-07	31.98° N, 112.76° E	larva
ZY-2013	Zaoyang, Hubei	2013-08-12	31.98° N, 112.76° E	larva
ZY-2014	Zaoyang, Hubei	2014-08-03	31.98° N, 112.76° E	larva
ZY-2015	Zaoyang, Hubei	2015-07-23	31.98° N, 112.76° E	larva
GA-2010	Gongan, Hubei	2010-08-01	30.05° N, 112.19° E	larva
GA-2011	Gongan, Hubei	2011-07-12	30.05° N, 112.19° E	larva
GA-2012	Gongan, Hubei	2012-07-22	30.05° N, 112.19° E	larva
GA-2013	Gongan, Hubei	2013-06-30	30.05° N, 112.19° E	larva
GA-2014	Gongan, Hubei	2014-07-23	30.05° N, 112.19° E	larva
GA-2015	Gongan, Hubei	2015-07-03	30.05° N, 112.19° E	larva
EZ-2010	Ezhou, Hubei	2010-07-20	30.35° N, 114.71° E	larva
EZ-2011	Ezhou, Hubei	2011-07-14	30.35° N, 114.71° E	larva
EZ-2012	Ezhou, Hubei	2012-06-20	30.35° N, 114.71° E	larva
EZ-2013	Ezhou, Hubei	2013-06-20	30.35° N, 114.71° E	larva
TM-2015	Tianmen, Hubei	2015-06-17	30.42° N, 114.46° E	larva
XG-2015	Xiaogan, Hubei	2015-06-03	30.95° N, 114.07° E	larva
CB-2015	Chibi, Hubei	2015-06-27	29.66° N, 113.85° E	larva
CS-2015	Changsha, Hunan	2015-06-15	28.18° N, 112.57° E	larva
XY-2015	Xinyang, Henan	2015-06-12	32.25° N, 113.88° E	larva
QJ-2020	Qianjiang, Hubei	2020-08-25	30.44° N, 112.98° E	larva
QJ-2021	Qianjiang, Hubei	2021-08-16	30.39° N, 112.66° E	larva
SZ-2020	Songzi, Hubei	2020-06-12	30.01° N, 111.90° E	larva
SZ-2021	Songzi, Hubei	2021-08-10	30.01° N, 111.90° E	larva
XT-2020	Xiantao, Hubei	2020-07-01	30.39° N, 113.17° E	larva
XT-2021	Xiantao, Hubei	2021-06-29	30.37° N, 113.35° E	larva
CD-2020	Changde, Hunan	2020-08-16	29.62° N, 111.78° E	larva
CD-2021	Changde, Hunan	2021-06-22	29.63° N, 111.74° E	larva

Hubei Kangbaotai Fine-Chemicals CO., Ltd., Hubei, China, and technical grade abamectin (97%) (CAS#: 71751-41-2) was procured from Chemtac Chemical CO., Ltd., Hebei, China. 1-naphtol (SCR®) (CAS#: 90-15-3), Coomassie brilliant blue G250 (Our-chem®) (CAS#: 6101-58-1), 1-phenyl-2-thiourea (Ourchem®) (CAS#: 103-85-5), 4-Nitroanisole (Ourchem®) (CAS#: 100-17-4) and Fast blue B salt (Ourchem®) (CAS#: 14263-94-6) were procured from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. 1-naphthyl acetate (CAS#: 830-81-9) and phenylmethanesulfonyl fluoride (PMSF) (CAS#: 329-98-6) were procured from Shanghai Macklin Biochemical Co. Ltd., Shanghai, China. Dithiothreitol (DTT) (CAS#: 3483-12-3) was procured from Beijing Solarbio Science & Technology Co., Ltd., Beijing, China.

Bioassays

Insecticide resistance to chlorpyrifos, triazophos and abamectin by *C. suppressalis* was assessed using topical application bioassays (He et al., 2007; Su et al., 2014a). All the insecticides were dissolved in acetone and then diluted into a series of acetone concentrations. Five to six doses of each insecticide were created, with each dose (concentration) made in triplicates. The control experiment was treated with acetone in place of an insecticide solution. Four filter papers were placed on the base of each petri dish (9 cm diameter) and hydrated by pipetting 5 ml of water onto the filter papers. Ten larvae were transferred onto rice stems (approximately 5–7 cm sections of the stem) in petri dishes (9 cm in diameter) for treatment with each replicate of a dose. The culture conditions for the treated larvae were controlled at 28 ± 1°C and a photoperiod of 16:8 h (L:D). Mortalities were checked 48 h later to determine the impact of chlorpyrifos and triazophos, and 72 h later for abamectin effect.

Enzyme assays

Detoxification enzyme sources were isolated from the third instar larvae of *C. suppressalis via* the following method. The 4th instar larvae were placed in a 2.0 ml homogenizer, inundated with 1 ml 0.1 mol/L phosphate buffer (at 4°C and pH 7.5), and homogenized in an ice-bath. The resulting solutions were collected and centrifuged at 18,000 r/min at 4°C for 30 min, and the obtained supernatants were used as detoxification enzyme solutions for enzyme assays. Protein content was determined utilizing the protein assay kit (Bio-Rad Laboratories, Inc., Hercules, CA, United States), employing bovine serum albumin as the standard. Enzymatic assays were conducted in three repetitions, and each assay was repeated at least twice.

Carboxylesterase (CarEs) activity was measured as demonstrated previously (Zhang et al., 2017a) but with a slight modification. 1 ml of the substrate solution of naphthyl acetate (1×10^{-6} mol/L physostigmine) was introduced into an EP-tube, preheated in a water bath at 37°C for 2 min, and doused with 0.20 ml of a diluted enzyme source. The mixture was then left to react at 37°C for 15 min, after which approximately 0.2 ml of the colorimetric reagent FAST Blue B was applied to terminate the reaction. Absorbance was measured with a microplate reader (Bio-Rad) at 600 nm after 30 min of incubation at room temperature.

Glutathione *S*-transferase activity was assessed using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate as described previously (Mao et al., 2019). 50 µl of an enzyme solution was added to a mixture of 790 µl of phosphate buffer (pH 6.5), 30 µl of a substrate (30 mM CDNB), and 30 µl of reduced glutathione (50 mM GSH), and the change in absorbance was measured at 340 nm at 5 s intervals for 2 min.

Cytochrome P450 monooxygenase activity was evaluated using p-nitroanisole (PNA) as the substrate as established previously (Zhang et al., 2017a). 675 µl of an enzyme source was added to a mixture of 750 µl of 2 µm PNA (p-nitroanisole) and 75 µl of 9.6 µM NADPH, and the change in absorbance was measured at 405 nm after 30 min of incubation at 34°C.

Statistical analysis

Mortality data were corrected using Abbott's formula, and LD_{50} values and 95% confidence interval (CI) were calculated employing the probit analysis. The resistance ratio (RR) was determined by dividing the LD_{50} value of a field population by the corresponding LD_{50} value of the susceptible baseline (Table 2). The degree of resistance was classified as demonstrated by Shao et al. (2013): resistance with RR ≤ 5 folds was classified as susceptibility, RR = 5–10 folds as a low resistance level, RR = 10–100 folds as a moderate resistance level and RR > 100 folds as a high resistance level. Correlations between variables were established using the Pearson method *via* the IBM SPSS Statistics 25 software package. $p < 0.05$ was considered statistically significant.

Results

Insecticide resistance

The field populations of *C. suppressalis* developed low to moderate levels of resistance to triazophos (Table 3; Figure 1, Figure 2). Specifically, from 2010 to 2014, 41.4% of the field populations of *C. suppressalis* were highly resistant to triazophos (RR = 101.6–250.0 folds), while 58.6% were moderately resistant (RR = 45.2–100.0 folds) (Table 3). In general, the field populations of *C. suppressalis* showed moderate levels of resistance from 2020 to 2021 (RR = 41.9–80.6 folds)

TABLE 2 The LD_{50} values of the susceptibility baseline of *C. suppressalis*.

Insecticide group	Insecticide	LD_{50} (95% CI[a]) μg/larva	Reference
Organophosphates	Chlorpyrifos	0.0084 (0.0073–0.0095)	Su et al. (2014a)
	Triazophos	0.0062 (0.0051–0.0074)	He et al. (2007)
Avermectins	Abamectin	0.00017 (0.00014–0.00020)	He et al. (2007)

[a]CI, confidence limit.

(Table 3). There were also high levels of resistance to triazophos in 2010 by the field populations of *C. suppressalis* collected from Zaoyang, the first population to highly resist the effects of triazophos in Hubei. The LD_{50} values ranged from 0.11 to 1.55 μg/larva, with a 14.1 folds variation, showing relatively inhomogeneous responses across the field populations. The results revealed that *C. suppressalis'* resistance to triazophos increased first and then decreased in the last decade (Figure 1). However, *C. suppressalis'* resistance ratio to triazophos in the same location fluctuated greatly across the years (Figure 2).

The field populations of *C. suppressalis* showed susceptibility and low to moderate levels of resistance to chlorpyrifos (Table 3; Figure 1, Figure 2). Forty-two of forty-six field populations of *C. suppressalis* maintained moderate resistance levels to chlorpyrifos from 2010 to 2021 (Table 3), with the other four collected from Zhijiang in 2011 and 2013, Zaoyang in 2012 and Xinyang in 2015 displaying low levels of resistance to chlorpyrifos (Table 3). The LD_{50} values ranged from 0.04 to 0.64 μg/larva, with a 16.0 folds variation, pointing to relatively inhomogeneous responses among the field populations. Meanwhile, there were variations in the levels of resistance to chlorpyrifos within the same regions of Centra China across the years (Figure 2). However, the tendency for the degree of resistance to chlorpyrifos to rise was not notable (Figure 1).

The field populations of *C. suppressalis* exhibited susceptibility, as well as low and moderate levels of resistance to abamectin (Table 3; Figure 1, Figure 2). Twenty-eight of thirty-one field populations of *C. suppressalis* gathered in 2010, 2011, 2013, 2014, 2020, and 2021 built moderate levels of resistance (RR = 11.2–36.3 folds) to abamectin, with the other three populations (Ezhou population in 2010 and Zaoyang and Gongan populations in 2013) displaying low levels of resistance (8.8–10 folds) against abamectin (Table 3). According to analyses of the 2012 impact, field populations from Jianli, Ezhou, and Gongan were moderately resistant to abamectin, and the remaining populations remained lowly resistant (Table 3). Six of nine field populations amassed from Central China in 2015 demonstrated moderate levels of resistance to abamectin, with the other three from Wuxue, Zaoyang and Chibi maintaining low levels of resistance (Table 3). Like *C. suppressalis'* resistance to chlorpyrifos, the tendency for the degree of resistance to abamectin to rise was not apparent; however, the resistance ratio to abamectin varied significantly within the same regions across the years (Figures 1, 2).

Enzyme activity

Enzyme activity, including carboxylesterase (CarE), glutathione *S*-transferase (GST) and cytochrome P450 monooxygenase (P450) differed between *C. suppressalis* populations (Figure 3). Findings fluctuated within the same regions across the years (Figure 3). Relative CarE activities ranged from 1.00 ± 0.06 (ZJ-2015) to 2.83 ± 0.18 (GA-2013), resulting in a 2.8-fold variation in esterase activity (Figure 3). There were also significant differences in relative glutathione *S*-transferase activities, ranging from 1.00 ± 0.05 (JL-2012) to 2.96 ± 0.13 (GA-2013), with a 3.0-fold variation (Figure 3). The relative cytochrome P450-dependent monooxygenase activity varied from 1.00 ± 0.07 (JL-2012) to 3.36 ± 0.20 (XY-2015), with a 3.4-fold variation (Figure 3).

Pair-wise correlation analysis

There were no correlations between insecticides (Table 4) and no significant correlations between enzyme activities (GST and P450) and the susceptibility of *C. suppressalis* populations to the insecticides evaluated (Table 4). Noteworthy correlations between enzyme activities, like esterase, and the susceptibility of *C. suppressalis* populations to triazophos were recorded ($r = 0.49$, $p = 0.030$) (Table 4). There were no meaningful correlations between the activity of CarE and other insecticides (Table 4).

Discussion

The extensive use of insecticides has resulted in the development of resistance by numerous important pest species. So far, 656 species have built insecticide resistance to 324 compounds, with 21870 cases of resistance having been reported (Sparks and Nauen, 2015; Mota-Sanchez and Wise, 2022). Agricultural productivity has been jeopardized by the widespread resistance to insecticides by crop pests (Roush and

TABLE 3 The resistance levels of *C. suppressalis* field populations to insecticides.

Population	Triazophos		Chlorpyrifos		Abamectin	
	LD_{50} (95%CI[a]) μg/larva	RR[b]	LD_{50} (95%CI) μg/larva	RR	LD_{50} (95%CI) μg/larva	RR
WX-2010	0.39 (0.28–0.54)	62.9	0.13 (0.10–0.18)	15.5	0.0034 (0.0026–0.0048)	20.1
WX-2011	0.45 (0.26–0.78)	72.6	0.19 (0.10–0.78)	22.6	0.0047 (0.0035–0.0065)	27.6
WX-2012	0.95 (0.72–1.25)	153.2	0.12 (0.08–0.21)	14.3	0.0014 (0.0010–0.0020)	8.2
WX-2013	1.55 (0.94–2.58)	250.0	0.40 (0.15–0.85)	47.6	0.0040 (0.0022–0.0074)	23.5
WX-2014	1.16 (0.76–2.58)	187.1	0.22 (0.10–0.99)	26.2	0.0022 (0.0014–0.0034)	13.9
WX-2015	nt		0.20 (0.13–0.30)	23.8	0.0007 (0.0002–0.0025)	4.1
ZJ-2010	0.29 (0.23–0.37)	46.8	0.15 (0.11–0.21)	17.9	0.0021 (0.0014–0.0030)	12.5
ZJ-2011	0.58 (0.43–0.79)	93.6	0.08 (0.05–0.14)	9.5	0.0021 (0.0013–0.0030)	12.4
ZJ-2012	0.41 (0.24–0.70)	66.1	0.35 (0.26–0.46)	41.7	0.0010 (0.0009–0.0012)	5.9
ZJ-2013	0.62 (0.56–0.68)	100.0	0.04 (0.02–0.08)	4.8	0.0020 (0.0011–0.0033)	11.8
ZJ-2014	0.63 (0.40–1.56)	101.6	0.11 (0.05–0.41)	13.1	0.0039 (0.0025–0.0069)	22.9
ZJ-2015	nt		0.44 (0.24–0.83)	52.4	0.0091 (0.0072–0.0116)	53.5
JL-2010	0.32 (0.23–0.44)	51.6	0.19 (0.13–0.29)	22.6	0.0040 (0.0030–0.0060)	23.6
JL-2011	0.63 (0.42–0.94)	101.6	0.16 (0.09–0.47)	19.0	0.0024 (0.0017–0.0030)	13.9
JL-2012	0.80 (0.60–1.08)	129.0	0.11 (0.08–0.20)	13.1	0.0023 (0.0015–0.0043)	13.5
JL-2013	0.72 (0.57–0.92)	116.1	0.12 (0.07–0.25)	14.3	0.0028 (0.0017–0.0042)	16.5
JL-2014	0.84 (0.55–1.73)	135.5	0.24 (0.13–0.84)	28.6	0.0037 (0.0021–0.0078)	21.8
ZY-2010	0.71 (0.52–0.96)	114.5	0.27 (0.19–0.55)	32.1	0.0062 (0.0041–0.0126)	36.3
ZY-2011	0.62 (0.54–0.72)	100.0	0.18 (0.11–0.43)	21.4	0.0045 (0.0035–0.0060)	26.6
ZY-2012	0.36 (0.26–0.49)	58.1	0.08 (0.06–0.14)	9.5	0.0007 (0.0004–0.0010)	3.9
ZY-2013	0.43 (0.28–0.65)	69.4	0.27 (0.13–1.06)	32.1	0.0017 (0.0009–0.0026)	10.0
ZY-2014	0.41 (0.27–0.84)	66.1	0.31 (0.15–1.71)	36.9	0.0019 (0.0012–0.0031)	11.2
ZY-2015	nt		0.27 (0.19–0.40)	32.1	0.0008 (0.0007–0.0011)	4.7
GA-2010	0.49 (0.40–0.60)	79.0	0.30 (0.18–1.02)	35.7	0.0045 (0.0033–0.0073)	26.6
GA-2011	0.48 (0.37–0.62)	77.4	0.21 (0.12–0.56)	25.6	0.0030 (0.0023–0.0038)	17.6
GA-2012	0.79 (0.63–0.99)	127.4	0.17 (0.11–0.40)	20.2	0.0019 (0.0014–0.0029)	10.9
GA-2013	1.26 (0.79–2.01)	203.2	0.19 (0.10–0.65)	22.6	0.0015 (0.0008–0.0025)	8.8
GA-2014	0.94 (0.58–2.14)	151.6	0.21 (0.12–0.62)	25.0	0.0046 (0.0030–0.0090)	27.1
GA-2015	nt		0.80 (0.33–1.95)	95.2	0.0090 (0.0041–0.0200)	52.9
EZ-2010	0.41 (0.27–0.67)	66.2	0.23 (0.16–0.40)	27.4	0.0017 (0.0011–0.0024)	10.0
EZ-2011	0.51 (0.42–0.62)	82.3	0.13 (0.09–0.25)	15.5	0.0034 (0.0026–0.0044)	19.8
EZ-2012	0.28 (0.25–0.30)	45.2	0.33 (0.27–0.41)	39.3	0.0027 (0.0021–0.0034)	15.8
EZ-2013	0.55 (0.35–0.85)	88.7	0.64 (0.55–0.74)	76.2	0.0019 (0.0015–0.0023)	11.2
TM-2015	nt		0.25 (0.19–0.32)	29.2	0.0019 (0.0013–0.0024)	11.0
XG-2015	nt		0.20 (0.13–0.35)	24.0	0.0052 (0.0034–0.0080)	30.5
CB-2015	nt		0.46 (0.35–0.66)	54.6	0.0013 (0.0010–0.0016)	7.4
CS-2015	nt		0.36 (0.24–0.54)	42.9	0.0051 (0.0029–0.012)	29.8
XY-2015	nt		0.03 (0.02–0.04)	3.4	0.0017 (0.0016–0.0018)	10.0
CD-2020	0.36 (0.26–0.51)	58.1	0.28 (0.18–0.39)	33.3	0.0058 (0.0042–0.0083)	34.1
CD-2021	0.48 (0.29–0.69)	77.4	0.31 (0.23–0.48)	36.9	0.0041 (0.0029–0.0064)	24.1
QJ-2020	0.44 (0.39–0.93)	71.0	0.36 (0.26–0.51)	42.9	0.0033 (0.0024–0.0044)	19.4
QJ-2021	0.34 (0.25–0.48)	54.8	0.27 (0.20–0.37)	32.1	0.0040 (0.0030–0.0057)	23.5
SZ-2020	0.26 (0.19–0.38)	41.9	0.17 (0.12–0.24)	20.2	0.0026 (0.0018–0.0038)	15.2
SZ-2021	0.50 (0.35–0.71)	80.6	0.25 (0.18–0.34)	29.8	0.0036 (0.0021–0.0052)	21.2
XT-2020	0.43 (0.32–0.63)	69.4	0.30 (0.21–0.40)	35.7	0.0018 (0.0014–0.0024)	10.6
XT-2021	0.29 (0.20–0.39)	46.7	0.23 (0.15–0.31)	27.4	0.0035 (0.0026–0.0049)	20.6

Nt, not test.

[a]CI, confidence limit.

[b]RR, resistance ratio was calculated by dividing the LD_{50} value of a field population by the corresponding LD_{50} value of the susceptibility baseline of *C. suppressalis*.

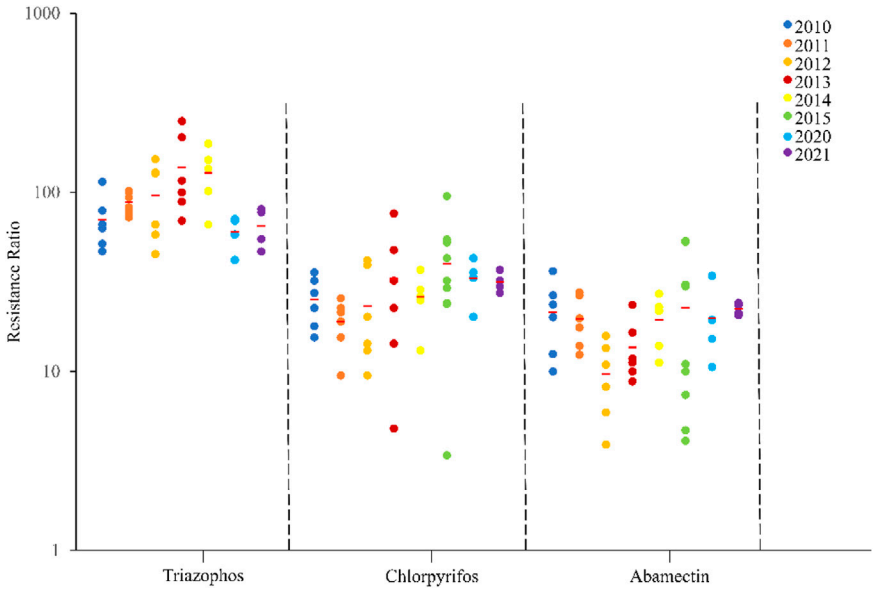


FIGURE 1
Resistance levels of *C. suppressalis* field populations collected in Centra China from 2010-2021 to 3 insecticides. The circle dots represent the resistance ratios of different populations of *C. suppressalis* to different insecticides. Red horizontal lines across the scatter diagram represent the mean values of the resistance ratios of the different populations.

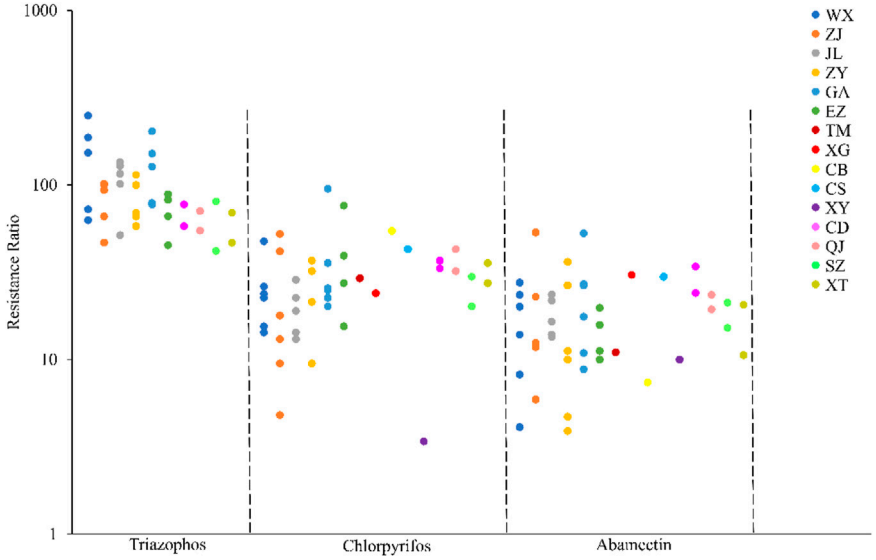


FIGURE 2
Comparison of the resistance levels of *C. suppressalis* field populations collected in Centra China to 3 insecticides. The circle dots represent the resistance ratios of different populations of *C. suppressalis* to different insecticides.

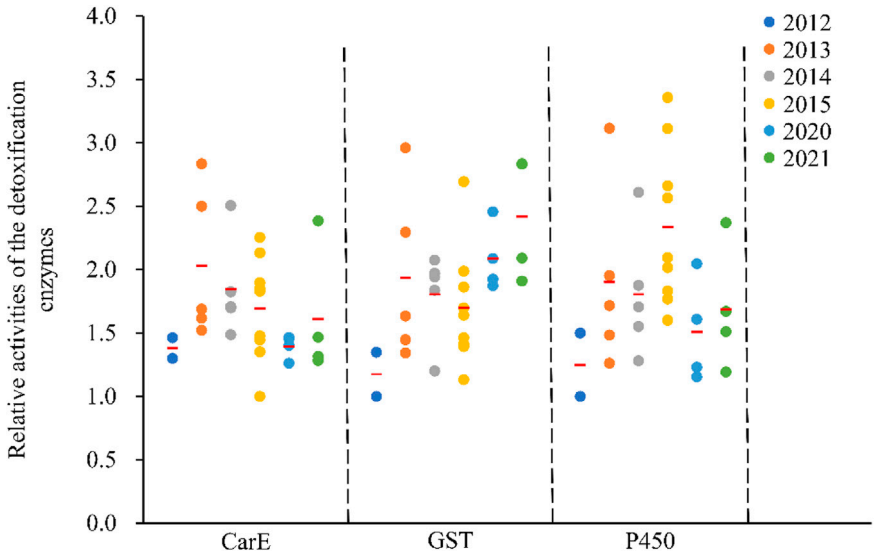


FIGURE 3
Relative activities of the detoxification enzymes in the field populations of *C. suppressalis* from 2012-2021. The relative activity of a detoxification enzyme corresponds to the ratio of the said detoxification enzyme activity and the minimum enzyme activity of the same enzyme. Red horizontal lines across the scatter diagram represent the mean values of the activities of the detoxification enzymes of the different populations.

TABLE 4 Correlation coefficients between the log LD_{50} values of the tested insecticides in the field populations of *Chilo suppressalis* from 2012-2021 and the enzyme activities.

	Triazophos	Abamectin	Chlorpyrifos	P450	GST	CarE
Triazophos	1					
Abamectin	−0.086 (0.72)	1				
Chlorpyrifos	−0.17 (0.47)	0.32 (0.09)	1			
P450	0.36 (0.12)	0.093 (0.63)	−0.12 (0.53)	1		
GST	−0.24 (0.32)	0.20 (0.32)	0.060 (0.76)	0.078 (0.69)	1	
CarE	0.49 (0.03)*	−0.033 (0.87)	−0.13 (0.51)	0.37 (0.05)	0.34 (0.073)	1

*Positive correlation between the LD_{50} value of insecticide and enzyme activity at the 95% significance level.

Tabashnik, 1990). Therefore, resistance to insecticides by insect pests is one of the most economically damaging circumstances that growers and pest control professionals face. Examining the sensitivity of *C. suppressalis* to insecticide each year can establish possible changes in susceptibility and is crucial to monitoring *C. suppressalis* resistance levels and banning ineffective insecticides that do not meet pest thresholds.

Triazophos, a broad-spectrum organophosphorus insecticide, was used to manage rice insect pests in China in the early 1990s (Jiang et al., 2001; Rani et al., 2001), becoming the most extensively used insecticide in crop protection against *C. suppressalis* in China thanks to its low cost and excellent efficacy. However, after prolonged commercial application for several years, elevated levels of resistance to triazophos were observed in the Zhejing province in 1999 (Jiang et al., 2001). And this resistance to triazophos continued to significantly increase in the years ahead (He et al., 2007; Hu et al., 2010; Zhang et al., 2011; He et al., 2012; He et al., 2013; Su et al., 2014a; Su et al., 2014b). Recent studies established that resistance to triazophos increased to high levels in numerous provinces in China due to extensive usage of the insecticide (Mao et al., 2019; Zhao, 2019). Since then, triazophos has not been recommended for use in the management of *C. suppressalis* anymore in the areas where *C. suppressalis* had developed high resistance to the insecticide. A comparison of data with those of preceding years revealed that pest insect resistance to triazophos decreased significantly over the years after the suspended application of triazophos (Su et al., 2014a). Additionally, the temporary fluctuations in localized resistance were probably caused by differences in the doses and varieties of insecticide applications, collection time of *C. suppressalis* and rice varieties (Zhao et al., 2021). Investigations in the past have registered low levels of cross-resistance to multiple

insecticides, including triazophos, chlorpyrifos, phoxim, isocarbophos, methamidophos, methomyl, abamectin and chlorantraniliprole (Qu et al., 2003; Cao, 2004; Mao et al., 2019). Findings, like triazophos resistance mechanisms in *C. suppressalis*, higher esterase activity, and microsomal O-demethylase and AChE insensitivity have also pointed to the possibility of cross-resistance to organophosphorus insecticides (Qu et al., 2003). However, unlike the results in a previous study (Mao et al., 2019), there were no significant correlations between the resistance to triazophos and chlorpyrifos or abamectin in this study, possibly because of the differences in collection sites, insecticide applications, operators, death standards and conditions during the bioassays. Regarding the increased susceptibility due to the suspended application of triazophos, the rotation of insecticides from different modes of action groups provides the best option for minimizing the development of resistance. For instance, rotating triazophos with abamectin could be an effective long-term resistance management strategy.

Chlorpyrifos was recommended as an alternative means of managing *C. suppressalis*, brown planthopper and rice leaf folder after the prohibition of high toxic insecticides (Xu et al., 2007). The development of resistance to chlorpyrifos by *C. suppressalis* has been rapid, with evolution from susceptibility and low (RR = 0.6–28.6 folds) to moderate resistance (RR = 2.3–78.4 folds) occurring between 2005 and 2011 (He et al., 2008; Su et al., 2014a). Moderate levels of resistance to chlorpyrifos have been noted in many provinces of China (Cheng et al., 2010; He et al., 2013; Su et al., 2014b). The increased resistance to chlorpyrifos in China from 2010-2015 was possibly associated with an escalated use of this insecticide against rice planthopper, striped rice borer and rice leaf folder and the cross-resistance to triazophos (Mao et al., 2019). Cross-resistance suggests that rotating triazophos with chlorpyrifos may not be an effective long-term resistance management strategy (Qu et al., 2003; Cao, 2004). Therefore, alternating with abamectin could slow the development of resistance to chlorpyrifos. However, susceptibility to chlorpyrifos must be monitored carefully to maintain control efficiency and successful resistance management.

Abamectin, an insecticide against arthropod pests, is of economic importance to horticulture and agriculture (Dybas, 1989). A wide range of insect types have developed resistance to abamectin around the world, including *C. suppressalis*, *Bactrocera dorsalis*, *Blattella germanica*, *Brontispa longissima*, *Deraeocoris brevis*, *Earias vittella*, *Frankliniella occidentalis*, *Helicoverpa armigera*, *Liriomyza trifolii*, *Metaseiulus occidentalis*, *Panonychus citri*, *Panonychus ulmi*, *Plutella xylostella*, *Spodoptera exigua*, *Spodoptera litura*, *Tetranychus cinnabarinus*, *Tetranychus turkestani*, *Tetranychus urticae*, and *Tuta absoluta* (Mota-Sanchez and Wise, 2022). Abamectin became an alternative to high toxic insecticides in 2006 after showing an excellent management efficacy against *C. suppressalis* and *Cnaphalocrocis medinalis* Guene (Gao, 2008; He et al., 2012). *C. suppressalis* subsequently slowly built resistance to abamectin from 2001 to 2011 (Su et al., 2014a). One inquiry reported that some field populations of *C. suppressalis* in China remained susceptible or displayed only low levels of resistance to abamectin in 2010 and 2011 after 10 more years of use (Su et al., 2014b). However, our analyses revealed that *C. suppressalis'* resistance to abamectin in China evolved from low to medium levels from 2010 to 2021. Growers' use of mixtures of abamectin to regulate *C. suppressalis* might not culminate in high resistance to abamectin by *C. suppressalis* because blending chemical groups with different MoAs slows down the process of selection for resistance (Su et al., 2014a). While abamectin has been used to manage *C. suppressalis* since 1998, it was only recommended as an alternative solution after *C. suppressalis* had become highly resistant to trizophos, monosultap and other insecticides (Zhang, 2012). Therefore, scientists must also carefully monitor susceptibility to abamectin to maintain control efficiency and successful resistance management.

Insecticide resistance is probably mediated by the metabolism of insecticides through detoxifying enzymes before they reach their targets (Heckel, 2012; Yang et al., 2020). And many past reports have found an association between insect resistance to insecticides and increases in the activities of these detoxifying enzymes (CarE, GST, and P450) (Zhao et al., 2021). Our investigation established a significant confirmatory correlation between resistance to triazophos and the activity of CarE, suggesting that CarE may be involved in the resistance of these field populations to triazophos. This finding is consistent with a revelation that enhancing the activity of EST (esterase) leads to resistance to triazophos (Qu et al., 2003; Mao et al., 2019). However, our study established no significant correlations between chlorpyrifos or abamectin and enzyme activity.

Conclusion

Our investigation found that field populations of *C. suppressalis* developed moderate to high levels of resistance to triazophos but still showed susceptibility, and low to moderate levels of resistance to chlorpyrifos and abamectin, indicating that insecticide resistance management programs are crucial to regulating pest insects. Hence, triazophos application should be suspended to control *C. suppressalis*, while the frequent use of chlorpyrifos and abamectin should be reduced in Central China. The fluctuations in resistance within the same regions across the years and within different regions in the same year could be the result of differences in insecticide applications and rice varieties. Because *C. suppressalis* is not a long-distance migratory insect pest, in theory, insecticide application practices and subsequent evolution of resistance to insecticide in one region would not influence the development of resistance in another region. Therefore, the geographical and temporal distribution of insecticide resistance must be scrutinized in detail. Resistance to insecticide by *C. suppressalis* should be

monitored continuously in Central China. However, resistance monitoring only determines shifts in susceptibility. To avoid resistance, biological control, crop rotation, transgenic plants and cultural practices must be implemented, along with insecticide application strategies, such as alternating the use of insecticides, changing mixtures of insecticides and reducing insecticide application frequencies.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

XZ, HW, and JhL conceived and designed the research. XZ, HM, and RH conducted the experiments. XZ, HM, and RH analyzed the data. HM and RH wrote the manuscript. XZ and JkL revised the manuscript. All authors have read and approved the manuscript.

Funding

This research was funded by the National Natural Science Foundation of China (Grant Number 32102263) and the Youth Talent Project of Hubei Education Department (Grant Number Q20211301).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Bhatt, P., Zhou, X. F., Huang, Y. H., Zhang, W. P., and Chen, S. H. (2021). Characterization of the role of esterases in the biodegradation of organophosphate, carbamate, and pyrethroid pesticides. *J. Hazard. Mat.* 411, 125026. doi:10.1016/j.jhazmat.2020.125026

Cao, M. (2004). *Monitoring of insecticide resistance, genetic analysis of triazophos and monosultap resistance and resistance risk assessment for fipronil in Chilo suppressalis (Walker)*. China, Jiangsu, Nanjing, Xuanwu: Nanjing Agricultural University, [dissertation/PhD thesis]. [Nanjing].

Cheng, X., Chang, C., and Dai, S. (2010). Responses of striped stem borer, *Chilo suppressalis* (Lepidoptera: Pyralidae), from taiwan to a range of insecticides. *Pest Manag. Sci.* 66, 762–766. doi:10.1002/ps.1939

Dybas, R. A. (1989). "Abamectin use in crop protection," in *Ivermectin and abamectin*. Editor W. C. Campbell (New York: Springer), 287–310. doi:10.1007/978-1-4612-3626-9_20

Enders, L. S., Rault, L. C., Heng-Moss, T. M., Siegfried, B. D., and Miller, N. J. (2020). Transcriptional responses of soybean aphids to sublethal insecticide exposure. *Insect biochem. Mol. Biol.* 118, 103285. doi:10.1016/j.ibmb.2019.103285

Gao, Z. (2008). *Laboratory screening for substitute insecticides of high toxic insecticides in control of rice leafroller, Cnaplocrocis medinalis (guenee) (Lepidoptera: Pyralidae)*. China, Jiangsu, Nanjing, Xuanwu: Nanjing Agricultural University. [dissertation/master's thesis]. [Nanjing].

He, Y., Gao, C., Cao, M., Chen, W., Huang, L., Zhou, W., et al. (2007). Survey of susceptibilities to monosultap, triazophos, fipronil, and abamectin in *Chilo suppressalis* (Lepidoptera: Crambidae). *J. Econ. Entomol.* 100, 1854–1861. doi:10.1603/0022-0493(2007)100[1854:sostmt]2.0.co;2

He, Y., Gao, C., Chen, W., Huang, L., Zhou, W., Liu, X., et al. (2008). Comparison of dose responses and resistance ratios in four populations of the rice stem borer, *Chilo suppressalis* (Lepidoptera: Pyralidae), to 20 insecticides. *Pest Manag. Sci.* 64, 308–315. doi:10.1002/ps.1522

He, Y., Zhang, J., Chen, J., and Shen, J. (2012). Using synergists to detect multiple insecticide resistance in field populations of rice stem borer. *Pestic. Biochem. Physiol.* 103, 121–126. doi:10.1016/j.pestbp.2012.04.008

He, Y., Zhang, J., Gao, C., Su, J., Chen, J., and Shen, J. (2013). Regression analysis of dynamics of insecticide resistance in field populations of *Chilo suppressalis* (Lepidoptera: Crambidae) during 2002–2011 in China. *J. Econ. Entomol.* 106, 1832–1837. doi:10.1603/ec12469

Heckel, D. G. (2012). Ecology. Insecticide resistance after Silent spring. *Science* 337, 1612–1614. doi:10.1126/science.1226994

Hu, J., Chen, W., Zhang, Z., Zheng, X. S., Jin, J. C., Su, J. Y., et al. (2010). Insecticide resistance monitoring of *Chilo suppressalis* in the drainage area of the yangtze river, China. *Chin. J. Rice Sci.* 24, 509–515. doi:10.3724/SP.J.1011.2010.01385

Huang, J. M., Rao, C., Wang, S., He, L. F., Zhao, S. Q., Zhou, L. Q., et al. (2020). Multiple target-site mutations occurring in lepidopterans confer resistance to diamide insecticides. *Insect biochem. Mol. Biol.* 121, 103367. doi:10.1016/j.ibmb.2020.103367

Huang, S. J., Chen, Q., Qin, W. J., Sun, Y., Qin, H. G., and John, T. (2017). Resistance monitoring of four insecticides and a description of an artificial diet incorporation method for *Chilo suppressalis* (Lepidoptera: Crambidae). *J. Econ. Entomol.* 110, 2554–2561. doi:10.1093/jee/tox266

Jiang, X., Zhang, Q., Hu, S., Xie, S., and Xu, X. (2001). The status of pesticide resistance of rice stalk borer in Zhejiang province and their management tactics. *Plant Prot. Technol. Ext.* 21, 27–29. doi:10.3969/j.issn.1672-6820.2001.03.016

Li, Z. L., Pan, D. D., Lu, M. X., and Du, Y. Z. (2020). Identification of qRT-PCR reference genes for *Chilo suppressalis* (Walker) during para-sitism by *Cotesia chilonis* (Matsumura). *J. Asia. Pac. Entomol.* 23, 107–112. doi:10.1016/j.aspen.2019.10.016

Lu, Y. H., Wang, G. R., Zhong, L. Q., Zhang, F. C., Bai, Q., Zheng, X. S., et al. (2017). Resistance monitoring of *Chilo suppressalis* (Walker) (Lepidoptera: Crambidae) to chlorantraniliprole in eight field populations from east and central China. *Crop Prot.* 100, 196–202. doi:10.1016/j.cropro.2017.07.006

Mao, K. K., Li, W. Z., Liao, X., Liu, C. Y., Qin, Y., Ren, Z. J., et al. (2019). Dynamics of insecticide resistance in different geographical populations of *Chilo suppressalis* (Lepidoptera: Crambidae) in China 2016–2018. *J. Econ. Entomol.* 112, 1866–1874. doi:10.1093/jee/toz109

Meng, X. K., Dong, F., Qian, K., Miao, L. J., Yang, X. M., Ge, H. C., et al. (2019). Transcriptome analysis reveals global gene expression changes of *Chilo suppressalis* in response to sublethal dose of chlorantraniliprole. *Chemosphere* 234, 648–657. doi:10.1016/j.chemosphere.2019.06.129

Meng, X. K., Wu, Z. L., Jiang, C. Y., Guan, D. J., Zhang, N., Jiang, H., et al. (2022). Identification and characterization of glutathione *S*-transferases and their potential roles in detoxification of abamectin in the rice stem borer, *Chilo suppressalis*. *Pestic. Biochem. Physiol.* 182, 105050. doi:10.1016/j.pestbp.2022.105050

Mota-Sanchez, D., and Wise, J. C. (2022). *The arthropod Pesticide resistance Database*. Michigan, United States: Michigan State University. http://www.pesticideresistance.com/display.php?page=ai&aiId=1 (accessed on August 1, 2022).

NATESC (2022). *Occurrence trend forecast of the major diseases and insect pests of main crops in China in 2022*. http://cb.natesc.gov.cn/sites/cb (accessed on August 1, 2022).

Nauen, R., Bass, C., Feyereisen, R., and Vontas, J. (2022). The role of cytochrome P450s in insect toxicology and resistance. *Annu. Rev. Entomol.* 67, 105–124. doi:10.1146/annurev-ento-070621-061328

Qian, L., Cao, G., Song, J., Yin, Q., and Han, Z. (2008). Biochemical mechanisms conferring cross-resistance between tebufenozide and abamectin in *Plutella xylostella*. *Pestic. Biochem. Physiol.* 91, 175–179. doi:10.1016/j.pestbp.2008.03.011

Qu, M., Han, Z., Xu, X., and Yue, L. (2003). Triazophos resistance mechanisms in the rice stem borer (*Chilo suppressalis* Walker). *Pestic. Biochem. Physiol.* 77, 99–105. doi:10.1016/j.pestbp.2003.09.003

Rani, S., Madan, V. K., and Kathpal, T. S. (2001). Persistence and dissipation behavior of triazophos in canal water under Indian climatic conditions. *Ecotoxicol. Environ. Saf.* 50, 82–84. doi:10.1006/eesa.2001.2103

Roush, R. T., and Tabashnik, B. E. (1990). *Pesticide resistance in arthropods*. New YorkNew York: Springer. doi:10.2307/3495418

Shao, Z. R., Feng, X., Zhang, S., Li, Z. Y., Huang, J. D., Chen, H. Y., et al. (2013). *Guideline for insecticide resistance monitoring of Plutella xylostella (L.) on cruciferous vegetables*. Beijing, China: China Agricultural Press.

Sparks, T. C., and Nauen, R. (2015). Irac: Mode of action classification and insecticide resistance management. *Pestic. Biochem. Physiol.* 121, 122–128. doi:10.1016/j.pestbp.2014.11.014

Su, J., Zhang, Z., Wu, M., and Gao, C. (2014a). Changes in insecticide resistance of the rice striped stem borer (Lepidoptera: Crambidae). *J. Econ. Entomol.* 107, 333–341. doi:10.1603/EC13285

Su, J., Zhang, Z., Wu, M., and Gao, C. (2014b). Geographic susceptibility of *Chilo suppressalis* walker (Lepidoptera: Crambidae), to chlorantraniliprole in China. *Pest Manag. Sci.* 70, 989–995. doi:10.1002/ps.3640

Sun, Y., Hu, Y. Y., Qin, W. Q., Wan, P., Zou, F., and Huang, S. J. (2021). Resistance of asiatic rice borer *Chilo suppressalis* (Lepidoptera: Crambidae) population to four insecticides in Jiangxi Province from 2017 to 2019. *J. Plant Prot.* 48, 396–406. doi:10.13802/j.cnki.zwbhxb.2021.2020049

Wei, Y. B., Yan, R., Zhou, Q. L., Qiao, L., Zhu, G., and Chen, M. (2019). Monitoring and mechanisms of chlorantraniliprole resistance in *Chilo suppressalis* (Lepidoptera: Crambidae) in China. *J. Econ. Entomol.* 112, 1348–1353. doi:10.1093/jee/toz001

Xu, D., Jin, M., Gu, Z., Xu, X., and Xu, G. (2007). Review of the application of chlorpyrifos in the control of rice pest insects. *Mod. Agrochem.* 6, 6.

Xu, H. X., Zheng, X. S., and Lv, Z. X. (2015). "*Chilo suppressali* walker," in *The institute of plant protection, Chinese academy of agricultural sciences and China society of plant protection*. 3rd Edition (Beijing, China: China Agriculture Press), Crop Diseases and Insect Pests in China.

Xu, L., Luo, G. H., Jing, Y. J., Xu, D. J., Xu, G. C., and Gu, Z. Y. (2021). Study on the synergistic mechanism of chlorpyrifos and ethofenprox mixture in poisoning *Chilo suppressalis* based on transcriptome sequencing. *Jiangsu J. Agr. Sci.* 37, 317–325. doi:10.3969/j.issn.1000-4440.2021.02.006

Xu, L. N., Zhou, Z. Y., Hu, F., and Hu, B. J. (2018). The insecticide susceptibility of *Chilo suppressalis* in Anhui Province. *J. Anhui Agric. Univ.* 45, 740–744. doi:10.13610/j.cnki.1672-352x.20180825.026

Yang, X., Deng, S., Wei, X. G., Yang, J., Zhao, Q. N., Yin, C., et al. (2020). MAPK-directed activation of the whitefly transcription factor *CREB* leads to P450-mediated imidacloprid resistance. *Proc. Natl. Acad. Sci. U. S. A.* 117, 10246–10253. doi:10.1073/pnas.1913603117

Yao, R. (2015). *Resistance monitoring to insecticides and primary study on resistance mechanism to diamide insecticides in Chilo suppressalis*. China, Jiangsu, Nanjing, Xuanwu: Nanjing Agricultural University. [dissertation/master's thesis]. [Nanjing].

Yao, R., Zhao, D. D., Zhang, S., Zhou, L. Q., Wang, X., Gao, C. F., et al. (2017). Monitoring and mechanisms of insecticide resistance in *Chilo suppressalis* (Lepidoptera: Crambidae) with special reference to diamides. *Pest Manag. Sci.* 73, 1169–1178. doi:10.1002/ps.4439

Zhang, S., Li, Y., Shao, Z., and Guo, J. (2011). Monitoring and management of insecticide resistance of Chilo suppressalis in China. *Plant Prot.* 37, 141–144. doi:10.3969/j.issn.0529-1542.2011.02.031

Zhang, S. Z., Zhang, X. L., Shen, J., Li, D. Y., Wan, H., You, H., et al. (2017a). Cross-resistance and biochemical mechanisms of resistance to indoxacarb in the diamondback moth, Plutella xylostella. *Pestic. Biochem. Physiol.* 140, 85–89. doi:10.1016/j.pestbp.2017.06.011

Zhang, X. L., Liao, X., Mao, K. K., Yang, P., Li, D. Y., Alia, E., et al. (2017b). The role of detoxifying enzymes in field-evolved resistance to nitenpyram in the brown planthopper *Nilaparvata lugens* in China. *Crop Prot.* 94, 106–114. doi:10.1016/j.cropro.2016.12.022

Zhang, Z. (2012). *Monitoring of insecticide resistance and baseline susceptibility of Chilo suppressalis (Lepidoptera: Pyralidae) to chlorantraniliprole*. China, Jiangsu, Nanjing, Xuanwu: Nanjing Agricultural University. [dissertation/master's thesis]. [Nanjing].

Zhao, S. Q. (2019). *Resistance monitoring and inheritance analysis of chlorantraniliprole resistance in the Chilo suppressalis (Lepidoptera: Pyralidae)*. China, Jiangsu, Nanjing, Xuanwu: Nanjing Agricultural University. [dissertation/mamaster's thesis]. [Nanjing].

Zhao, X., Xu, X., Wang, X. G., Yin, Y., Li, M. L., Wu, Y. Q., et al. (2021). Mechanisms for multiple resistances in field populations of rice stem borer, *Chilo suppressalis* (Lepidoptera: Crambidae) from Sichuan Province, China. *Pestic. Biochem. Physiol.* 171, 104720. doi:10.1016/j.pestbp.2020.104720

Zhu, Z., and Cheng, J. (2013). The evolution and perspective of rice insect pest management strategy in China. *Plant Prot.* 39, 25–32. doi:10.3969/j.issn.0529-1542.2013.05.004

frontiers | Frontiers in Physiology

TYPE Original Research
PUBLISHED 29 November 2022
DOI 10.3389/fphys.2022.1048208

OPEN ACCESS

EDITED BY
Shun-Fan Wu,
Nanjing Agricultural University, China

REVIEWED BY
Yueping He,
Huazhong Agricultural University, China
Lu Xu,
Jiangsu Academy of Agricultural Sciences (JAAS), China

*CORRESPONDENCE
Xiaoming Xia,
xxm@sdau.edu.cn

†These two authors contributed equally to the present study

SPECIALTY SECTION
This article was submitted to Invertebrate Physiology, a section of the journal Frontiers in Physiology

RECEIVED 19 September 2022
ACCEPTED 12 October 2022
PUBLISHED 29 November 2022

CITATION
Wen S, Liu C, Wang X, Wang Y, Liu C, Wang J and Xia X (2022), Resistance selection of triflumezopyrim in Laodelphax striatellus (fallén): Resistance risk, cross-resistance and metabolic mechanism.
Front. Physiol. 13:1048208.
doi: 10.3389/fphys.2022.1048208

Resistance selection of triflumezopyrim in *Laodelphax striatellus* (fallén): Resistance risk, cross-resistance and metabolic mechanism

Shengfang Wen[1,2†], Chang Liu[1†], Xueting Wang[1], Youwei Wang[1], Chao Liu[1], Jinhua Wang[2] and Xiaoming Xia[1*]

[1]College of Plant Protection, Shandong Agricultural University, Taian, China, [2]College of Resources and Environment, Shandong Agricultural University, Taian, China

The risk assessment and resistance mechanisms of insecticide resistance are critical for resistance management strategy before a new insecticide is widely used. Triflumezopyrim (TFM) is the first commercialized mesoionic insecticide, which can inhibit nicotinic acetylcholine receptor with high-performance against the small brown planthopper (SBPH), *Laodelphax striatellus* (Fallén). In our study, the resistance of SBPH to TFM increased 26.29-fold, and the actual heritability of resistance was 0.09 after 21 generations of continuous selection by TFM. After five generations of constant feeding under insecticide-free conditions from F_{16} generation, the resistance level decreased 2.05-fold, and the average resistance decline rate per generation was 0.01, but there were no statistical decline. The TFM resistant strains had no cross-resistance to imidacloprid, nitenpyram, thiamethoxam, dinotefuran, flonicamid, pymetrozine, and chlorfenapyr. The third and fifth nymphal stage duration, pre-adult stage, adult preoviposition period, longevity, emergence rate, and hatchability of the resistant strain were significantly lower than those of the susceptible strain, while the female-male ratio was considerably increased. The fitness cost was 0.89. Further, cytochrome P450 monooxygenase (P450) and carboxylesterase (CarE) activities were markedly increased, but only the enzyme inhibitor piperonyl butoxide (PBO) had a significant synergistic effect on the resistant strain. The expression of *CYP303A1*, *CYP4CE2,* and *CYP419A1v2* of P450 genes was significantly increased. SBPH has a certain risk of resistance to TFM with continuous application. The TFM resistance may be due to the increased activity of P450 enzyme regulated by the overexpression of P450 genes.

KEYWORDS

***Laodelphax striatellus*, triflumezopyrim, resistance risk, biological fitness, detoxification enzyme**

1 Introduction

The small brown planthopper (SBPH), *Laodelphax striatellus* (Fallén), is one of the most economically important pests widely distributed in China (Zheng et al., 2017). SBPH can damage rice, corn, and wheat by sucking plants, ovipositing, and spreading viral diseases, leading to severe crop yield reduction (Mu et al., 2016; Wei et al., 2017; Wu et al., 2018). Currently, insecticides are the primary measure to control SBPH in the field (Miah et al., 2018). However, SBPH has developed varying degrees of resistance to several insecticides, including chlorpyrifos, ethiprole, buprofezin, and imidacloprid, as a result of their long-term, extensive, and unreasonable application (Gao et al., 2008; Wang et al., 2008; Xu et al., 2014; Elzaki et al., 2015; Liu et al., 2015).

Triflumezopyrim (TFM) is a new mesoionic insecticide that acts on nicotinic acetylcholine receptors (nAChRs) (Cordova et al., 2016; Wen et al., 2021a). TFM is the only available acetylcholine receptor inhibitor (Cordova et al., 2016). TFM has a wide insecticidal spectrum with a long-lasting effect. Further, it exerts an excellent insecticidal effect on rice planthoppers (Zhang K. L et al., 2020; Wen et al., 2021b). TFM has great potential in the integrated control and resistance management of SBPH. Previous researches reported that sublethal doses of TFM could significantly affect population development and detoxification enzyme activities of *Sogatella furcifera* (Horvath) (Chen et al., 2020) and SBPH (Wen et al., 2021b). The new study reported that SBPH had a certain risk of resistance to TFM after a short-term continuous selection (Zhang et al., 2022).

The risk assessment of insecticide resistance is crucial before continuous application. This will provide in-depth knowledge about applying new insecticides to control a specific pest comprehensively. It can be assessed by the realistic heritability (h^2), fitness cost, resistance stability, and cross-resistance (Wang et al., 2021). The h^2 can estimate the genetic ability and risk of resistance (Afzal and Shad, 2016). Fitness cost refers to the disadvantage of the fitness of resistance genes, manifested in the ecological, physiological, or biochemical changes of individual organisms and populations to adapt to the adverse effects of pesticides, such as decreased fecundity and survival rate. It may affect the development of insecticide resistance (Kliot and Ghanim, 2012; Tieu et al., 2017). Several studies have reported the fitness cost of planthopper against imidacloprid, chlorfluazuron, buprofezin, etc (Liu and Han, 2006; Liao et al., 2019b; Zeng et al., 2022). Resistance stability can predict the development trend of resistance. The more stable the resistance is, the more difficult it is to manage (Afzal et al., 2015). The determination of cross-resistance can provide ideas for alternation of pesticides and the resistance mechanism (Wang R. et al., 2020).

Therefore, we screened SBPH strain resistant to TFM after long-term continuous selection, evaluated the resistance risk of SBPH to TFM, and determined the cross-resistance with other insecticides. Further, the metabolic mechanism of resistance SBPH for TFM was preliminarily studied. This research provides a theoretical reference for resistance mechanism study and SBPH resistance management for TFM.

2 Materials and methods

2.1 Insects

The susceptible strain (SS) of SBPH was obtained from Yutai, Shandong Province (N35° 1′ 13.00″ E116° 38′ 3.01″) in October 2016. The SS strain was reared on 'Wuyujing 3' rice seedlings without exposure to insecticides for more than 2 years. The rice-seedling spraying approach was used to continuously select the resistant strain (RS) of SBPH from the SS to 21 generations. The insects were raised at 25 ± 1°C, 70%–80% relative humidity, and a 16:8 h L:D photoperiod.

2.2 Insecticides and synergists

TFM (96%) and cyantraniliprole (94%) were supplied by DuPont Company (Shanghai, China). Imidacloprid (96%) was provided by Shandong Weifang Rainbow Chemical Co., Ltd (Weifang, China). Dinotefuran (99.1%), thiamethoxam (98%), flonicamid (98.5%), pymetrozine (98%), and nitenpyram (98%) were purchased from Shandong United Pesticide Industry Co., Ltd (Jinan, China). Piperonyl butoxide (PBO, 95%), triphenyl phosphate (TPP, 98%), and diethyl maleate (DEM, 96%) were purchased from Shanghai Macklin Biochemical Co., Ltd (Shanghai, China).

2.3 Bioassay

The rice-seedling dip method described by Xu et al. (2019) and Liao et al. (2019b), with minor changes, was used to test TFM toxicity for SBPH. TFM was dissolved in acetone and diluted to different concentrations with deionized water containing 0.1% Triton X-100. Deionized water containing 0.1% Triton X-100 was employed as a control. Rice seedlings with roots were cut to a length of approximately 10 cm, rinsed with water, and air-dried in a shady environment. The rice seedlings were soaked in insecticide for 30 s, retrieved and drained until the liquid stopped dripping, and then dried naturally in the shade. Wet absorbent cotton was used to cover the roots of the rice seedlings, which were then put in glass test tubes (2 cm × 20 cm) with five seedlings per tube. The third instar nymph of SBPH was sedated with CO_2 (10–15 s) before being put in a test tube containing rice seedlings, with 20 insects in each tube and three replicates for each concentration. Afterwards, the glass test tubes were sealed with four layers of gauze (200-mesh). The feeding conditions of all treatments were the same as those in Section 2.1, and the

mortality was recorded after 72 h. If the insects did not move after being lightly touched with a small writing brush, they were pronounced dead.

2.4 Resistance selection

SBPH resistance to TFM was selected with minor changes of Mao et al.'s (2016) rice-seedling spraying approach. The LC_{50} value measured by the previous generation bioassay was used as the concentration of TFM in the spray to screen the resistance of the next generation. Rice seeds were placed on two moistened layer paper towels in plastic boxes (35 cm × 25 cm × 15 cm). When the rice seedlings were approximately 10 cm tall, the sufficient TFM solution prepared following above methods was uniformly sprayed onto rice seedlings by using Matabi sprayer (style 7) until the dripping water, which were then dried in a cool and dry place. Then more than 1,000 third instar nymphs were moved into the boxes containing treated rice seedlings with insecticide, and covered with a mesh cloth for feeding at the above condition. More than 4,000 third instar nymphs were treated in each screening generation. After 72 h, the surviving insects were moved into a new box containing fresh rice seedlings without insecticide for continuous routine feeding. The toxicity of TFM to SBPH was measured by rice-seedling dip method described above in each generation to monitor the development of resistance.

$$\text{Resistance ratio (RR)} = \frac{LC_{50} \text{ of TFM to RS}}{LC_{50} \text{ of TFM to SS}}$$

2.5 Estimation of the resistance realistic heritability and prediction of the resistance development rate

Tabashnik and Mcganghey (1994) and Tabashnik (1992) provided approaches for determining the h^2 and resistance development rates of TFM resistant strain, respectively. The specific calculation method is in Supplementary Figure S1.

2.6 Resistance stability

A portion of the RS-F_{16} strain was isolated individually and reared continuously for five generations without exposure to any pesticide to evaluate TFM resistance stability in SBPH, similar to the approach described by Tabashnik and Mcganghey (1994). R was calculated to determine the average selection response:

$$R = \frac{\lg(\text{finale } LC_{50}) - \lg(\text{initial } LC_{50})}{N}$$

where N is the response of the number of generations reared without being exposed to pesticides.

2.7 Cross-resistance

The sensitivity of the third instar nymphs of SS and RS (F_{20}) strains to neonicotinoids insecticide (imidacloprid, dinotefuran, thiamethoxam, flonicamid, nitenpyram), pyridine azomethine insecticide (pymetrozine) and anthranilic diamide insecticide (cyantraniliprole) were determined using the rice-seedling dip method described in Section 2.3 to clarify the cross-resistance of TFM with these insecticides. The following formula was used to compute the cross-resistance ratio (CR):

$$\text{CR} = \frac{LC_{50} \text{ of test insecticide to RS}}{LC_{50} \text{ of test insecticide to SS}}$$

2.8 Biological fitness

The biological fitness of the SS and RS strains of SBPH was evaluated using a modified version of the age-stage, two-sex life table technique reported by Liao et al. (2019a). Fifty nymphs (fifth instar) were randomly selected from the SS and RS (F_{20}) strains, fed separately in glass test tubes. When the adults emerged, single female and male were paired and fed separately with fresh rice seedlings, which were inspected every half-day (8:00 and 20:00) to check whether there were nymphs on the seedlings. One hundred newly hatched nymphs were collected from the SS and RS strains on the same day. Each of the 100 nymphs was considered a replicate for each strain and individually fed in separate test tubes. The nymph's molting and death were examined and recorded twice daily (8:00 and 20:00). The withered rice seedlings were replaced promptly to insure sufficient nutrition. The adults were separately paired when they emerged. The rice seedlings were superseded and retained every half-day (8:00 and 20:00) until all the insects died. Based on the method by Shao et al. (2012), the quantity of newly hatched nymphs was observed and documented until no nymphs hatched for 10 days. All the rice seedlings were boiled for 10 min and 48-h submerged in 95% ethanol. Then the rice seedlings were dissected, and the unhatched eggs were documented under the anatomical microscope.

2.9 Synergism of the enzyme inhibitors

The approach for determining the synergism of the enzyme inhibitors is similar to that described by Liao et al. (2019a), with minor variations. The enzyme synergist triphenyl phosphate (TPP), diethyl maleate (DEM), and piperonyl butoxide (PBO) were dissolved in acetone and prepared in different concentrations. The fifth instar nymphs from the RS (F_{17}) and SS strains were selected as test insects. There were 20 insects in each treatment, and the experiment was repeated thrice for each concentration. SBPH was anaesthetized with CO_2 for 20 s, and

TABLE 1 The resistance development of the susceptible strain of SBPH to TFM.

Selected generations	No.	Slope±SE	LC_{50} (mg·L^{-1})	95% CI[a]	χ^2 (*df*)	Resistance ratio (RR)
F_0	360	1.40 ± 0.27	0.55	0.39–0.88	3.64 (5)	-
F_1	360	1.38 ± 0.27	0.63	0.44–1.07	2.10 (5)	1.15
F_2	360	1.71 ± 0.29	1.37	0.93–1.86	2.46 (5)	2.49
F_3	360	0.86 ± 0.26	1.06	0.58–5.50	1.28 (5)	1.93
F_4	360	0.89 ± 0.26	1.32	0.70–8.20	2.63 (5)	2.40
F_5	360	1.36 ± 0.27	2.23	1.55–3.61	2.95 (5)	4.05
F_6	360	1.46 ± 0.38	2.33	1.54–5.89	2.33 (4)	4.24
F_7	360	1.72 ± 0.35	1.87	1.25–2.49	4.17 (5)	3.40
F_8	360	1.62 ± 0.35	1.80	1.15–2.43	5.19 (5)	3.27
F_9	360	1.10 ± 0.42	3.18	1.77–9.84	1.20 (5)	5.78
F_{10}	360	1.53 ± 0.44	3.91	2.65–8.74	3.64 (5)	7.11
F_{11}	360	1.11 ± 0.25	6.02	3.53–10.56	3.10 (6)	10.95
F_{12}	360	1.29 ± 0.21	5.52	3.80–7.99	7.83 (6)	10.40
F_{13}	360	1.39 ± 0.33	6.40	4.10–11.45	0.89 (5)	11.64
F_{14}	360	1.47 ± 0.22	7.74	5.63–11.29	4.18 (6)	14.07
F_{15}	360	1.44 ± 0.34	6.50	4.25–12.91	0.66 (5)	11.82
F_{16}	360	1.26 ± 0.32	7.18	4.31–11.32	2.88 (5)	13.05
F_{17}	360	1.20 ± 0.26	8.34	5.38–13.17	3.77 (5)	15.16
F_{18}	360	1.63 ± 0.23	9.31	7.23–12.31	7.13 (5)	16.93
F_{19}	360	1.07 ± 0.26	11.66	6.17–18.37	4.57 (5)	21.20
F_{20}	360	1.01 ± 0.31	13.30	5.67–25.21	2.49 (5)	24.18
F_{21}	360	1.37 ± 0.27	14.46	9.63–20.97	2.44 (5)	26.29

[a]95% CI = 95% confidence interval.

0.04 μL of a synergist was applied to the insects pronotum by a micropipette. Acetone treatment was used as a control. Preliminary tests were undertaken to establish the maximal dose (TPP 0.2 μg/insect; DEM 2.5 μg/insect; PBO 0.2 μg/insect) of the synergists that had no apparent adverse impact on the fifth instar nymphs of SBPH for 72 h. After pretreatment with the synergist for 1 h, the effect of the synergists on the efficacy of TFM was determined by the rice-seedling dip method described in Section 2.3. The following formula was used to compute the synergistic ratio (SR):

$$SR = \frac{LC_{50} \text{ of TFM alone}}{LC_{50} \text{ of TFM with the synergist}}$$

2.10 Assays for the activity of detoxification enzymes

The extraction method of enzyme solution was according to the conventional method of Ding et al. (2021). Three replicates were used for each strain, and each replicates contained thirty third instar nymphs (90 insects per strain).

The activities of carboxylesterase (CarE), glutathione-*S*-transferase (GST), and cytochrome P450 monooxygenase (P450) were determined according to conventional methods. CarE activity was evaluated according to the methods reported by Han et al. (1998) and Ding et al. (2021). Activity determination of GST and P450 refers to the practices of Kao et al. (1989) and Aitio (1978), respectively. According to Wen et al. (2021b), the protein concentrations were measured using the Enhanced BCA (Bicinchoninic acid) Protein Assay Kit (Beyotime Biotechnology). The specific operation steps are in Supplementary Figure S2.

2.11 Determination of the expression levels of P450 genes

The third instar nymphs of the SS and RS (F_{20}) strains were collected, and each strain was divided into three groups with 30 insects in each group. The total RNA was extracted using the RNA-esay™ Isolation Reagent (Vazyme Biotech Co., Ltd, Nanjing, China). After that, reverse transcription of RNA to first-strand cDNA was performed using HiScript®III RT SuperMix for qPCR (+gDNA wiper) reagent kit (Vazyme Biotech Co., Ltd, Nanjing, China). The qRT-PCR reaction was carried out using ChamQ universal SYBR® qPCR Master Mix reagent kit (Vazyme Biotech Co., Ltd, Nanjing, China). The

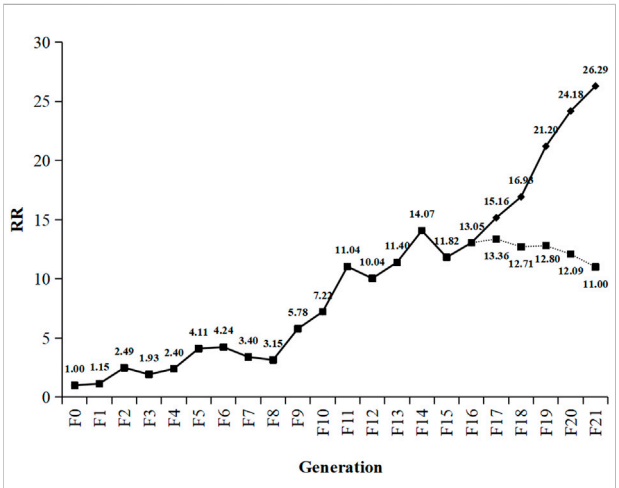


FIGURE 1
Change trend of triflumezopyrim resistance ratio from F0 to F21. Generations of *Laodelphax striatellus*. The dotted line represents the trend in resistance stability of RS strain (F16) of SBPH after continuously reared 5 generations without TFM. RR (resistance ratio) = $\frac{LC_{50}\text{ of TFM to RS}}{LC_{50}\text{ of TFM to SS}}$.

primers used in qRT-PCR analyses were provided in Supplementary Table S1, and the level of P450 gene transcripts was normalized to that of GAPDH. Relative quantification was performed using the $2^{-\Delta\Delta CT}$method (Livak and Schmittgen, 2021).

2.12 Data analysis

SPSS 16.0 was used to calculate the LC_{50} value, slope, 95% confidence interval (CI), and χ^2 and performed to analyze the differences in emergence rate, female-male ratio, hatchability, metabolic enzyme activities, and expression levels of P450 genes in SS and RS strains by one-way ANOVA with Tukey's test ($p < 0.05$). The toxicity differences in different treatments for resistance stability and synergism test were compared by Poloplus software. The difference of toxicity is significant if the 95% confidence limit for the median lethal dose ratio is greater than 1 and the p value for the equality hypothesis is less than 0.05. TWOSEX-MSChart software was performed on analyzed SBPH life table data, including the intrinsic rate of increase (r), finite rate of increase (λ), net reproductive rate (R_0), and mean generation time (T) (Chi 1988; Chi, 2020) and to compare the differences in developmental duration, single female egg production, life span, and other life table parameters of SBPH in SS and RS strains at different stages by bootstrapping (100,000 times) (Huang and Chi, 2013; Akca et al., 2015). The formulas are as follows.

$$R_0 = \sum_{x=0}^{\infty} l_x m_x$$

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1$$

$$\lambda = e^r$$

$$T = \frac{\ln R_0}{r}$$

$$\text{Relative fitness}\left(R_f\right) = \frac{R_0\ (\text{RS})}{R_0\ (\text{SS})}$$

3 Results

3.1 Triflumezopyrim selection pressure on small brown planthopper

The RS strain was derived from the SS strain of SBPH by continuous TFM selection of 21 generations in the laboratory (Table 1; Figure 1). The LC_{50} value of TFM for SBPH increased from 0.55 mg L^{-1}–14.46 mg L^{-1}. The resistance slowly increased after selection from F_0 to F_8 generations. The resistance ratio increased only by approximately 4-fold. However, from F_8 to F_{21} generations, the resistance development rapidly increased, the resistance ratio of the RS strain was increased 26.29-fold. Base on the resistance levels criterion: low (RR = 5–10-fold), medium (RR = 10–100-fold), and high (>100-fold) (Mu et al., 2016; Xue et al., 2022), the resistance level was intermediate.

3.2 Estimation of the realistic heritability of resistance and prediction of the resistance development rate

After continuous TFM resistance selection for 21 generations, the average selection response (R) was 0.07,

TABLE 2 Realized heritability of SBPH to TFM.

Mean selection response per generation				Mean selection differential per generation				Realistic heritability (h^2)
Initial LC_{50} (mg·L^{-1})	Final LC_{50} (mg·L^{-1})	Selective response (R)	Survival rate (p)	Select intensity (i)	Average slope	Phenotypic standard deviation (σ_p)	Selection differential (S)	0.09
0.55	14.46	0.07	40.1	0.97	1.33	0.75	0.73	

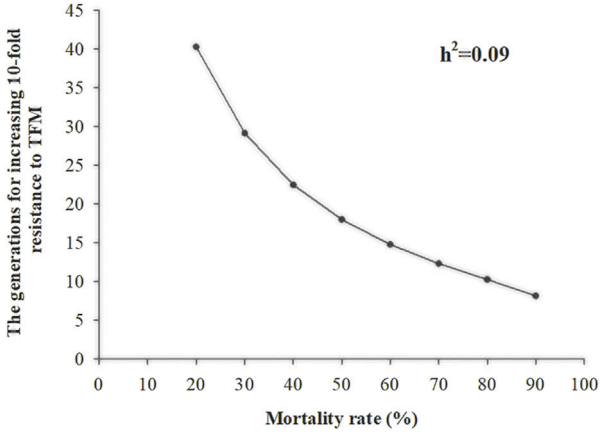


FIGURE 2
The number of generations of *Laodelphax striatellus* required for 10-fold in LC_{50} of triflumezopyrim (h^2 = 0.09) at different selection intensities.

the average selection differential (*S*) was 0.73, and the h^2 was 0.09 (Table 2). As the selection pressure increased in each generation (survival rate after selection was from 20% to 90%), the generation that showed a 10-fold increase in resistance to TFM decreased from 40 to 8 generations (Figure 2).

3.3 Resistance stability of the RS strain

To investigate the resistance stability of TFM, the RS strain (F_{16}) of SBPH was continuously reared for 5 generations without TFM exposure. The RS strain resistance decreased from 13.05-fold (F_{16}) to 11.00-fold (RD_5), and the average selection response (*R*) was -0.01 (Table 3). However, there is no statistical decline in resistance from F_{16} to $RD_{1\text{-}5}$.

3.4 Cross-resistance of the RS strain

The results of cross-resistance between TFM and other insecticides for the RS strain of SBPH are shown in Table 4. Compared to SS strain, the cross-resistance ratio of the RS strain for imidacloprid, nitenpyram, thiamethoxam, dinotefuran, flonicamid, pymetrozine, and cyantraniliprole were 1.48, 1.28, 1.10, 0.90, 0.95, 1.39, and 1.08-fold, respectively. All cross-resistance ratio were lower than 2. The TFM had no cross-resistance with these insecticides.

3.5 Biological fitness comparison between the RS and susceptible strain strains

The development duration and life table parameters of the SS and RS strains of SBPH are shown in Table 5; Figure 3, respectively. The development time of third instar, fifth instar and pre-adult, adult preoviposition period (APOP), longevity of the RS strain were significantly reduced by 0.39, 0.59, 0.99, 0.85, and 3.93 days, respectively ($p < 0.05$) as compared to SS strain (Table 5). Further, the emergence rate and hatchability were significantly decreased in the RS strain by 5.6% and 5.7%, respectively ($p < 0.05$). In contrast, the female-male ratio was substantially increased by 5.3% ($p < 0.05$) compared to that of the SS strain (Figure 3). No statistically substantial differences were

TABLE 3 The resistance stability of RS strain of SBPH without TFM.

Generations	No.	Slope±SE	LC_{50} mg·L^{-1}	95% CI (mg·L^{-1})	χ^2 (*df*)	Resistance ratio (RR)	The average selection response (*R*)	LCR_{50}[a] (95% CI)[c]	Hypothesis of equality (χ^2, *p*)[d]
F_{16}	360	1.26 ± 0.32	7.18	4.31–11.32	2.88 (5)	13.05	−0.01		
RD_1[b]	360	1.64 ± 0.28	7.35	5.24–10.08	2.08 (5)	13.36		0.98 (0.57–1.68)	1.85.0.40
RD_2	360	1.34 ± 0.27	6.99	4.56–10.21	7.29 (5)	12.71		1.03 (0.58–1.83)	0.34.0.84
RD_3	360	1.44 ± 0.27	7.04	4.76–10.02	6.17 (5)	12.80		1.02 (0.58–1.79)	0.74.0.69
RD_4	360	1.13 ± 0.26	6.65	3.90–10.34	5.19 (5)	12.09		1.08 (0.58–2.01)	0.06.0.97
RD_5	360	1.28 ± 0.27	6.05	3.69–8.89	4.14 (5)	11.00		1.19 (0.66–2.15)	0.60.0.74

[a]LCR_{50} means LC_{50} of F_{16}/LC_{50} of RDn.
[b]RD, means Resistance decline strain.
[c]The difference is significant if the 95% confidence limit is greater than 1 and the *p* values for the equality hypothesis is less than 0.05.

TABLE 4 Cross-resistance of SS and RS strains of SBPH to seven insecticides.

Insecticides	Strains	No.	Slope±SE	LC_{50} (95%CI) ($mg{\cdot}L^{-1}$)	χ^2 (*df*)	Cross-resistance ratio (CR)
Imidacloprid	SS	360	1.21 ± 0.21	18.79 (11.65–27.34)	10.57 (6)	-
	RS	360	1.16 ± 0.20	27.73 (18.32–41.74)	7.57 (6)	1.48
Nitenpyram	SS	360	1.27 ± 0.32	1.90 (1.09–3.22)	1.78 (5)	-
	RS	360	1.38 ± 0.33	2.44 (1.56–4.29)	2.57 (5)	1.28
Thiamethoxam	SS	360	1.35 ± 0.33	5.37 (2.71–8.41)	3.24 (5)	-
	RS	360	1.34 ± 0.33	5.92 (3.13–9.34)	2.95 (5)	1.10
Dinotefuran	SS	360	1.45 ± 0.27	3.89 (2.68–5.59)	4.71 (5)	-
	RS	360	1.33 ± 0.27	3.51 (2.29–5.13)	6.25 (5)	0.90
Flonicamid	SS	360	1.20 ± 0.36	8.16 (3.86–13.76)	1.73 (5)	-
	RS	360	1.54 ± 0.38	7.76 (4.46–11.54)	2.93 (5)	0.95
Pymetrozine	SS	360	1.26 ± 0.32	28.40 (16.15–48.16)	1.61 (5)	-
	RS	360	1.577 ± 0.34	39.60 (26.73–66.29)	2.06 (5)	1.39
Cyantraniliprole	SS	360	1.17 ± 0.32	16.02 (7.86–27.34)	2.43 (5)	-
	RS	360	1.32 ± 0.33	17.25 (9.75–27.91)	2.23 (5)	1.08

TABLE 5 Duration of the development and life table parameters for the SS and RS strains of SBPH[a].

Stages		Susceptible strain (SS)	Resistance strain (RS)
Nymphal stage duration (d)	1st instar	2.89 ± 0.05[a]	2.78 ± 0.05[a]
	2nd instar	2.38 ± 0.06[a]	2.36 ± 0.05[a]
	3rd instar	2.70 ± 0.07[a]	2.31 ± 0.06[b]
	4th instar	3.26 ± 0.13[a]	3.22 ± 0.10[a]
	5th instar	4.78 ± 0.16[a]	4.19 ± 0.10[b]
	Pre-adult	15.87 ± 0.22[a]	14.88 ± 0.22[b]
Adult stage duration (d)		17.75 ± 0.84[a]	17.45 ± 0.81[a]
APOP[b] (d)		5.44 ± 0.23[a]	4.59 ± 0.19[b]
TPOP[c] (d)		19.96 ± 0.25[a]	20.38 ± 0.23[a]
Longevity(d)		29.76 ± 1.24[a]	25.83 ± 1.25[b]
Fecundity (eggs/female)		133.95 ± 9.11[a]	122.92 ± 7.32[a]
Emergence rate[d] (%)		79.63 ± 1.27[a]	74.03 ± 0.93[b]
Female-male ratio[d] (%)		48.87 ± 1.8[b]	54.17 ± 1.64[a]
Hatchability[d] (%)		94.2 ± 1.17[a]	88.5 ± 0.80[b]
r (d^{-1})		0.15 ± 0.01[a]	0.15 ± 0.01[a]
λ (d^{-1})		1.17 ± 0.01[a]	1.16 ± 0.01[a]
R_0 (offspring individual-1)		53.24 ± 7.44[a]	47.52 ± 6.66[a]
T (d)		25.61 ± 0.47[a]	25.45 ± 0.44[a]
R_f		-	0.89

[a]The values (mean ± SE) followed by different letters in the same row indicate the significant difference at $p < 0.05$ using the paired bootstrap test.
[b]APOP, means adult preoviposition period.
[c]TPOP, means total preoviposition period.
[d]The values (mean ± SE) followed by different letters in the same row are significantly different at 0.05 level using the Tukey's test.

observed in any other parameters ($p > 0.05$). Though no significant differences of R_0 were found, R_0 (47.52) of the RS strain was lower than the R_0 (53.24) of the SS strain, and the relative fitness R_f was 0.89 (Table 5).

3.6 Synergism of the enzyme inhibitors

The synergism results of enzyme inhibitors showed that (Table 6), PBO exhibited significant synergistic action on the

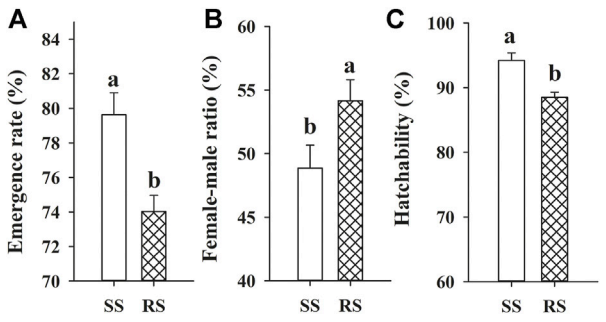


FIGURE 3
Means (±SE) of emergence rate **(A)**, female-male ratio **(B)** and hatchability **(C)** of SBPH in the SS and RS strains. Different letters above error bars are significantly different at 0.05 levels using the Tukey's test.

RS strain with synergistic ratios of 1.88-fold, but no significant synergistic action on the SS strain with synergistic ratios of 1.52-fold. TPP showed no significant synergistic action on the two strains, with a low synergistic ratio1.18- and 1.28-fold, respectively. DEM also had no synergism on two strains, only with synergistic ratios of 1.02- and 1.07-fold, respectively.

3.7 Detoxification metabolic enzyme activity

The activity of the three detoxification metabolic enzymes (CarE, GST, and P450) in the SS and RS strains of SBPH was shown in Figure 4. Compared to the SS strain, the activity of P450 in the RS strain increased significantly by 1.71-fold ($p < 0.01$), CarE in the RS strain also increased by 1.16-fold ($p < 0.05$). However, the GST activity showed no significant differences between in the SS and RS strains. According to the enzyme activity and synergism results, P450 and CarE may be both contribute to metabolic resistance of SBPH to TFM, and P450 may be one major metabolic factor.

3.8 Expression levels of P450 genes in RS and susceptible strain strains

To further clarify the association between P450 genes and the TFM resistance mechanism of SBPH, we determined and compared the differences in the expression levels of 53 P450 genes between the SS and RS strain. The up-regulated expression of nine genes (*CYP303A1*, *CYP304H1v4,* and *CYP305A13v2* from Clade 2; *CYP6CW3v2* and *CYP6ER2* from Clade 3; *CYP4C72*, *CYP4C78*, and *CYP4CE2* from Clade 4; *CYP419A1v2* from the mitochondrial clade) was more than 2-fold in the RS strain. In particular, the expression levels of *CYP303A1*, *CYP4CE2,* and *CYP419A1v2* were 4.82-, 8.92-, and 7.41- fold, respectively (Figure 5).

4 Discussion

SBPH has developed resistance to many insecticides due to the indiscriminate application of pesticides in the field (Elzaki et al., 2015; Liu et al., 2015). TFM is a novel insecticide and the only member of the 4E group to target nAChR (Cordova et al., 2016; Wen et al., 2021a). TFM is highly effective in controlling SBPH (Zhang Y. C et al., 2020). However, the resistance risk and underlying mechanism of SBPH against TFM have few studies reported yet.

After 21 generations of continuous selection with TFM, SBPH developed a 26.29-fold resistance to TFM. The resistance development showed a fluctuating upward trend. The SBPH slowly developed resistance against TFM till the F_8 generation. But the resistance was rapidly increased to 26.29-fold after the F_8 generation, reaching the middle resistance level. However, previous study showed SPBH could increase 45.1-fold

TABLE 6 Synergism of three enzyme inhibitors on TFM of SBPH.

Strain	Treatment	No.	Slope±SE	LC_{50} ($mg{\cdot}L^{-1}$) (95% CI)	χ^2 (*df*)	Synergistic ratios[a] (SR) (95% CI)[b]	Hypothesis of equality (χ^2, *p*)[c]
Sensitive strain (SS)	TFM	360	1.61 ± 0.34	0.57 (0.38–0.90)	3.20 (5)	-	
	TFM + TPP	360	1.34 ± 0.27	0.49 (0.33–0.72)	4.96 (5)	1.18 (0.69–2.02)	0.78.0.68
	TFM + DEM	360	1.64 ± 0.28	0.56 (0.40–0.77)	6.47 (5)	1.02 (0.62–1.69)	0.01.0.99
	TFM + PBO	360	1.37 ± 0.27	0.38 (0.25–0.55)	5.05 (5)	1.52 (0.89–2.60)	2.7.0.26
Resistance strain (RS)	TFM	360	1.44 ± 0.27	8.25 (5.74–11.99)	4.71 (5)	-	
	TFM + TPP	360	1.13 ± 0.29	6.46 (4.07–10.64)	5.08 (5)	1.28 (0.73–2.24)	1.56.0.46
	TFM + DEM	360	1.24 ± 0.26	7.70 (5.17–12.70)	3.62 (5)	1.07 (0.63–1.84)	0.39.0.82
	TFM + PBO	360	1.36 ± 0.33	4.39 (2.73–7.45)	3.62 (5)	1.88[b] (1.06–3.33)	4.59.0.04

[a]SR (synergism ratio) = LC_{50} of TFM/LC_{50} of TFM + synergist.
[b]The difference is significant if the 95% confidence limit is greater than 1 and the *p* values for the equality hypothesis is less than 0.05.
[c]Significant synergistic action.

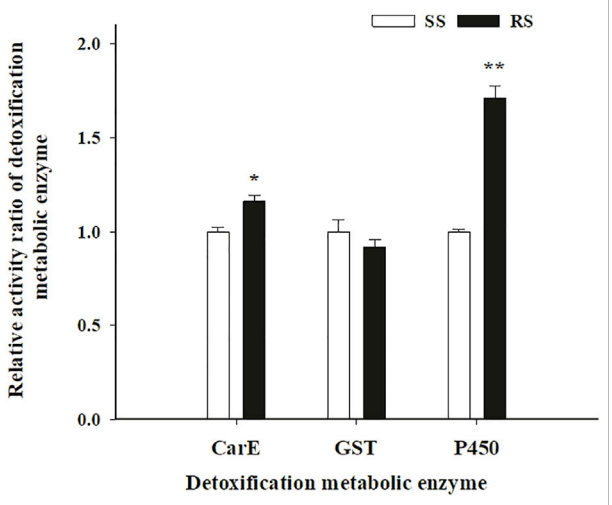


FIGURE 4
Means (±SE) of activity ratio of detoxification metabolic enzyme in SS and RS strains of SBPH. *Significant at 0.05 level and **Significant at 0.01 level using the Tukey's test.

to TFM only after 16 generations selection (Zhang et al., 2022). This different result may be due to the differences of selection method. Because the insecticide treated times were 96 h in previous study, which gave more selection pressure to SBPH than in our study, in which the treated times were only 72 h.

In the previous research, the resistance development trend of SBPH for buprofezin showed an "S" curve. During buprofezin resistance selection, the resistance increased slowly in the early stage and then increased exponentially after reaching a critical value. The resistance remained relatively stable after reaching a certain level (Zhang et al., 2012; Mao et al., 2016). The differences between our findings and those of other studies may be still due to insufficient time for resistance selection in our experiment. Therefore, the resistance selection of SBPH will need to be continued to obtain more realistic development trends and laws for resistance of SBPH to TFM.

Evaluating the resistance risk of new insecticides can provide critical theoretical references for scientific use and resistance prevention. The h^2 for resistance represents the ability of insect resistance to be inherited by the next generation and the risk of resistance development. Diptaningsari et al. (2019) reported that the h^2 of *Nilaparvata lugens* (Stål) for imidacloprid was 0.0893. Zhang Y. C et al. (2020) estimated that during the development of TFM resistance in *N. lugens*, h^2 was 0.0451. Recent studies have reported that the h^2 of TFM resistance in SBPH was 0.13 (Zhang et al., 2022). When the TFM selection pressure was used with a survival rate of 50% in each generation, the LC_{50} values could increase 10-fold only in 6.72 generations. However, we found that the h^2 of TFM resistance in SBPH was 0.09, which was lower than Zhang et al.'s results. The resistance could increase 10-fold in just 18.00 generations when the survival rate was 50% for each generation. Therefore, if TFM is consistently used in the field, SBPH will develop a specific TFM resistance.

Fu et al. (2018) determined the resistance stability of the spinetoram-selected strain of Thrips hawaiiensis and found that without insecticide exposure, the resistance decreased from 19.42-fold to 12.35- and 9.50-fold after two and five generations, respectively, and the average reaction rates were -0.0982 and -0.0621, respectively. In this study, the resistance level decreased 2.05-fold after 5 generations without TFM, but there were no statistical decline in resistance level from F_{16} to $RD_{1\text{-}5}$. The TFM resistance stability of SBPH was related high, which may be able to lead to problems for future resistance management of TFM.

TFM generally has no cross-resistance with other insecticides, including nAChR competitive regulators. Several studies have reported that TFM has no cross-resistance with sulfoxaflor, nitenpyram, clothianidin, and buprofezin (Mao et al., 2018; Liao et al., 2019a; Jin et al., 2019; Zeng et al., 2022). Our results showed that there was no evidence of cross-resistance between TFM and tested five neonicotinoids insecticides, pyridine azomethine insecticide and anthranilic diamide insecticide. However, in Zhang et al.'s study (2022), the triflumezopyrim-resistant strain of SBPH showed minor cross-

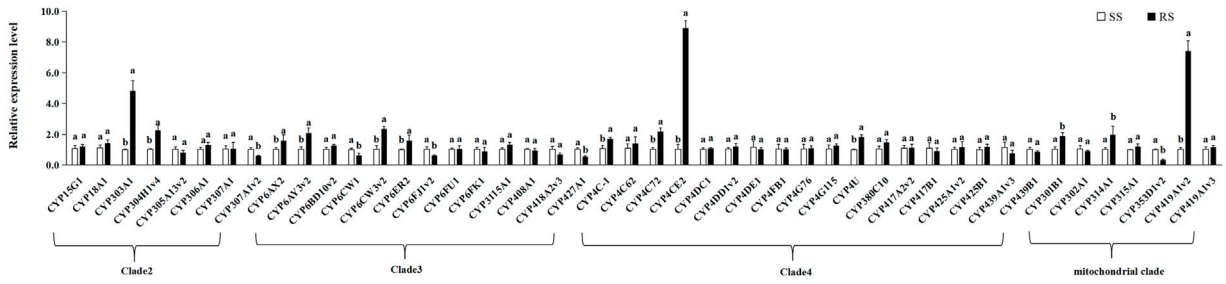


FIGURE 5
Means (±SE) of relative expression levels of P450 genes. Different letters above error bars for the same gene indicates significant differences between the RS and SS strains at 0.05 levels using the Tukey's test.

resistance to dinotefuran, in which may be due to the resistant level of previous study (45.1-fold) were higher than our research (26.29-fold). These results indicate that TFM may have a certain risk of cross-resistance to neonicotinoids insecticides in future.

Determining fitness cost and the relationship between resistance fitness and resistance are significant for clarifying the law of resistance development. Several studies have shown that resistant insect strains display fitness costs under insecticide selection pressure. Jin et al. (2021) showed that the R_f of clothianidin-resistant strains of N. lugens was 0.78. Compared with the SS strain, the APOP of clothianidin-resistant strains increased significantly, while fecundity decreased significantly. Zhang et al. (2018) studied the difference in life table between nitenpyram-resistant *N. lugens* and its susceptible strain by an age-stage, two-sex life table method and found that the fitness cost of the resistant strain was 0.55. Moreover, the r and R_0 values were lower than those of the susceptible strain. The developmental duration of the resistant strain was increased, whereas life span and hatchability were significantly decreased. In our study, the third instar, fifth instar, pre-adult, APOP, longevity, emergence rate, and hatchability of the RS strain were significantly reduced compared to the SS strain. The R_f value of the RS strain was 0.89. Therefore, the RS strain has disadvantages in development and reproduction. These adverse effects of fitness cost are important factors limiting resistance evolution, which may be valuable in formulating effective resistance management strategies (Kliot and Ghanim., 2012).

The metabolic resistance mechanism of SBPH to TFM can be revealed by comparing the activities of three detoxification metabolic enzymes and the synergism of enzyme inhibitors between the SS and RS strains. Several studies have demonstrated that increased activity of metabolic detoxification enzymes contributes significantly to insects' resistance to insecticides (Zhang S. R et al., 2020). Previously, Lu et al. (2017) found that TPP can effectively reduce the resistance level of *N. lugens* to chlorpyrifos, which demonstrated that the resistance of *N. lugens* to chlorpyrifos was related to an increase in CarE activity. The resistance of *N. lugens* to nitenpyram has also been associated with increased CarE activity (Zhang et al., 2017). However, previous study found that CarE activity was not associated with TFM resistance in SBPH (Zhang et al., 2022). In this study, although CarE enzyme activity in resistant strain was higher than that in susceptible strain, TPP had no obvious synergistic effect on the two strains.

The increase in GST activity was related to the resistance of insect pests to pyrethroids, neonicotinoids, and other insecticides (Vontas et al., 2001; Yang et al., 2016; He et al., 2018). However, based on the results of enzyme inhibitor synergism and activity, GST did not play a role in the resistance development in SBPH for TFM, similar to Zhang et al results (2022).

P450 is an important enzyme in detoxification. Many studies have shown that P450 can mediate the resistance of insects to insecticides (Tabashnik. 1992; Wen et al., 2009; Ding et al., 2013; Garrood et al., 2016). Mao et al. (2016) studied the resistance mechanism of SBPH for buprofezin and found that the enhanced P450 activity plays an essential role in the resistance of SBPH to buprofezin. Zhang et al. (2022) also found that P450 contribute to triflumezopyrim resistance in *L. striatellus*. Similar to previous studies, both the increases of P450 activities and higher synergistic ratio of PBO were found in resistant strain. The enzyme activity and synergism results indicated that P450 and CarE may be both contribute to metabolic resistance of SBPH to TFM, and P450 may be one major metabolic factor. More studies will need to be performed to confirm if CarE contributed to TFM resistance in SBPH in future.

To further clarify the detoxification enzymes involved in developing TFM resistance in SBPH, we selected 53 P450 genes for their expression analysis based on the enzyme activity and enzyme inhibitor results. The overexpression of the P450 genes generally causes the enhancement of P450 activity in resistant insects (Elzaki et al., 2016). Previous studies reported overexpression of *CYP6AY3v2*, *CYP353D1v2*, and *CYP4C71* in resistant SBPH strains. These genes encode the P450 enzymes, which can metabolize imidacloprid and mediate the generation of resistance to imidacloprid (Elzaki et al., 2016; Elzaki et al., 2017; Wang et al., 2017; Xiao et al., 2019). Zhang et al. (2022) found that seven P450 genes were up-regulated more than 1.5-fold in TFM-resistance strain of SBPH, but only three genes were up-regulated more than 2-fold. In our study, compared with SS strain, nine genes were more than 2-fold overexpressed in the RS strain, which *CYP303A1*, *CYP4CE2*, and *CYP419A1v2* were significantly up-regulated.

Previous studies have shown that the resistance of *Bemisia tabaci* field population to imidacloprid may be related to the increased expression of *CYP303A1-like* gene. (Wang Q. et al., 2020). The significant overexpression of *CYP303* gene has a significant correlation with the formation of resistance of *Bemisia tabaci* to imidacloprid in the field. (Ilias et al., 2015). Therefore, *CYP303A1* may play a role in the resistance of SBPH to TFM. However, Zhang et al. (2012) found that the expression levels of *CYP303A1* and *CYP419A1v2* genes were not related to the resistance of *L. striatellus* to thiamethoxam. Also, there were no significant differences in *CYP4CE2* expression between deltamethrin-resistant population and sensitive population of *L. striatellus* (Xu et al., 2013). Next, further studies are still needed to prove whether these three genes are associated with TFM resistance in *L. striatellus*. In summary, the results of this study on the resistance risk and metabolic resistance mechanism of SBPH for TFM could assist in the rational application and prolong the insecticide service life in the field context. Moreover, these findings will provide an essential theoretical reference for delaying the development of resistance to TFM and resistance management.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

SW and CL conceived the research idea and designed the methodology. SW, XW, YW, and CL conducted the experiments and collected data. CL and XW analyzed the data. SW wrote the manuscript. XX and JW edited the manuscript. All authors approved the final version of the manuscript.

Funding

This research was sponsored by the National Natural Science Foundation of China (No. 32072459 and 31301695), the National Key Research and Development Program (No. 2018YFD0200604), and Funds of Shandong "Double Tops" Program (No. SYL2017XTTD11).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.1048208/full#supplementary-material

References

Afzal, M. B. S., Shad, S. A., Abbas, N., Ayyaz, M., and Walker, W. B. (2015). Cross-resistance, the stability of acetamiprid resistance and its effect on the biological parameters of cotton mealybug, *Phenacoccus solenopsis* (Homoptera: Pseudococcidae), in Pakistan. *Pest Manag. Sci.* 71, 151–158. doi:10.1002/ps.3783

Afzal, M. B. S., and Shad, S. A. (2016). Genetic analysis realized heritability and synergistic suppression of indoxacarb resistance in *Phenacoccus solenopsis Tinsley* (Homoptera: Pseudococcidae). *Crop Prot.* 84, 62–68. doi:10.1016/j.cropro.2016.02.009

Aitio, A. (1978). A simple and sensitive assay of 7-ethoxycoumarin deethylationt. *Anal. Biochem.* 85, 488–491. doi:10.1016/0003-2697(78)90245-2

Akca, I., Ayvaz, T., Yazici, E., Smith, C. L., and Chi, H. (2015). Demography and population projection of *Aphis fabae* (Hemiptera: Aphididae): With additional comments on life table research criteria. *J. Econ. Entomol.* 108, 1466–1478. doi:10.1093/jee/tov187

Chen, L., Wang, X. G., Zhang, Y. Z., Yang, R., Zhang, S. R., Xu, X., et al. (2020). The population growth, development and metabolic enzymes of the white-backed planthopper, *Sogatella furcifera* (Hemiptera: Delphacidae) under the sublethal dose of triflumezopyrim. *Chemosphere* 247, 125865. doi:10.1016/j.chemosphere.2020.125865

Chi, H. (2020). TWOSEX-MSChart: A computer program for the age-stage, two-sex lifetable analysis. Available at: http://140.120.197.173/Ecology/(accessed March 27, 2020).

Chi, H. (1988). Life-table analysis incorporating both sexes and variable development rates among individuals. *Environ. Entomol.* 17, 26–34. doi:10.1093/ee/17.1.26

Cordova, D., Benner, E. A., Schroeder, M. E., Holyoke, C. W., Jr., Zhang, W. M., Pahutski, T. F., et al. (2016). Mode of action of triflumezopyrim: A novel mesoionic insecticide which inhibits the nicotinic acetylcholine receptor. *Insect biochem. Mol. Biol.* 74, 32–41. doi:10.1016/j.ibmb.2016.04.008

Ding, J., Wang, Y. W., Wang, S., Wang, X. T., Wang, Z. W., Liu, C., et al. (2021). Effects of host plants on the population parameters and activities of detoxification enzymes in *Laodelphax striatellus*. *Entomol. Exp. Appl.* 169, 264–274. doi:10.1111/eea.13014

Ding, Z. P., Wen, Y. C., Yang, B. J., Zhang, Y. X., Liu, S. H., Liu, Z. W., et al. (2013). Biochemical mechanisms of imidacloprid resistance in *Nilaparvata lugens*: Over-expression of cytochrome P450 *CYP6AY1*. *Insect biochem. Mol. Biol.* 43, 1021–1027. doi:10.1016/j.ibmb.2013.08.005

Diptaningsari, D., Trisyono, Y. A., Purwantoro, A., and Wijonarko, A. (2019). Inheritance and realized heritability of resistance to imidacloprid in the Brown planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae), from Indonesia. *J. Econ. Entomol.* 112, 1831–1837. doi:10.1093/jee/toz090

Elzaki, M. E. A., Miah, M. A., Wu, M., Zhang, H., Pu, J., Jiang, L., et al. (2017). Imidacloprid is degraded by *CYP353D1v2*, a cytochrome P450 overexpressed in a resistant strain of *Laodelphax striatellus*. *Pest Manag. Sci.* 73, 1358–1363. doi:10.1002/ps.4570

Elzaki, M. E. A., Zhang, W., Feng, A., Qiou, X., Zhao, W., and Han, Z. (2016). Constitutive overexpression of cytochrome P450 associated with imidacloprid resistance in *Laodelphax striatellus* (Fallen). *Pest Manag. Sci.* 72, 1051–1058. doi:10.1002/ps.4155

Elzaki, M. E. A., Zhang, W., and Han, Z. (2015). Cytochrome P450 *CYP4DE1* and *CYP6CW3v2* contribute to ethiprole resistance in *Laodelphax striatellus* (Fallén). *Insect Mol. Biol.* 24, 368–376. doi:10.1111/imb.12164

Fu, B. L., Li, Q., Qiu, H. Y., Tang, L. D., Zeng, D. Q., Liu, K., et al. (2018). Resistance development, stability, cross-resistance potential, biological fitness and biochemical mechanisms of spinetoram resistance in *Thrips hawaiiensis* (Thysanoptera: Thripidae). *Pest Manag. Sci.* 74, 1564–1574. doi:10.1002/ps.4887

Gao, B. L., Wu, J., Huang, S. J., Mu, L. F., and Han, Z. J. (2008). Insecticide resistance in field population of *Laodelphax striatellus* (fallén) (Hemiptera: Delphacidae) in China and its possible mechanisms. *Int. J. Pest Manag.* 54, 13–19. doi:10.1080/09670870701553303

Garrood, W. T., Zimmer, C. T., Gorman, K. J., Nauen, R., and Davies, T. (2016). Field-evolved resistance to imidacloprid and ethiprole in populations of Brown planthopper *Nilaparvata lugens* collected from across south and East Asia. *Pest Manag. Sci.* 72 (1), 140–149. doi:10.1002/ps.3980

Han, Z. J., Moores, G. D., Denholm, I., and Devonshire, A. L. (1998). Association between biochemical markers and insecticide resistance in the cotton aphid, *Aphis gossypii* Glover. *Pestic. Biochem. Physiol.* 62, 164–171. doi:10.1006/pest.1998.2373

He, C., Xie, W., Yang, X., Wang, S. L., Wu, Q. J., and Zhang, Y. J. (2018). Identification of glutathione S-transferases in *Bemisia tabaci* (Hemiptera: Aleyrodidae) and evidence that *GSTd7* helps explain the difference in insecticide susceptibility between *B. tabaci* Middle East-Minor Asia 1 and Mediterranean. *Insect Mol. Biol.* 27, 22–35. doi:10.1111/imb.12337

Huang, Y. B., and Chi, H. (2013). Life tables of *Bactrocera cucurbitae* (Diptera: Tephritidae): With an invalidation of the jackknife technique. *J. Appl. Entomol.* 137, 327–339. doi:10.1111/jen.12002

Ilias, A., Lagnel, J., Kapantaidaki, D. E., Roditakis, E., Tsigenopoulos, C. S., Vontas, J., et al. (2015). Transcription analysis of neonicotinoid resistance in Mediterranean (MED) populations of *B. tabaci* reveal novel cytochrome P450s, but no nAChR mutations associated with the phenotype. *BMC Genomics* 16, 939. doi:10.1186/s12864-015-2161-5

Jin, R. H., Mao, K. K., Liao, X., Xu, P. F., Li, Z., Ali, E., et al. (2019). Overexpression of CYP6ER1 associated with clothianidin resistance in *Nilaparvata lugens* (Stål). *Pestic. Biochem. Physiol.* 154, 39–45. doi:10.1016/j.pestbp.2018.12.008

Jin, R. H., Mao, K. K., Xu, P. F., Wang, Y., Liao, X., Wan, H., et al. (2021). Inheritance mode and fitness costs of clothianidin resistance in Brown planthopper, *Nilaparvata lugens* (Stål). *Crop Prot.* 140, 105414. doi:10.1016/j.cropro.2020.105414

Kao, C. H., Hung, C. F., and Sun, C. N. (1989). Parathion and methyl parathion resistance in diamondback moth (Lepidoptera: Plutellidae) larvae. *J. Econ. Entomol.* 82, 1299–1304. doi:10.1093/jee/82.5.1299

Kliot, A., and Ghanim, M. (2012). Fitness costs associated with insecticide resistance. *Pest Manag. Sci.* 68, 1431–1437. doi:10.1002/ps.3395

Liao, X., Jin, R. H., Zhang, X. L., Ali, E., Mao, K. K., Xu, P. F., et al. (2019a). Characterization of sulfoxaflor resistance in the Brown planthopper, *Nilaparvata lugens (Stål). Pest Manag. Sci.* 75, 1646–1654. doi:10.1002/ps.5282

Liao, X., Mao, K. K., Ali, E., Jin, R. H., Li, Z., Li, W., et al. (2019b). Inheritance and fitness costs of sulfoxaflor resistance in *Nilaparvata lugens* (stål). *Pest Manag. Sci.* 75, 2981–2988. doi:10.1002/ps.5412

Liu, S. H., Shi, B. F., Ge, S. S., Yang, B. J., and Liu, Z. W. (2015). Iimidacloprid resistance in *Laodelphax striatellus* (Fallén) populations from different areas in China. *Plant Prot.* 41, 181–186. doi:10.3969/j.issn.0529-1542.2015.02.035

Liu, Z. W., and Han, Z. J. (2006). Fitness costs of laboratory-selected imidacloprid resistance in the Brown planthopper, *Nilaparvata lugens* Stål. *Pest Manag. Sci.* 62, 279–282. doi:10.1002/ps.1169

Livak, K. J., and Schmittgen, T. D. (2021). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25, 402–408. doi:10.1006/meth.2001.1262

Lu, K., Wang, Y., Chen, X., Zhang, Z. C., Li, W. R., Zhou, Q., et al. (2017). Characterization and functional analysis of a carboxylesterase gene associated with chlorpyrifos resistance in *Nilaparvata lugens* (Stål). *Comp. Biochem. Physiol. C. Toxicol. Pharmacol.* 203, 12–20. doi:10.1016/j.cbpc.2017.10.005

Mao, K. K., Zhang, X. L., Ali, E., Liao, X., Jin, R. H., Ren, Z. J., et al. (2018). Characterization of nitenpyram resistance in *Nilaparvata lugens* (Stål). *Pestic. Biochem. Physiol.* 157, 26–32. doi:10.1016/j.pestbp.2019.03.001

Mao, X. L., Liu, J., Li, X. K., Chi, J. J., and Liu, Y. J. (2016). Resistance risk, cross-resistance and biochemical resistance mechanism of *Laodelphax striatellus* to buprofezin. *Chin. J. Appl. Ecol.* 27, 255–262. doi:10.13287/j.1001-9332.201601.036

Miah, M. A., Elzaki, M. E. A., Husna, A., and Han, Z. J. (2018). An overexpressed cytochrome P450 CYP439A1v3 confers deltamethrin resistance in *Laodelphax striatellus* (fallén) (Hemiptera: Delphacidae). *Arch. Insect Biochem. Physiol.* 100, e21525. doi:10.1002/arch.21525

Mu, X. C., Zhang, W., Wang, L. X., Zhang, S., Zhang, K., Gao, C. F., et al. (2016). Resistance monitoring and cross-resistance patterns of three rice planthoppers, *Nilaparvata lugens, Sogatella furcifera* and *Laodelphax striatellus* to dinotefuran in China. *Pestic. Biochem. Physiol.* 134, 8–13. doi:10.1016/j.pestbp.2016.05.004

Shao, L. Y., Sun, F. Y., Li, Z. Q., Yu, F. Q., Yang, M., and Wang, X. Q. (2012). An accurate and simple method for egg stripping and detection of Laodelphax striatellus. *Liaoning Agric. Sci.* 3, 61–62. doi:10.3969/j.issn.1002-1728.2012.03.018

Tabashnik, B. E., and Mcganghey, W. H. (1994). Resistance risk assessment for single and multiple insecticides: Responses of Indian meal moth (Lepidoptera: Pyralidae) to *Bacillus thuringiensis*. *J. Econ. Entomol.* 87, 834–841. doi:10.1093/jee/87.4.834

Tabashnik, B. E. (1992). Resistance risk assessment: Realized heritability of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae), *tobacco budworm* (Lepidoptera: Noctuidae) and *Colorado potato beetle* (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* 85, 1551–1559. doi:10.1093/jee/85.5.1551

Tieu, S., Chen, Y. Z., Woolley, L. K., Collins, D., Barchia, I., Lo, N., et al. (2017). A significant fitness cost associated with ACE1 target site pirimicarb resistance in a field isolate of *Aphis gossypii* Glover from Australian cotton. *J. Pest Sci. (2004).* 90, 773–779. doi:10.1007/s10340-016-0803-2

Vontas, J. G., Small, G. J., and Hemingway, J. (2001). Glutathione S-transferases as antioxidant defence agents confer pyrethroid resistance in *Nilaparvata lugens*. *Biochem. J.* 357, 65–72. doi:10.1042/0264-6021:3570065

Wang, F., Liu, J., Shuai, S., Miao, C. L., Chi, B. J., Chen, P., et al. (2021). Resistance of *Bemisia tabaci* mediterranean (Q-biotype) to pymetrozine: Resistance risk assessment, cross-resistance to six other insecticides and detoxification enzyme assay. *Pest Manag. Sci.* 77, 2114–2121. doi:10.1002/ps.6240

Wang, L. H., Fang, J. Z., and Liu, B. S. (2008). Relative toxicity of insecticides to *Laodelphax striatellus* (fallén) (Homoptera: Delphacidae) and the resistance of field populations from different areas of east China. *Acta Entomol. Sin.* 51, 930–937. doi:10.3321/j.issn:0454-6296.2008.09.006

Wang, Q., Wang, M. N., Jia, Z. Z., Ahmat, T., Xie, L. J., and Jiang, W. H. (2020a). Resistance to neonicotinoid insecticides and expression changes of eighteen cytochrome P450 genes in field populations of *Bemisia tabaci* from Xinjiang, China. *Entomol. Res.* 50, 205–211. doi:10.1111/1748-5967.12427

Wang, R., Qu, C., Wang, Z. Y., and Yang, G. F. (2020). Cross-resistance, biochemical mechanism and fitness costs of laboratory-selected resistance to pyridalyl in diamondback moth, *Plutella xylostella*. *Pestic. Biochem. Physiol.* 163, 8–13. doi:10.1016/j.pestbp.2019.10.008

Wang, R., Zhu, Y., Deng, L., Zhang, H., Wang, Q., Yin, M., et al. (2017). Imidacloprid is hydroxylated by *Laodelphax striatellus CYP6AY3v2. Insect Mol. Biol.* 26, 543–551. doi:10.1111/imb.12317

Wei, Q., Wu, S. F., and Gao, C. F. (2017). Moleular characterization and expression pattern of three GABA receptor-like subunits in the small Brown planthopper *Laodelphax striatellus* (Hemiptera: Delphacidae). *Pestic. Biochem. Physiol.* 136, 34–40. doi:10.1016/j.pestbp.2016.08.007

Wen, S. F., Liu, C., Wang, Y. W., Xue, Y. N., Wang, X. T., Wang, J. H., et al. (2021a). Oxidative stress and DNA damage in earthworm (*Eisenia fetida)* induced by triflumezopyrim exposure. *Chemosphere* 264, 128499. doi:10.1016/j.chemosphere.2020.128499

Wen, S. F., Xue, Y. N., Du, R. S., Liu, C., Wang, X. T., Wang, Y. W., et al. (2021b). Toxicity and sublethal effects of triflumezopyrim on the development and detoxification enzymatic activities in the small Brown planthopper (SBPH), *Laodelphax striatellus* (Fallen). *Crop Prot.* 150, 105813. doi:10.1016/j.cropro.2021.105813

Wen, Y. C., Liu, Z. W., Bao, H. B., and Han, Z. J. (2009). Imidacloprid resistance and its mechanisms in field populations of Brown planthopper, *Nilaparvata lugens* Stål in China. *Pestic. Biochem. Physiol.* 94, 36–42. doi:10.1016/j.pestbp.2009.02.009

Wu, Y., Ding, J., Xu, B., You, L. L., Ge, L. Q., Yang, G. Q., et al. (2018). Two fungicides alter reproduction of the small Brown planthopper *Laodelphax striatellus* by influencing gene and protein expression. *J. Proteome Res.* 17, 978–986. doi:10.1021/acs.jproteome.7b00612

Xiao, Q. Q., Deng, L., Elzaki, M. E. A., Zhu, L., Xu, Y. F., Han, X. Y., et al. (2019). The inducible *CYP4C71* can metabolize imidacloprid in *Laodelphax striatellus* (Hemiptera: Delphacidae). *J. Econ. Entomol.* 113, 399–406. doi:10.1093/jee/toz292

Xu, L., Wu, M., and Han, Z. J. (2014). Biochemical and molecular characterisation and cross-resistance in field and laboratory chlorpyrifos-resistant strains of *Laodelphax striatellus* (Hemiptera: Delphacidae) from eastern China. *Pest Manag. Sci.* 70, 1118–1129. doi:10.1002/ps.3657

Xu, L., Wu, M., and Han, Z. J. (2013). Overexpression of multiple detoxification genes in deltamethrin resistant *Laodelphax striatellus* (Hemiptera:Delphacidae) in China. *PLoS ONE* 8, e79443. doi:10.1371/journal.pone.0079443

Xu, P. F., Shu, R. H., Gong, P. P., Li, W. H., Wan, H., and Li, J. H. (2019). Sublethal and transgenerational effects of triflumezopyrim on the biological traits of the Brown planthopper, *Nilaparvata lugens* (stål) (Hemiptera: Delphacidae). *Crop Prot.* 117, 63–68. doi:10.1016/j.cropro.2018.11.010

Xue, Y. N., Liu, C., Liu, D. M., Ding, W. J., Li, Z. G., Cao, J. L., et al. (2022). Sensitivity differences and biochemical characteristics of *Laodelphax striatellus* (Fallén) to seven insecticides in different areas of Shandong, China. *Insects* 13, 780. doi:10.3390/insects13090780

Yang, X., He, C., Xie, W., Liu, Y. T., Xia, J. X., Yang, Z. Z., et al. (2016). Glutathione S-transferases are involved in thiamethoxam resistance in the field whitefly *Bemisia tabaci* Q (Hemiptera: Aleyrodidae). *Pestic. Biochem. Physiol.* 134, 73–78. doi:10.1016/j.pestbp.2016.04.003

Zeng, B., Liu, Y. T., Zhang, W. J., Feng, Z. R., Wu, S. F., Gao, C. F., et al. (2022). Inheritance and fitness cost of buprofezin resistance in a near-isogenic, field-derived strain and insecticide resistance monitoring of *Laodelphax striatellus* in China. *Pest Manag. Sci.* 78 (5), 1833–1841. doi:10.1002/ps.6801

Zhang, K. L., Li, Z. Q., Ding, J., Du, R. S., Zhao, X. Y., and Xia, X. M. (2020). Sensitivity detection of cyantraniliprole to *Laodelphax striatellus* in Shandong and effect of sublethal cyantraniliprole on detoxification enzyme activities. *J. Environ. Entomology* 1, 212–220. doi:10.3969/j.issn.1674-0858.2020.01.27

Zhang, S. R., Gu, F. C. A., Du, Y., Li, X. Y., Gong, C. W., Pu, J., et al. (2022). Risk assessment and resistance inheritance of triflumezopyrim resistance in *Laodelphax striatellus*. *Pest Manag. Sci.* 78, 2851–2859. doi:10.1002/ps.6909

Zhang, S. R., Wang, X. G., Gu, F. C., Gong, C. W., Chen, L., Zhang, Y. M., et al. (2020). Sublethal effects of triflumezopyrim on biological traits and detoxification enzyme activities in the small Brown planthopper *Laodelphax striatellus* (Hemiptera: Delphacidae). *Front. Physiol.* 167, 261. doi:10.3389/fphys.2020.00261

Zhang, X. L., Liao, X., Mao, K. K., Yang, P., Li, D. Y., Ali, E., et al. (2017). The role of detoxifying enzymes in field-evolved resistance to nitenpyram in the Brown planthopper *Nilaparvata lugens* in China. *Crop Prot.* 94, 106–114. doi:10.1016/j.cropro.2016.12.022

Zhang, X. L., Mao, K. K., Liao, X., He, B. Y., Jin, R. H., Tang, T., et al. (2018). Fitness cost of nitenpyram resistance in the Brown planthopper *Nilaparvata lugens*. *J. Pest Sci. (2004).* 91 (3), 1145–1151. doi:10.1007/s10340-018-0972-2

Zhang, Y. C., Feng, Z. R., Zhang, S., Pei, X. G., Zeng, B., Zheng, C., et al. (2020). Baseline determination, susceptibility monitoring and risk assessment to triflumezopyrim in *Nilaparvata lugens* (Stål). *Pestic. Biochem. Physiol.* 167, 104608. doi:10.1016/j.pestbp.2020.104608

Zhang, Y. L., Guo, H. F., Yang, Q., Li, S., Wang, L. H., Zhang, G. F., et al. (2012). Over expression of a P450 gene (*CYP6CW1*) in buprofezin-resistant *Laodelphax striatellus* (Fallén). *Pestic. Biochem. Physiol.* 104, 277–282. doi:10.1016/j.pestbp.2012.10.002

Zheng, X. M., Tao, Y. L., Chi, H., Wan, F. H., and Chu, D. (2017). Adaptability of small Brown planthopper to four rice cultivars using life table and population projection method. *Sci. Rep.* 7, 42399. doi:10.1038/srep42399

frontiers | Frontiers in Physiology

TYPE Original Research
PUBLISHED 22 December 2022
DOI 10.3389/fphys.2022.1107045

OPEN ACCESS

EDITED BY
Xiaoming Xia,
Shandong Agricultural University, China

REVIEWED BY
Hu Wan,
Huazhong Agricultural University, China
Shun-Fan Wu,
Nanjing Agricultural University, China

*CORRESPONDENCE
Yao Tan,
850310.tanhuaf4@163.com

SPECIALTY SECTION
This article was submitted to Invertebrate Physiology, a section of the journal Frontiers in Physiology

RECEIVED 24 November 2022
ACCEPTED 12 December 2022
PUBLISHED 22 December 2022

CITATION
Ren H, Zhang H, Ni R, Li Y, Li L, Wang W, Tian Y, Pang B and Tan Y (2022), Detection of ryanodine receptor G4911E and I4754M mutation sites and analysis of binding modes of diamide insecticides with RyR on *Galeruca daurica* (Coleoptera: Chrysomelidae). *Front. Physiol.* 13:1107045. doi: 10.3389/fphys.2022.1107045

Detection of ryanodine receptor G4911E and I4754M mutation sites and analysis of binding modes of diamide insecticides with RyR on *Galeruca daurica* (Coleoptera: Chrysomelidae)

Hao Ren[1], Hongling Zhang[1], Ruoyao Ni[2], Yanyan Li[1], Ling Li[1], Wenhe Wang[3], Yu Tian[4], Baoping Pang[1] and Yao Tan[1,2]*

[1]Research Center for Grassland Entomology, Inner Mongolian Agricultural University, Hohhot, China, [2]State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, China, [3]Forestry station of Ar Horqin Banner, Chifeng, China, [4]Grassland Station of Xianghuang Banner, Xilinhot, China

In recent years, the leaf beetle *Galeruca daurica* has broken out in the northern grasslands of Inner Mongolia, its management still mainly depends on chemical control using traditional insecticides or with novel action. The study was aim to identify mutation locus associated with resistance to diamide insecticides in field population of *G. daurica*, to provide a reference for rational selection of insecticides and to avoid the rapid resistance development to diamide insecticides. We cloned the full length of the ryanodine receptor gene of *G. daurica* (*GdRyR*), constructed 3D model and transmembrane regions by homologous modeling based on deduced amino acid sequence. Two potential mutation loci (Gly4911Glu and Ile4754Met) and allelic mutation frequencies were detected in individuals of *G. daurica*. In addition, their binding patterns to two diamide insecticides (chlorantraniliprole, cyantraniliprole) were analyzed separately using a molecular docking method. The full-length cDNA sequence of *GdRyR* (GenBank accession number: OP828593) was obtained by splicing and assembling, which is 15,399 bp in length and encodes 5,133 amino acids. The amino acid similarity of *GdRyR* with that of other Coleopteran insects were 86.70%–91.33%, which possessed the typical structural characteristics. An individual resistance allelic mutation frequency test on fifty field leaf beetles has identified 12% and 32% heterozygous individuals at two potential mutation loci Gly4911Glu and Ile4754Met, respectively. The affinity of the I4754M mutant model of *GdRyR* for chlorantraniliprole and cyantraniliprole was not significantly different from that of the wild type, and all had non-covalent interactions such as hydrogen bonding, hydrophobic interactions and π-cation interactions. However, the G4911E mutant model showed reduced affinity and reduced mode of action with two diamide insecticides, thus affecting the binding stability of the ryanodine receptor to the diamide insecticides. In conclusion, the G4911E mutation in *GdRyR* may be a potential mechanism for the development of resistance to diamide insecticides on *G. daurica* and

should be a key concern for resistance risk assessment and reasonable applications of diamide insecticides for control in future. Moreover, this study could provide a reference for ryanodine receptor structure-based insecticides design.

KEYWORDS

Galeruca daurica, ryanodine receptor, diamide insecticide, homologous modeling, molecular docking, binding modes

Introduction

Galeruca daurica (Coleoptera: Chrysomelidae), a serious grassland pest since its sudden outbreaks on the Inner Mongolia grasslands in 2009 (Zhou et al., 2016, Zhou et al., 2019), has continually spread and caused great losses to pasture on the Inner Mongolia grasslands (Ma et al., 2019, Ma et al., 2021), and levels of damage has increased year by year (Duan et al., 2022). The application of traditional chemical insecticides to control outbreaking pests in desert steppes has the advantages of a good insecticidal effect with rapid results, especially organophosphorus, pyrethroids, chloronicotinyl insecticide (Dong et al., 2016; Gao et al., 2022). Currently, the management of *G. daurica* is dependent on above-mentioned insecticides (Chang et al., 2015; Zhang et al., 2022). Besides, plant-derived insecticides have been reported to provide good protection against the key pest *G. daurica*, such as: neem, matrine and nicotine analogues (Chang et al., 2015; Du et al., 2016). In recent years, the chemical insecticides with novel action, high efficiency, high selectivity, low toxicity and low residue are being chosen to control this outbreaking insect in desert steppe. Among them, chlorantraniliprole has been reported to have strong insecticidal activity against the *G. daurica* and recommended for pest management (Zhang et al., 2022).

Diamide insecticides are a new class of highly effective, broad-spectrum, low toxicity, good systemic absorption and highly selective insecticides with O-amido benzamide chemical structure, mainly with stomach toxicity and high effectiveness against Coleoptera, Diptera and Lepidoptera insects (Tohnishi et al., 2005; Temple et al., 2009; Hughes et al., 2013; Huang et al., 2021). The main products currently on the market include: Chlorantraniliprole, Fluorobenzamide, Cyantraniliprole, Cyclaniliprole, Tetrachlorantraniliprole, Broflanilide, and Tetraniliprole (Sparks and Nauen, 2014). These chemical ligands can open calcium channels in insects by binding to the Ryanodine receptor (RyR), causing the freeing of large amounts of Ca^{2+} from the sarcoplasmic reticulum and endoplasmic reticulum, therefore, a significant reduction in the Ca^{2+} content triggers an imbalance in the insect's internal environment, resulting in the cessation of feeding, functional muscle disorders, neurological disorders, and even death (Sattelle et al., 2008; Jeanguenat, 2013; Guo et al., 2021). RyR is a calcium channel protein distributed in the endoplasmic reticulum and sarcoplasmic reticulum of animal myocytes, and consists of a homotetrameric structure with four subunits containing 5,000 amino acid residues (Amador et al., 2009). Currently, diamide insecticides have been widely used against key agricultural insects, such as: diamond back moth *Plutella xylostella* (Guo et al., 2014, Guo et al., 2021), beet armyworm *Spodoptera exigua* (Zuo et al., 2017), *Spodoptera frugiperda* (Lv et al., 2021), rice stem borer Chilo suppressalis (Gao et al., 2013; Huang et al., 2020) and so on. Among them, chlorantraniliprole and flubendiamide were reported the most widely-used diamide insecticides with high insecticidal activity, low toxicity and safety to mammals and humans (Troczka et al., 2015).

In recent years, it has been continuously reported that the overuse of diamide insecticides led to the occurrence of resistance, with the resistance mechanism of the diamond back moth *P. xylostella* as a lepidopteran model pest being more intensively studied. Troczka et al. (2015) reported that the G4946E mutation in RyR was detected in resistant *P. xylostella* populations in Thailand and the Philippines, and was confirmed to affect the binding of diamide insecticides to RyR by *in vitro* expression in Sf9 cells. More recent studies have demonstrated that the I4790M/K mutation in PxRyR is associated with resistance to diamide insecticides in field populations of diamond back moth (Guo et al., 2014; Jouraku et al., 2020; Wang et al., 2020; Jiang et al., 2021; Richardson et al., 2021). It was reported that the high frequencies of mutations were found among six Chinese field populations collected from 2016 to 2019 resulting in RyR I4743M substitutions on *Spodoptera exigua*, however, no significant correlation was found between chlorantraniliprole resistance level and SeRyR I4743M allele frequency but CYP9A186 F116V substitutions (Teng et al., 2022). Boaventura et al. (2019) reported that a I4734M mutation of the FAW ryanodine receptor (RyR) C-terminal domains II to VI has recently been described to confer target-site resistance to diamides in Lepidopteran insect *Spodoptera frugiperda*. Among the reported mutant loci of the insect ryanodine receptor involving resistance to diamide insecticides, G4946E and I4790M on *P. xylostella* are the classical allele substitutions with the highest frequency of resistance, corresponding to G4911 and I4754 of RyR in *G. daurica*, respectively. It has not been reported that resistance occurrence, resistance levels of diamide insecticides on *G. daurica*, whether there are amino acid changes in *GdRyR* in field populations and whether they may contribute to resistance to diamide insecticides.

With the development of bioinformatics and computer simulation technology, protein homology modeling, molecular docking and other technical tools are becoming increasingly sophisticated. Novel ideas for studying pest resistance mechanisms to insecticides are proposed by predicting the binding pattern of receptors and ligands (Chen et al., 2021). Homology modeling uses the crystal structure of a protein molecule with high homology to its amino acid sequence as a template for sequence alignment, and predicts the structure of an unknown, homologous protein from the conserved domain of known crystal protein molecule (Boris and Ke, 2022). At present, the crystal structure of the *GdRyR* protein has not been identified, but the structure analysis of some mammalian ryanodine receptors is more mature and can be used as a reference approach for structural simulation and molecular docking (Ma et al., 2020). The deduced amino acid sequence was obtained from the cloned *GdRyR* gene sequence, and homology modeling and molecular docking were applied to construct the 3D model and analyze its affinity conformation, respectively.

In the study, we cloned the full-length *GdRyR* gene, examined the two classic mutation allele frequency of *GdRyR* associated with resistance to diamide insecticides in individuals of field populations of *G. daurica*, and applied homology modeling to construct a three-dimensional model of *GdRyR* and its transmembrane region. The binding patterns of *GdRyR* and diamide insecticides were studied by molecular docking, and the relationship between potential mutation sites of *GdRyR* and resistance was analyzed. The aim of this study was to explore the mechanism of resistance to diamide insecticides from the perspective of structural biology, and to lay a theoretical foundation for further analysis of the resistance mechanism of diamide insecticides.

Materials and methods

Insect rearing and sample preparation

The overwintering eggs of *G. daurica* were collected from the Xianghuang Banner grasslands (44°62′N, 115°80′E) of Inner Mongolia, China on September 2021, were brought back to the Research Center for Grassland Entomology (40°48′N, 111°42′E), Inner Mongolia Agricultural University (Hohhot, Inner Mongolia, China), and were maintained in intelligent light artificial incubator (PRX-350C, Ningbo HaiShuSaiFu experimental instrument Co., Ltd.) (25°C ± 1°C, RH = 70 ± 10%, L14:D10). After hatching out, the larvae were fed on *Allium mongolicum* in a incubator under the same conditions just as described by Tan et al. (2018). The healthy third instar larvae in consistent physiological state were collected for further experiments.

Bioassay of chlorantraniliprole

Bioassays were conducted using a leaf-dip method based on methods of He et al. (2012) and Zhang et al. (2022) with minor modifications. 95% chlorantraniliprole (DuPont) was dissolved in acetone solution, and then diluted into five to six concentrations with distilled water containing .05% Triton X-100 at a triple gradient dose. The .05% Triton X-100 solution was used as blank control. The fresh leek cultured in the laboratory measuring 5 cm × .5 cm were immersed in the prepared various concentrations of chlorantraniliprole for 15 s, and taken out in ventilated cupboard to air dry and then placed into a Petri dish lined with filter paper. Twenty healthy 2-day third instar larvae were placed into each Petri dish with six pieces of leek leaves with four replications for toxicity assessment bioassays. Mortality was assessed after 48 h of exposure, larvae that did not move when gently pushed with a fine hair brush were considered dead. The control groups was below 5%, mortality was corrected using Abbott's formula (Abbott, 1925). The LC_{50} value and slope were calculated by regression probit analysis conducted with the POLO-Plus software (2002).

Cloning of *GdRyR* gene

Sequences annotated as ryanodine receptor genes were screened from the transcriptome data published in Genbank (Bioproject No. 785282), and specific primers listed in Table 1 were designed for segmental cloning. The Total RNA was extracted from the whole body using RNAiso reagent (TaKaRa, Dalian, China) based on the manufacturer's instructions, cDNA was synthesized by reverse transcription using the PrimeScript® RT Reagent Kit with gDNA Eraser (TaKaRa, Dalian, China) and as the PCR templates. Total PCR reaction system (50 μl) included cDNA template (2 μl), forward and reverse primers (1 μl, 0.2 μmol/L), amplification enzyme mix (25 μl), RNAse Free ddH_2O (21 μl). 2 × Vazyme LAmp® Master Mix was purchased online from Vazyme Co., Ltd. and used for high fidelity and rapid PCR, the annealing velocity is high to 1 kb/s. PCR amplification conditions were performed as follows: 98°C for 3 min, followed by 35 cycles of 98°C for 10 s, 60°C for X s (X: each primer used a different annealing time), and 72°C for 3 min. Finally, it was extended for 5–10 min at 72°C (Table 1). The expected-size fragments were purified, ligated into pMD19-T vector (TaKaRa, code: D102A), and positive transformants were selected for plasmid isolation using MiniBEST Plasmid Purification Kit (TaKaRa, Dalian, China) and sent to Sangon Biotech company for sequencing. The fragments were overlapped and aligned with the annotated *GdRyR* gene identified from the transcriptome database using DNAMAN software, and the spliced complete CDS sequence of the *GdRyR* gene was submitted to the NCBI database, the Genbank accession number is OP828593.

TABLE 1 Primers used in the study.

Primer name	Primer sequences (5′-3′)	Target fragment size (bp)	PCR working procedure	Purpose
RyR1-F	TACAAAAAAAAAACCTAA ACCTCC	4,892	98°C, 3°min; (98°C, 10 s	Amplification of *RyR* gene fragment
RyR1-R	CTGTCAACGCCATCAATA ACCCTA		60°C, 30°s; 72°C, 3 min)*35	
			72°C, 10 min	
RyR2-F	AATCCTACTGCTACTCAA CCACTC	5,664	98°C, 3°min; (98°C, 10 s	
RyR2-R	GCTGTAAGTCTTCTCGCT ATCAAC		60°C, 45°s; 72°C, 3 min)*35	
			72°C, 10 min	
RyR3-F	GTCTTTGTTTTATGTGAA TACCGT	4,325	98°C, 3°min; (98°C, 10 s	
RyR3-R	GGAATGCCTTTGCTACCA CTGCCG		60°C, 30°s; 72°C, 3 min)*35	
			72°C, 10 min	
RyR4-F	CATAGGGCAGTGTCATTC CTCGCT	1,544	98°C, 3°min; (98°C, 10 s	
RyR4-R	TTGCTGTTGGGGGCTCGT AAAGTT		60°C, 20°s; 72°C, 1 min)*35	
			72°C, 5 min	
S1-F	CATAGGGCAGTGTCATTC CTCGCT	234	98°C, 3°min; (98°C, 10 s	Mutation site frequency detecting
S1-R	TAAATCGTCATCTTCTGG CTGTTC			
S2-F	AGATAATGCTTTCCTGTA TTCCTT	250	60°C, 15°s; 72°C, 20°s)*30	
S2-R	GTCTTCTTCTTGAACATA AAACTT		72°C,5 min	

Bioinformatics analysis of *GdRyR* gene

ORF Finder was applied to search the open reading frame of the *GdRyR* gene, and sequence integrity was verified by NCBI's Blastx tool to predict the gene isoelectric point, molecular mass, atomic composition, protein structural domain, signal peptide, and transmembrane region. Phylogenetic tree was constructed using the BLAST tool and DNAMAN V6.0 software to align amino acid sequence homology, using the neighbor-joining method in MEGA software and repeatedly running 1,000 times.

Detection the G4911E and I4754M Allelic Mutation Frequencies in the Field Population

The total RNA was isolated from individuals of *G. daurica* population collected from the Xianghuang Banner grasslands for cDNA preparation and PCR. The two pairs of specific primers containing the potential mutation sites G4911 and I4754 were designed based on the sequence of the C-terminal transmembrane region (Table 1). The specific bands obtained by PCR amplification were purified and sent to company for sequencing, and the sequences from different individuals were compared in the point mutation region to detect the frequency of allelic mutation sites.

Homologous modeling of *GdRyR* and molecular docking

Homologous modeling and model evaluation

The *GdRyR* amino acid sequence was submitted to the SWISS-MODEL server, and the predicted rabbit RyR cryo-electron microscopy structure (PDB: 7CF9) with 61.8% amino acid sequence homology to *GdRyR* reported by Ma et al. (2020) was used as a template to construct a model of *GdRyR* using Modeller 10.2. The Align2D module was used to compare *GdRyR*

with the template sequence, and then the Model-single module was used to build 100 candidate models. The stability of the models was measured by the Dope parameter, and the model with the lowest energy was selected as the optimal model for homology modeling, which was named as WT *GdRyR*. To obtain more accurate 3D structure for molecular docking, the amino acid sequence of the C-terminus of *GdRyR* was submitted on the AlphaFold2 platform on the Wemol online website, and the AlphaFold2 tool was used to construct a protein model of the C-terminus of *GdRyR* (WT *GdRyR*-C). The 3D structures of protein crystals obtained from Modeller and AlphaFold2 simulations were verified for stereochemical plausibility and energetic stability using the Molprobity tool. The rationality of the *Pis* and *Phi* angles between residues was demonstrated by the Ramachandran Plot, and the rationality of the protein model was evaluated based on the percentage of optimal regions, generally allowed regions, *etc.* in the Ramachandran Plot (Williams et al., 2018).

Construction of *GdRyR* mutants

The amino acid sequence of *GdRyR* was compared with domains containing mutation loci for *P. xylostella* RyR (G4946E, I4790M), *S. frugiperda* for (G4900E, I4743M) and *S. exigua* (G4891E, I4743M) (Zou et al., 2019), which correspond to G4900E, I4754M mutation sites of *GdRyR*. Mutation of "Ile" was replaced by "Met" in I4754 in *GdRyR* using AlphaFold2, the mutation model *GdRyR*-C-M4754 was constructed. Similarly, "Gly" was substituted into "Glu" in G4900 in *GdRyR*, and the mutation model *GdRyR*-C-E4900 was constructed. The models WT *GdRyR*-C, *GdRyR*-C-M4754 and *GdRyR*-C-E4911were processed separately using Autodock Tool-1.5.7 for energy minimization and dehydrogenation as molecular docking receptors.

Ligand Construction

The 3D structures of chlorantraniliprole and cyanobacteriamide were downloaded from the PubChem website, and converted into a suitable format for molecular docking using Open Babel-2.4.1, and used as ligands for molecular docking using Autodock Tool-1.5.6 for energy minimization *etc.*

Molecular docking and binding mModel analysis

Based on the reported binding sites of the rabbit RyR complex crystal structure with chlorantraniliprole, the active pockets of the constructed 3D model were predicted and the docking-box was constructed. The box parameters used for the docking of *GdRyR*s were as follows: center-x = 8.18, center-y = −1.56, center-z = .877; size-x = 99.75, size-y = 99.75, size-z = 99.75. The processed protein receptors were docked to the ligands using Autodock vina1.1.2 software. Computing platform was as follows: Microsoft-PC: Intel(R) Core(TM) i5-1035G4CPU@1.10GHz 1.50 GHz.

By molecular docking, the two ligands were selected for optimal conformation to construct complexes with the ryanodine receptor, and the binding modes were analyzed using the PLIP online tool, including: hydrogen bonding, hydrophobic interactions, π-π, π-cation, halogen bonding and non-covalent interaction forces such as interactions. Pymol-2.1.0 was used to visualize the binding modes.

Computing software and online tools

Homologous sequence alignment: https://blast.ncbi.nlm.nih.gov/Blast.cgi; Predicting protein properties such as gene isoelectric point, molecular mass and atomic composition: ProtParam online tool; Protein domain prediction: https://www.ebi.ac.uk/interpro/result/InterProScan/(InterProscan); Signal peptide prediction: http://www.cbs.dtu.dk/services/SigRyRlP/; Homologous modeling: https://swissmodel.expasy.org/(SWISS-MODEL) (Waterhouse et al., 2018); Protein quality evaluation: https://Main page-MolProbity (duke.edu) (Molprobity) (Williams et al., 2018); Protein crystal structure PDB data: http://www1.rcsb.org/; NCBI databse: https://www.ncbi.nlm.nih.gov/; Transmembrane region prediction (TMHMM): http://www.cbs.dtu.dk/services/TMHMM/(Möller et al., 2001); Action force analysis tools (PLIP): https://projects.biotec.tu-dresden.de/plip-web/plip (Melissa et al., 2021); Small molecular crystal structure: PubChem (nih.gov).

MEGA X was applied to construct phylogenetic tree (Kumar et al., 2018); Open Babel-2.4.1 was used to convert the file format of the chemical structure type (O'Boyle et al., 2011); The 3D protein crystal structure was visualized by Pymol-2.1.0 (Delano, 2002); Modeller (Version-10.2) was used for homologous modeling (Chen et al., 2021); Autodock Tool-1.5.7 (Morris et al., 2009) and Autodock vina 1.1.2 (Trott and Olson., 2010) were used for molecular docking; AlphaFold2 was applied for deep learning modeling (Jumper et al., 2021).

Results

Toxicity of chlorantraniliprole

Linear regression of the dose–mortality relationship (Y = −2.557 ± 2.291 X) was fitted to the observed data (i.e. no significant deviation between the observed and the expected data; $\chi^2 = 1.81$, $df = 3$) and LC_{50} was considered valid (inf *lim* < LC_{50} < sup *lim*: 21.25 < 44.10 mg/L < 80.33).

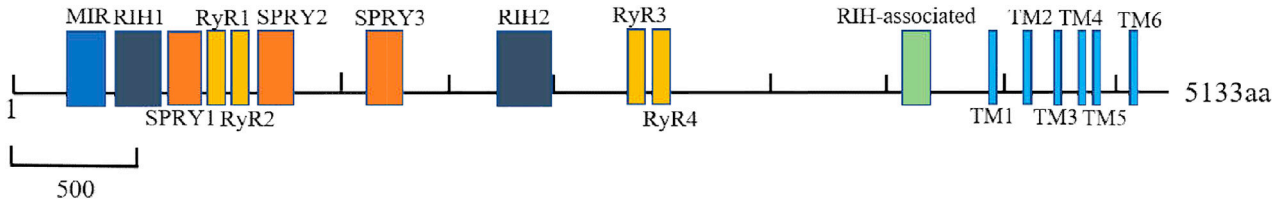


FIGURE 1
The schematic diagrams of conserved gene domains of *GdRyR*.

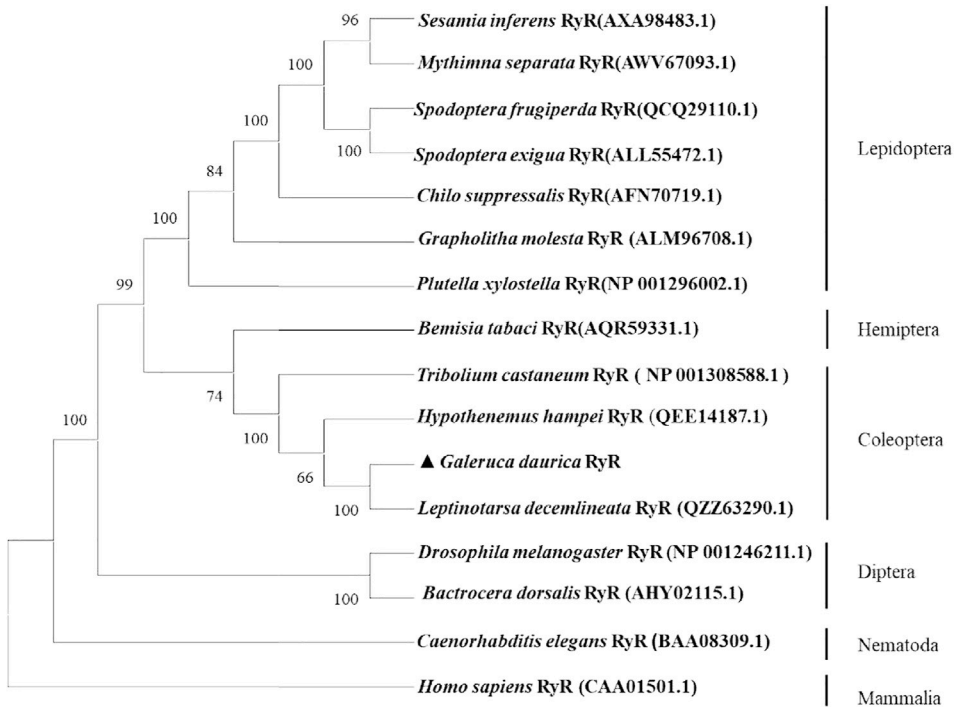
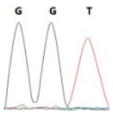
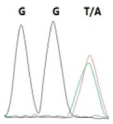
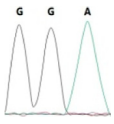
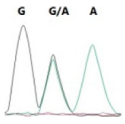
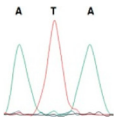
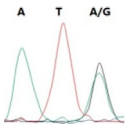
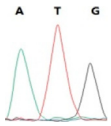


FIGURE 2
Phylogenetic tree of ryanodine receptor amino acid sequences from *G. daurica* and other insects. Note: The tree was generated by MEGA X using the Neighbor-Joining (NJ) method, using model p-distance. The topology was tested using bootstrap analyses (1,000 replicates). Numbers at nodes are bootstrap values.

Gene cloning and sequence analysis of *GdRyR* gene

Based on the *GdRyR* sequence information in the transcriptome, a total of four target fragments were amplified. After overlapped, a complete open reading frame (ORF) of 15,399 bp, encoding 5,133 amino acids, was obtained by BlastX alignment and sequence splicing. The molecular weight of the protein is 582.53 kDa, and the isoelectric point (pI) is 5.51. The *GdRyR* consists of five elements: carbon (C), hydrogen (H), Nitrogen (N), Oxygen (O) and Sulphur (S), and the total number of atoms is 81,490, the chemical formula is $C_{258925}H_{40538}N_{6992}O_{7799}S_{236}$, it has six transmembrane structure regions and no signal peptide. As shown in Figure 1, the N-terminal part of the gene contains one MIR domain (Mannosyltransferase, IP3R and RyR) located between amino acid sites 220-397, two RIH domains (RyR and IP3R Homology) located at amino acid sites 447-642 and 2254-2484, respectively, and three SPRY domains (SPla and RyR) located at amino acid sites 664-802, 1091-1214 and 1563-1706, respectively. The gene also has four RyR domains located at amino acid sites 854-944, 967-1056, 2861-2952, 2979-3062, and one highly conserved RIH-associated domain

TABLE 2 Allelic frequencies of point mutations on *GdRyR* from field population.

	G4911E				I4754M		
Genotype	GGT, 	GGT/A, 	GGA, 	GG/AA, 	ATA, 	ATA/G, 	ATG, 
Amino acid	Gly(G)	Gly(G)	Gly(G)	Gly/Glu (G/E)	Ile(I)	Ile/Met (I/M)	Met(M)
Number of test leaf beetles	22	13	9	6	33	16	1
Point mutation frequency	44%	26%	18%	12%	66%	32%	2%

located at amino acid sites 4021–4137 before the transmembrane helix; The C-terminus has six transmembrane helices (TM1 to TM6) located at amino acid sites 4474-4496, 4658-4680, 4739-4761, 4881-4903, 4929-4951, and 5009-5028, respectively.

Sequence homology analysis of *GdRyR* gene

The amino acid sequences encoded by the *GdRyR* gene were blasted with RyR of other insect species researched in the NCBI database, and a phylogenetic tree was constructed using the neighbour-joining (NJ) method. Figure 2 showed that *GdRyR* has the highest homology with RyR of potato beetle *Leptinotarsa decemlineata* (GenBank Accession No: QZZ63290.1), and amino acid sequence identity is 91.33%. The amino acid homology with *Hypothenemus hampei* RyR (QEE14187.1) and *Tribolium castaneum* RyR (NP 001308588.1) were 88.05% and 86.70%, respectively, and the RyR of the four Coleopteran species clustered as one clade. The clustering results indicated that the *GdRyR* gene is highly similar to taxonomically similar insect taxa.

Analysis of the G4911E and I4754M allelic mutation frequencies

The two potential allelic mutation sites and the occurrence frequency of Gly4911Glu and Ile4754Met were examined by individual extraction of RNA, using reverse-transcribed cDNA as a template, respectively. Fifty beetles collected from the grasslands of Xilingol Xianghuang Banner, Inner Mongolia, were conducted to gene sequence testing, the mutation loci and allelic frequencies of the *GdRyR* are summarized in Table 2. The results showed that only the heterozygous genotype G4911E was present in the test population, accounting for approximately 12% of the population; the heterozygous and homozygous genotypes of I4754M were present in 32% and 2% of the population, respectively.

Analysis and evaluation of homologous modeling

The Modeller and AlphaFold2 are applied to construct the *GdRyR* 3D crystal structure (WT *GdRyR*), the C-terminal wild-type 3D structure of *GdRyR* (WT *GdRyR*-C) and the mutant structure (*GdRyR*-C-M4754) (*GdRyR*-C-E4911), respectively. Figure 3A showed the 3D structure of *GdRyR* consists of multiple α-helices, β-folds, β-turns, irregular curls, and some extended extension structures. In lateral view, the protein crystal structure resembles the letter "Y," with a wide N-terminus and a narrow C-terminus, separated by a small angle at the C-terminus. As it can be seen from Figures 3C, E, G that the 3D structure of the C-terminal region of *GdRyR* is mainly composed of several parallel sets of α-helices, exhibiting obvious transmembrane protein properties. The 3D structural model of Ramachandran Plot diagram and the test results are shown in Figure 3 and Table 3, respectively. The percentages of residues in the optimal regions for WT *GdRyR*, WT *GdRyR*-C and *GdRyR*-C-M4754 and *GdRyR*-C-E4911 were 86.9%, 88.3%, 87.8%, and 88.1%, respectively. The proportions of amino acid residues in the acceptable region were 95.8%, 94.6%, 94.9%, and 94.6% respectively, all are greater than 90%, indicating that the constructed models were all reasonable.

Analysis of binding modes of diamide insecticides with *GdRyR*

The binding affinities of WT *GdRyR*-C to the optimal conformation of chlorantraniliprole and cyantraniliprole were −31.38, −33. 89 kJ/mol, respectively. The binding pattern was analyzed using PLIP and drawn by Pymol. As Figure 4 shows: Y4660 (Tyr-220), K4663 (Lys-223) form a hydrophobic interaction with the benzene and pyrazole rings of chlorantraniliprole, K4663 forms a hydrogen bond with the oxygen atom on the carbonyl group, K4762 (Lys-322) forms a hydrogen bond with the nitrogen atom of the pyrazole ring, G4911 (Gly-471) forms a hydrogen bond with the NH of the

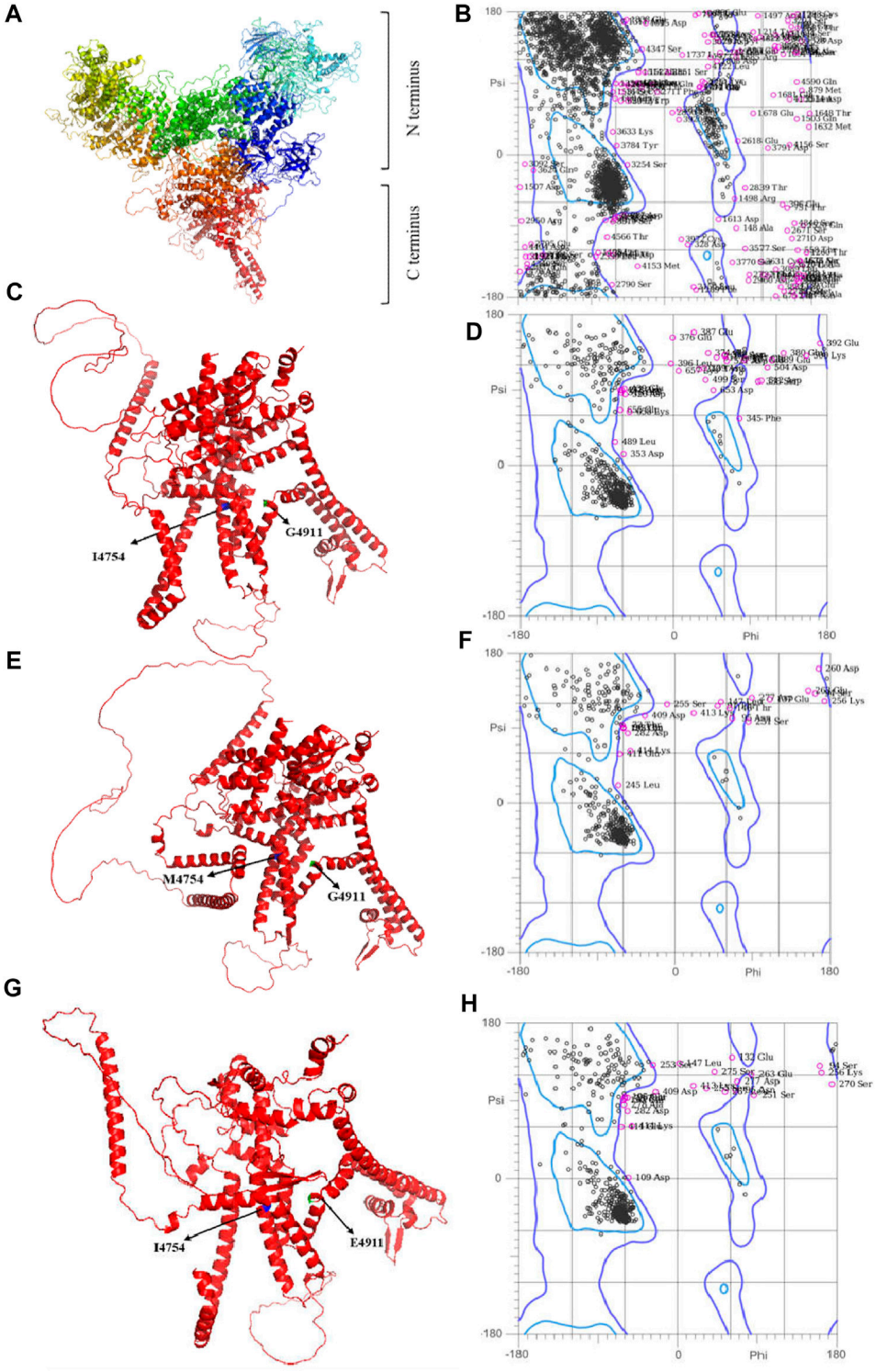


FIGURE 3
The models and evaluation results of WT *GdRyR*, WT *GdRyR*-C, *GdRyR*-C-M4754, and *GdRyR*-C-E4911. Note: **(A)** Three-dimension RyR model of WT *GdRyR*; **(B)** The result of Molprobity evaluation of WT *GdRyR*; **(C)** Three-dimension RyR model of the C-terminal transmembrane region of WT *GdRyR*-C; **(D)** The result of Molprobity evaluation of WT *GdRyR*-C; **(E)** Three-dimension RyR model of *GdRyR*-C-M4954; **(F)** The results of Molprobity evaluation of *GdRyR*-C-M4954. **(G)** Three-dimension RyR model of *GdRyR*-C-E4911; **(H)** The results of Molprobity evaluation of *GdRyR*-C-E4911.

TABLE 3 The results of Molprobity evaluation of *GdRyR*.

3D model	Favored regions (98 (%)	Allowed regions (>99.8 (%)
WT *GdRyR*	86.9	95.8
WT *GdRyR*-C	88.3	94.6
GdRyR-C-M4754	87.8	94.9
GdRyR-C-E4911	88.1	94.6

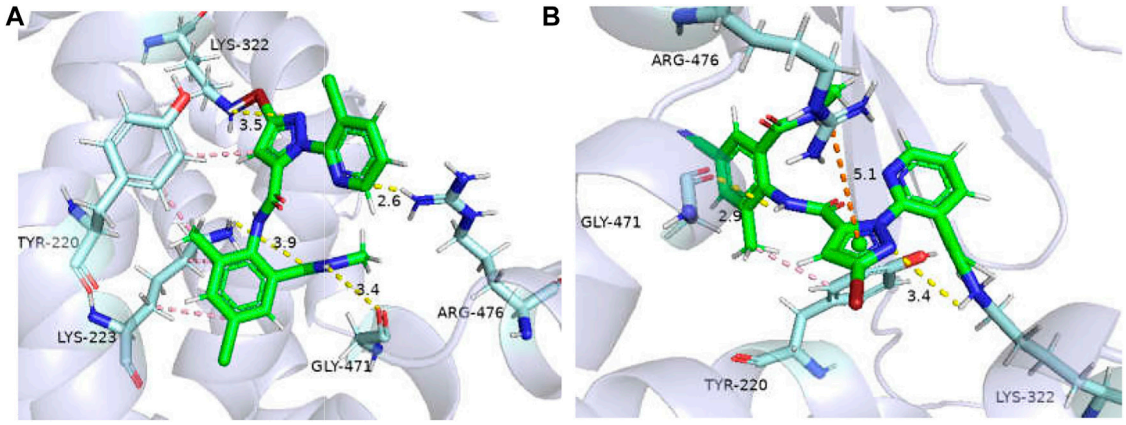


FIGURE 4
Binding modes of WT *GdRyR* and chlorantraniliprole **(A)** and cyanobacteriamide **(B)**. Note: Hydrophobic effect: pink; Hydrogen bond: yellow; π-cation: orange; Hydrogen: white; Oxygen: red; Nitrogen: blue; Selphur: dark orange.

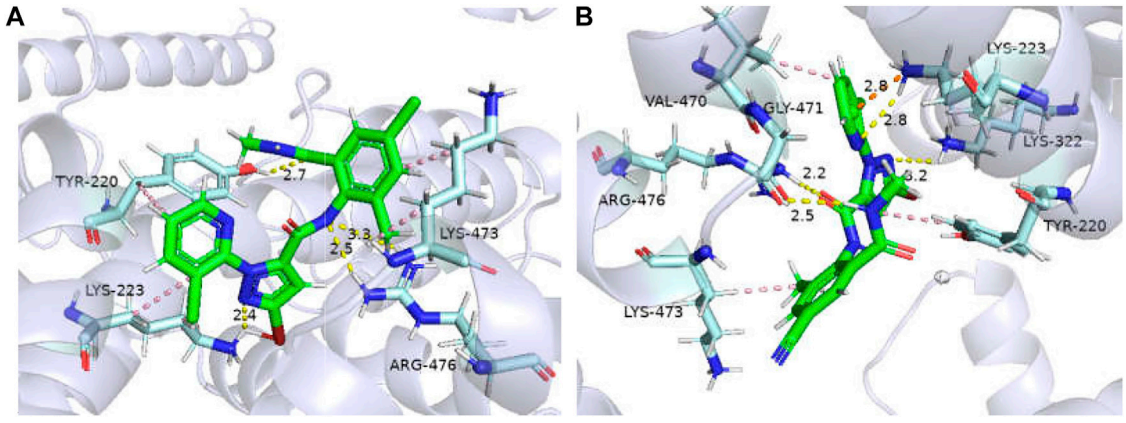


FIGURE 5
Binding modes of *GdRyR*-M4754 and chlorantraniliprole **(A)** and cyanobacteriamide **(B)**. Note: Hydrophobic effect: pink; Hydrogen bond: yellow; π-cation: orange; Hydrogen: white; Oxygen: red; Nitrogen: blue; Selphur: dark orange.

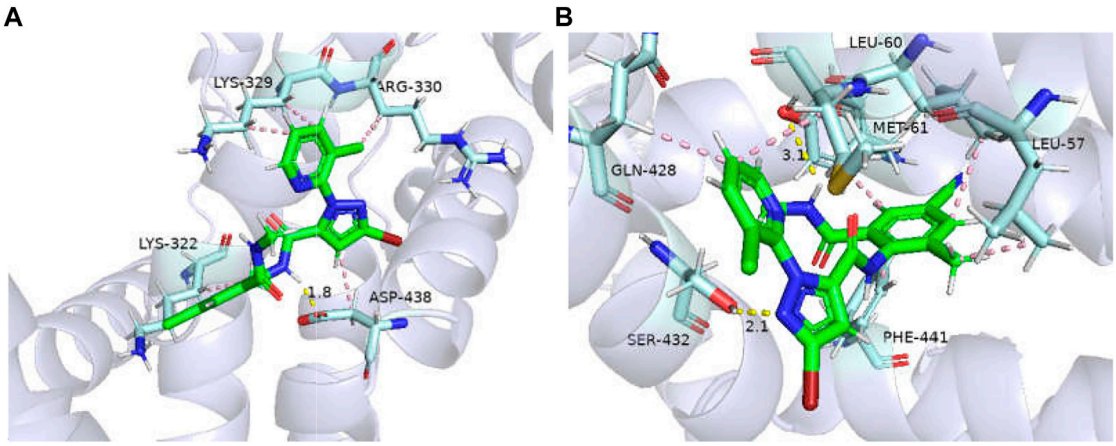


FIGURE 6
Binding modes of *GdRyR*-E4911 and chlorantraniliprole **(A)** and cyanobacteriamide **(B)**. Note: Hydrophobic effect: pink; Hydrogen bond: yellow; π-cation: orange; Hydrogen: white; Oxygen: red; Nitrogen: blue; Selphur: dark orange.

methylamino structure on the benzene ring, R4916 (Arg-476) forms a hydrogen bond with the nitrogen atom of the pyridine ring (Figure 4A). Y4660 forms a hydrophobic interaction with the methyl group on the benzene ring of cyantraniliprole, K4762 forms a hydrogen bond with the nitrogen atom of the pyrazole ring, G4911 forms a hydrogen bond with the NH of the amide structure on the side of the pyrazole ring, the nitrogen atom of R4916 forms a π-cation with the pyrazole ring of cyantraniliprole (Figure 4B).

The binding affinities of *GdRyR*-M4754 to the optimal conformation of chlorantraniliprole and cyantraniliprole were −32.23, −33. 91 kJ/mol, respectively. Combined pattern analysis diagram was seen in Figure 5: Y4660 (Tyr-220) and K4663 (Lys-223) form hydrophobic interactions with the pyridine ring of chlorantraniliprole, K4913 (Lys-473) forms hydrophobic interactions with the benzene ring as well as the methyl group, Y4660 forms a hydrogen bond with the oxygen atom on the carbonyl group of the benzene ring, K4663 forms a hydrogen bond with the nitrogen atom of the pyrazole ring, R4916 (Arg-476) forms a hydrogen bond with the NH of the amide structure on the pyrazole side (Figure 5A). Y4660, V4910 (Val-470) and K4663 form hydrophobic interactions with the pyridine ring, pyrazole ring and methyl group of cyantraniliprole, respectively. K4663 forms a hydrogen bond with nitrogen on the pyridine ring, K4762 (Lys-332) forms a hydrogen bond with the nitrogen atom of the pyrazole ring, G4911 (Gly-471) forms a hydrogen bond with NH of the amide structure on the benzene ring side, R4916 forms a hydrogen bond with the oxygen atom of the carbonyl group on the pyrazole ring side, the nitrogen atom of K4663 forms a π-cation interaction with the pyridine ring of cyantraniliprole (Figure 5B).

The binding affinities of *GdRyR*-E4911 to the optimal conformation of chlorantraniliprole and cyantraniliprole were −25.89, −27.54 kJ/mol, respectively. Combined pattern analysis diagram was seen in Figure 6: K4762 (Lys-329) forms hydrophobic interactions with methyl of chlorantraniliprole, K4769 (Lys-322) and R4770 (Arg-330) form hydrophobic interactions with the pyridine ring and the chlorine ion on the pyridine ring, respectively. D4878 (Asp-438) forms hydrophobic interactions and hydrogen bonds with the pyrazole ring and the NH of the pyrazole side amide structure, respectively (Figure 6A). L4497 (Leu-60) and L4500 (Leu-57) form hydrophobic interactions with the methyl and benzene rings of cyantraniliprole, respectively. M4501 (Met-61) and Q4868 (Gln-428) form hydrophobic interactions with the pyridine ring, Y4759 (Tyr-341) and F4881 (Phe-441) form hydrophobic interactions with the benzene ring, Y4759 forms a hydrogen bond with the NH of the amide structure on the side of the benzene ring, Q4868 (Gln-428) forms a hydrogen bond with the nitrogen atom of the pyrazole ring (Figure 6B).

Analysis of mutation sites of *GdRyR*

As shown in Table 4: The mutation of isoleucine (Ile) to methionine (Met) at position 4754 in *GdRyR* showed no decrease in binding affinity to diamide insecticides; the mutation of glycine (Gly) to glutamic acid (Glu) at position 4911 in *GdRyR* showed a significant decrease in binding affinity to diamide insecticides. The binding modes of two diamide insecticides with WT *GdRyR*, *GDRYR*-M4754 and *GDRYR*-E4911 are shown in Figures 4–6: After mutating isoleucine (Ile, position 4754) of *GdRyR* to methionine (Met), the mode of interaction with diamide insecticides, the residues involved in the formation of the action force and WT *GdRyR* are essentially the same, mainly because the isoleucine (Ile) at position 4754 of

TABLE 4 The binding affinity changes of the wild-type (WT) and mutant *GdRyR*s and diamides.

Diamide insecticide	Binding affinity ±SE/(kJ/mol)		
	WT *GdRyR*	*GdRyR*-M4754	*GdRyR*-E4911
Chlorantraniliprole	−31.38 ± 0.14a	−32.32 ± 0.24a	−25.89 ± 0.29 b
Cyantraniliprole	−33.89 ± 032a	−33.91 ± 0.46a	−27.54 ± 0.32 b

Each value represents the mean (±SE) of ten replicates. The values (Mean ± SE) followed by different letters in the same column are significantly different between mutation at 5% significance level using ANOVA, followed by Dunnett T3 correction.

WT *GdRyR* is not involved in the formation of the force action between ligands and receptors. The mutation to glutamic acid (Glu) in WT *GdRyR*, which has a hydrogen bonding interaction between glycine (Gly) at position 4911 and the diamide insecticide, prevents the formation of hydrogen bonds with the diamide insecticide, resulting in a reduced receptor-ligand interaction and decreased affinity. The mutations of amino acid from Gly to Glu at position 4911 in WT *GdRyR* may lead to resistance to diamide insecticides on *G. daurica*, while further studies are needed to determine whether the I4734M mutation is associated with resistance.

Disscussion

The insect ryanodine receptor, one of the largest Ca^{2+} releasing channels, has an important role in muscle excitation-contraction coupling and is an important action target of diamide insecticides. Currently, in addition to the model insect *Drosophila*, the RyR genes of *P. xylostella* (Sun et al., 2012), *Cnaphalocrocis medinalis* (Wang et al., 2012), *Ostrinia furnacalis* (Cui et al., 2013), *S. exigua* (Zuo et al., 2017), *C. suppressalis* (Gao et al., 2013; Huang et al., 2021) and *S. frugiperda* (Li et al., 2021) have been successfully cloned, and different mutant sites have been reported to be associated with diamide insecticide resistance. In this study, the full-length cDNA sequence of the *GdRyR* gene was obtained by segmental cloning technique, which was assembled by splicing. Sequence homology analysis showed that *GdRyR* had 86.70%, 88.05%, and 91.33% amino acid similarity with the ryanodine receptors of the other Coleopteran insects, *T. castaneum* (Herbst), *H. hampei* (Ferrari) and *L. decemlineata* (Say), respectively. The structural domains were predicted to have one MIR structural domain, two RIH structural domains, three SPRY structural domains, four RyR structural domains and one RIH-associated structural domain at the N-terminal part of *GdRyR*, and six hydrophobic transmembrane motifs at the C-terminal end of *GdRyR*, this result is highly similar to the reported positions of the functional structural domains of insects such as *Carposina sasakii* (Sun et al., 2012), *C. suppressalis* (Peng et al., 2017). The MIR structural domain functions as a transferring ligand in mammals, and the RIH and MIR structural domains are involved in the formation of IP3 binding sites in IP3Rs (Ponting, 2000). The SPRY structural domain is often considered to have a function in regulating protein interactions (Woo et al., 2006; Cui et al., 2009). These structural features are similar to those of *Drosophila* DmRyR, suggesting that the *GdRyR* gene can encode a functional Ca^{2+} channel protein (Xu et al., 2000; Bouakaz et al., 2002).

Diamide insecticides, a class of chemical insecticides acting on insect ryanodine receptors, have good efficacy against a wide range of pests and are widely used in the chemical control on Lepidoptera, Coleoptera and Diptera. In recent years, with the widespread use of diamide insecticides, many insects have developed a high level of resistance (Lai et al., 2011; Guo et al., 2014; Wang et al., 2018). Resistance mechanism studies suggested that the diamide insecticide resistance is associated with target mutation site of the ryanodine receptor (Troczka et al., 2015; Nauen and Steinbach, 2016). Field populations of *S. exigua* with a homozygous mutation in the ryanodine receptor gene I4743M have developed up to 154-fold higher levels of resistance to chlorantraniliprole (Zuo et al., 2019). Teng et al. (2022) found that the key mutation was I4743M in the resistant population of *S. exigua* in six regions of eastern China by sequencing the transmembrane region of RyR, and the mutation frequency was determined to be as high as 70%–100%. In this study, two potential mutation loci (G4911 and I4754) of the ryanodine receptor were examined in individuals from field populations of *G. daurica*, there were 12% of the heterozygous mutants G4911E in the population, while 32% of the heterozygous and 2% of heterozygous mutants were I4754M. The study showed that the G4946E and I4790M/K mutations in RyR were detected in resistant populations of *P. xylostella* (Guo et al., 2014; Troczka et al., 2015; Jouraku et al., 2020). It was confirmed that the G4946E and I4790M/K mutations could affect the binding of diamide insecticides to RyR by *in vitro* expression in Sf9 cells and construction of UAS-PxRyR transgenic *Drosophila* strain (Jouraku et al., 2020; Jiang et al., 2021; Richardson et al., 2021). Resistance to diamide insecticides in *S. frugiperda* is associated with mutations in the I4743M and G4891E loci of RyR (Boaventura et al., 2019). Therefore, the two amino acid sites G4911 and I4754 in *GdRyR* may be potential target binding sites for diamide insecticides, and mutations at the

two sites could also be a potential resistance mechanism to diamide insecticides. In order to verify the inference, we analyzed the binding pattern of diamide insecticides to *GdRyR* and its mutants through protein homology modeling and molecular docking techniques.

The binding pattern analysis of insect RyR with diamide insecticides has been a hot topic of research in structural biology. Steinbach et al. (2015) had constructed a homology model of PxRyR, on which the G4946E and I4790M mutations were introduced, and the binding mode of diamide insecticides to PxRyR were analyzed, suggesting that the binding site of RyR to diamide insecticides may be close to the two mutation sites, but the exact binding mode, interaction force and residues forming the force are not clear. Studies on the mechanisms of resistance to diamide insecticides in Coleoptera are poorly reported. Based on the previous study, the rabbit ryanodine receptor (PDB: 7CF9) was selected as the template for constructing a homologous 3D model of *GdRyR*. In addition, *GdRyR*-E49111 and *GdRyR*-M4754 mutant models were constructed based on potential resistance allelic frequency detection by introducing the G4911E and I4754M mutations, and analyzed for their binding patterns to diamide insecticides. The results showed that mutating Gly of *GdRyR* to Glu resulted in reduced affinity to diamide insecticides. The current study showed that both of the G4946E mutation in *PxRyR* and the G4900E mutation in *SeRyR* can lead to resistance to diamide insecticides. Therefore, a substitution from Gly to Glu at 4911 position of *GdRyR* may lead to resistance to diamide insecticides on *G. daurica*. The frequency of mutations in *GdRyR* at G4911E should be a key concern for resistance risk assessment and reasonable applications of diamide insecticides for *G. daurica* control in future. The I4790M mutation of PxRyR and I4734M of SfRyR are also involved in insect resistance to diamide insecticides (Guo et al., 2014; Boaventura et al., 2019). The amino acid at position I4754 of *GdRyR* was not involved in its binding to diamide insecticides, and the substitution from Ile to Met may not reduce the affinity of *GdRyR* for diamide insecticides. Our result is similar with that of Teng et al. (2022), who reported that no significant correlation was found between chlorantraniliprole resistance level and RyR I4743M allele frequency in the six field populations of *S. exigua*. Therefore, the I4754M mutation may not be involved in the resistance to diamide insecticides on *G. daurica*, but whether the I4754M mutation can lead to resistance needs to be further explored in*in vitro* functional experiments.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/genbank/, OP828593. http://www.wwpdb.org/, PDB: 7CF9.

Ethics statement

The animal subject used in this study is a kind of leaf beetle in northern steppe, which is an invertebrate and exempt from this requirement. No specific permits were required for the collection from the field and for maintenance in laboratory. This study did not involve any endangered species, protected species, or protected areas.

Author contributions

YTA and HR conceived research; HR, HZ, RN, WW, and YTI conducted experiment; HR, RN, YL, and LL analyzed data and conducted statistical analyses; YTA secured funding; HR, B-PP, and YTA wrote the manuscript; All authors read and approved the Manuscript.

Funding

This work was supported by the Inner Mongolian Science and Technology Key Project-2021 grant funded by the Inner Mongolian Chamber of Science and Technology (2021GG0108), Higher Education Young Scientific and Technology Talent Plan Fund (NJYT-19-B22), and the Project of Central Support for Local Development (2022-184).

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18, 265–267. doi:10.1093/jee/18.2.265a

Amador, F. J., Liu, S., Ishiyam, N., Plevin, M. J., Wilson, A., MacLennan, D. H., et al. (2009). Crystal structure of type I ryanodine receptor amino-terminal -trefoil domain reveals a disease-associated mutation "Hot Spot" Loop. *P Natl. Acad. Sci. U. S. A.* 106, 11040–11044. doi:10.1073/pnas.0905186106

Boaventura, D., Bolzan, A., Padovez, F., Okuma, D., Omoto, C., and Nauen, R. (2019). Detection of a ryanodine receptor target-site mutation in diamide insecticide resistant fall armyworm, *Spodoptera frugiperda*. *Pest Manag. Sci.* 76, 47–54. doi:10.1002/ps.5505

Boris, S. Z., and Ke, D. (2022). Pyrethroids in an AlphaFold2 model of the insect sodium channel. *Insects* 13, 745. doi:10.3390/insects13080745

Bouakaz, A., De, J. N., Gerfault, L., and Cachard, C. (2002). Regulation of mammalian ryanodine receptors. *Front. Biosci-landmrk.* 35, d2072–d2080. doi:10.2741/A899

Chang, J., Zhou, X. R., Li, H. P., and Pang, B. P. (2015). Synergistic effects of *metarhizium anisopliae* mixed with three pesticides against *Galeruca daurica*. *Chin. J. Pest. Sci.* 17, 54–59.

Chen, X. Y., Wang, Y., Ma, Y., Wang, R. N., and Zhao, D. X. (2021). To explore the Radix Paeoniae Rubra-Flos Carthami herb pair's potential mechanism in the treatment of ischemic stroke by network pharmacology and molecular docking technology. *Medicine* 100, 27752. doi:10.1097/MD.0000000000027752

Cui, L., Yang, D. B., Yan, X. J., Rui, C., Wang, Z. Y., and Yuan, H. Z. (2013). Molecular cloning, characterization and expression profiling of a ryanodine receptor gene in Asian corn borer, *Ostrinia furnacalis* (Guenee). *PLOS One* 8, e75825. doi:10.1371/journal.pone.0075825

Cui, Y. F., Shen, H. T., Norris, N. C., Karunasekara, Y., Pouliquin, P., Board, P. G., et al. (2009). A dihydropyridine receptor α 1s loop region critical for skeletal muscle contraction is intrinsically unstructured and binds to a SPRY domain of the type 1 ryanodine receptor. *Int. J. Biochem. Cell. B* 41, 677–686. doi:10.1016/j.biocel.2008.08.004

Delano, W. L. (2002). The Pymol molecular graphics system. *Proteins* 30, 442–454.

Dong, W., Zhang, X. B., Zhang, X. Y., Wu, H. H., Zhang, M., Ma, E. B., et al. (2016). Susceptibility and potential biochemical mechanism of *Oedaleus asiaticus* to beta-cypermethrin and deltamethrin in the Inner Mongolia, China. *Pestic. Biochem. Phys.* 132, 47–52. doi:10.1016/j.pestbp.2015.11.011

Du, G. L., Ma, C. Y., Hong, J., Zhang, Z. R., Li, X. Y., and Yu, C. (2016). Occurrence trend of *Galeruca daurica* Joannis in grassland and control efficiency of botanical pesticides. *Plant Prot. Sci.* 42, 253–256.

Duan, T. F., Gao, S. J., Wang, H. C., Li, L., Li, Y. Y., Tan, Y., et al. (2022). MicroRNA let-7-5p targets the juvenile hormone primary response gene Krüppel homolog 1 and regulates reproductive diapause in Galeruca daurica. *Insect Biochem. Molec* 142, 103727. doi:10.1016/j.ibmb.2022.103727

Gao, C. F., Yao, R., Zhang, Z. Z., Wu, M., Zhang, S., and Su, J. Y. (2013). Susceptibility baseline and chlorantraniliprole resistance monitoring in *Chilo suppressalis* (Lepidoptera: Pyralidae). *J. Econ. Entomol.* 106, 2190–2194. doi:10.1603/ec13058

Gao, S. J., Tan, Y., Han, H. B., Guo, N., Gao, H. Y., Wang, N., et al. (2022). Resistance to beta-cypermethrin, azadirachtin, and matrine, and detoxification enzyme activity in *Oedaleus asiaticus* (Bey-Bienko) in Inner Mongolia, northern China. *J. Insect Sci.* 22, 1–8. doi:10.1093/jisesa/ieac063

Guo, L., Li, C., Coupland, G., Liang, P., and Chu, D. (2021). Up-regulation of calmodulin involved in the stress response to cyantraniliprole in the whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Insect Sci.* 28, 1745–1755. doi:10.1111/1744-7917.12887

Guo, L., Wang, Y., Zhou, X. G., Li, Z. Y., Liu, S. Z., Liang, P., et al. (2014). Functional analysis of a point mutation in the ryanodine receptor of *Plutella xylostella* (L.) associated with resistance to chlorantraniliprole. *Pest Manag. Sci.* 70, 1083–1089. doi:10.1002/ps.3651

He, Y. X., Zhao, J. W., Zheng, Y., Desneux, N., and Wu, K. M. (2012). Lethal effect of imidacloprid on the coccinellid predator Serangium japonicum and sublethal effects on predator voracity and on functional response to the whitefly Bemisia taboci. *Ecotoxicol.* 21, 1291e1300.

Huang, J. M., Rao, C., Wang, S., He, F. L., Zhao, S. Q., Zhou, L. Q., et al. (2020). Multiple target-site mutations occurring in Lepidopterans confer resistance to diamide insecticides. *Insect Biochem. Molec* 121, 103367. doi:10.1016/j.ibmb.2020.103367

Huang, J. M., Sun, H., He, L. F., Liu, C., Ge, W. c., Ni, H., et al. (2021). Double ryanodine receptor mutations confer higher diamide resistance in rice stem borer, Chilo suppressalis. *Chilo suppressalis* 77, 4971–4979. doi:10.1002/ps.6539

Hughes, K. A., Lahm, G. P., Selby, T. P., and Stevenson, T. M. (2013). *Cyano anthranilamide insecticides*. Patent: USPTO TOSUS8475819B2.

Jeanguenat, A. (2013). The story of a new insecticidal chemistry class: The diamides. *Pest Manag. Sci.* 69, 7–14. doi:10.1002/ps.3406

Jiang, D., Qian, C., Wang, D. H., Wang, F. L., Zhao, S., Yang, Y. H., et al. (2021). Varying contributions of three ryanodine receptor point mutations to diamide insecticide resistance in *Plutella xylostella*. *Pest Manag. Sci.* 77, 4874–4883. doi:10.1002/ps.6534

Jouraku, A., Kuwazaki, S., Miyamoto, K., Uchiyama, M., Kurokawa, T., Mori, E., et al. (2020). Ryanodine receptor mutations (G4946E and I4790K) differentially responsible for diamide insecticide resistance in diamondback moth, *Plutella xylostella* L. *Insect Biochem. Molec* 118, 103308. doi:10.1016/j.ibmb.2019.103308

Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., et al. (2021). Highly accurate protein structure prediction with AlphaFold. *Nature* 596, 583–589. doi:10.1038/s41586-021-03819-2

Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. (2018). Mega X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* 35, 1547–1549. doi:10.1093/molbev/msy096

Lai, T. C., Li, J., and Su, J. Y. (2011). Monitoring of beet armyworm *Spodoptera exigua* (Lepidoptera: Noctuidae) resistance to chlorantraniliprole in China. *Pestic. Biochem. Phys.* 101, 198–205. doi:10.1016/j.pestbp.2011.09.006

Li, K., Wang, K., Tao, L., Li, L., Li, J., Wu, W. W., et al. (2021). Analysis of binding modes and resistance risk between ryanodine receptor of *Spodoptera frugiperda* and diamide insecticides. *Chin. J. Pest Sci.* 23, 856–868.

Lv, S. L., Shi, Y., Zhang, J. C., Liang, P., Zhang, L., and Gao, X. W. (2021). Detection of ryanodine receptor target-site mutations in diamide insecticide-resistant *Spodoptera frugiperda* in chiRyR. *Insect Sci.* 28, 639–648. doi:10.1111/1744-7917.12896

Ma, H. Y., Li, Y. Y., Li, L., Tan, Y., and Pang, B. P. (2021). Juvenile hormone regulates the reproductive diapause through Methoprene-tolerant gene in *Galeruca daurica*. *Insect Mol. Biol.* 30, 446–458. doi:10.1111/imb.12710

Ma, H. Y., Zhou, X. R., Tan, Y., and Pang, B. P. (2019). Proteomic analysis of adult *Galeruca daurica* (Coleoptera: Chrysomelidae) at different stages during summer diapause. *Comp. Biochem. Phys. D.* 29, 351–357. doi:10.1016/j.cbd.2019.01.007

Ma, R. F., Haji-Ghassemi, O., Ma, D., Jiang, H., Lin, L. Y., Yao, L., et al. (2020). Structural basis for diamide modulation of ryanodine receptor. *Nat. Chem. Biol.* 16, 1246–1254. doi:10.1038/s41589-020-0627-5

Melissa, F. A., Katja, L. L., Sarah, N. B., Florian, K., Sebastian, S., Joachim, V. H., et al. (2021). Plip 2021: Expanding the scope of the protein-ligand interaction profiler to DRYR and RRYR. *Nucleic Acids Res.* 49, 530–534.

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., et al. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J. Comput. Chem.* 30, 2785–2791. doi:10.1002/jcc.21256

Nauen, R., and Steinbach, D. (2016). "Resistance to diamide insecticides in lepidopteran pests," in *Advances in insect control and resistance management*. Editors A. R. Horowitz and I. Ishaaya (Basel: Springer Nature Switzerland AG), 219–240.

O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., and Hutchison, G. R. (2011). Open Babel: An open chemical toolbox. *J. Cheminformatics* 3, 33. doi:10.1186/1758-2946-3-33

Peng, Y. C., Sheng, C. W., Casida, J. E., Zhao, C. Q., and Han, Z. J. (2017). Ryanodine receptor genes of the rice stem borer, *Chilo suppressalis*: Molecular cloning, alternative splicing and expression profiling. *Pestic. Biochem. Phys.* 135, 69–77. doi:10.1016/j.pestbp.2016.06.002

Ponting, C. P. (2000). Novel repeats in ryanodine and IP 3 receptors and protein O-mannosyltransferases. *Trends Biochem. Sci.* 25, 48–50. doi:10.1016/s0968-0004(99)01513-3

Richardson, W., Homem, R. A., Troczka, B. J., George, C. H., Ebbinghaus-Kintscher, U., Williamson, M. S., et al. (2021). Diamide insecticide resistance in transgenic Drosophila and Sf9-cells expressing a full-length diamondback moth ryanodine receptor carrying an I4790M mutation. *Pest Manag. Sci.* 78, 869–880. doi:10.1002/ps.6730

Sattelle, D. B., Cordova, D., and Cheek, T. R. (2008). Insect ryanodine receptors: Molecular targets for novel pest control chemicals. *Invertebr. Neurosci.* 8, 107–119. doi:10.1007/s10158-008-0076-4

Software, LeOra. (2002). *PoloPlus: Probit and logit analysis*. Berkeley, CA: LeOra Software.

Sparks, T. C., and Nauen, R. (2014). Irac: Mode of action classification and insecticide resistance management. *Pestic. Biochem. Phys.* 121, 122–128. doi:10.1016/j.pestbp.2014.11.014

Steinbach, D., Gutbrod, O., Lummen, P., Matthiesen, S., Schorn, C., and Nauen, R. (2015). Geographics spread, genetics and functional characteristics of ryanodine based targer-site resistance to diamide insecticides in diamondback moth, *Plutella xylostella*. *Insect Biochem. Mol. Biol.* 63, 14–22.

Sun, L. N., Cui, L., Rui, C. H., Yan, X. J., Yang, D. B., and Yuan, H. Z. (2012). Modulation of the expression of ryanodine receptor from *Plutella xylostella* as a result of diamide insecticide application. *Gene* 511, 265–273. doi:10.1016/j.gene.2012.09.027

Tan, Y., Zhang, Y., Huo, Z. J., Zhou, X. R., and Pang, B. P. (2018). Molecular cloning of heat shock protein 10(Hsp10) and 60 (Hsp60) cDNAs from *Galeruca daurica* (Coleoptera: Chrysomelidae) and their expression analysis. *B Entomol. Res.* 108, 510–522. doi:10.1017/S0007485317001079

Temple, J. H., Pommireddy, P. L., Cokk, D. R., and Leonard, B. R. (2009). Arthropod M AnAgeMent susceptibility of selected Lepidopteran pests to rynaxypyr®, a novel insecticide. *J. Cotton Sci.* 13, 23–31.

Teng, H. Y., Zuo, Y. Y., Yuan, J., Fabrick, J. A., Wu, Y. D., and Yang, Y. H. (2022). High frequency of ryanodine receptor and cytochrome P450 *CYP9A186* mutations in insecticide-resistant field populations of *Spodoptera exigua* from China. *Pestic. Biochem. Phys.* 186, 105153. doi:10.1016/j.pestbp.2022.105153

Tohnishi, M., Nakao, H., Furuya, T., Seo, A., Kodama, H., Tsubata, K., et al. (2005). Flubendiamide, a novel insecticide highly active against lepidopterous insect pests. *J. Pestic. Sci.* 30, 354–360. doi:10.1584/jpestics.30.354

Troczka, B. J., Williams, A. J., Williams, M. A., Field, L. M., Luemmen, P., and Davies, T. G. E. (2015). Stable expression and functional characterisation of the diamondback moth ryanodine receptor G4946E variant conferring resistance to diamide insecticides. *Sci. Rep-uk.* 5, 14680. doi:10.1038/srep14680

Trott, O., and Olson, A. J. (2010). AutoDock vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* 31, 455–461. doi:10.1002/jcc.21334

Wang, J. J., Li, Y. Q., Han, Z. J., Zhu, Y. L., Xie, Z. J., Wang, J., et al. (2012). Molecular characterization of a ryanodine receptor gene in the rice leaffolder, *Cnaphalocrocis medinalis* (Guenée). *PLOS One* 7, e36623. doi:10.1371/journal.pone.0036623

Wang, R., Wang, J. D., Che, W. N., and Luo, C. (2018). First report of field resistance to cyantraniliprole, a new anthranilic diamide insecticide, on *Bemisia tabaci* MED in China. *J. Integr. Agric.* 17, 158–163. doi:10.1016/s2095-3119(16)61613-1

Wang, X. L., Cao, X. W., Jiang, D., Yang, Y. M., and Wu, Y. D. (2020). CRISPR/Cas9 mediated ryanodine receptor I4790M knock-in confers unequal resistance to diamides in *Plutella xylostella*. *Insect Biochem. Molec* 125, 103453. doi:10.1016/j.ibmb.2020.103453

Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., et al. (2018). SWISS-MODEL: Homology modelling of protein structures and complexes. *Nucleic Acids Res.* 46, W296-W303–W303. doi:10.1093/nar/gky427

Williams, C. J., Headd, J. J., Moriarty, N. W., Prisant, M. G., Videau, L. L., Deis, L. N., et al. (2018). MolProbity: More and better reference data for improved all-atom structure validation. *Protein Sci.* 27, 293–315. doi:10.1002/pro.3330

Woo, J. S., Suh, H. Y., Park, S. Y., and Oh, B. H. (2006). Structural basis for protein recognition by B30.2/SPRY domains. *Mol. Cell.* 24, 967–976. doi:10.1016/j.molcel.2006.11.009

Xu, X., Bhat, M. B., Nishi, M., Takeshima, H., and Ma, J. (2000). Molecular Cloning of cDNA encoding a *Drosophila* ryanodine receptor and functional studies of the Carboxyl-terminal calcium release channel. *Biophys. J.* 78, 1270–1281. doi:10.1016/S0006-3495(00)76683-5

Zhang, W. B., Ren, H., Sun, F. L., Shen, T. T., Yuan, S., Gao, X. W., et al. (2022). Evaluation of the toxicity of chemical and biogenic insecticides to three outbreaking insects in desert steppes of northern China. *Toxins* 14, 546. doi:10.3390/toxins14080546

Zhou, X. R., Gao, J. C., and Pang, B. P. (2016). Effects of temperature on the termination of egg diapause and post-diapause embryonic development of *Galeruca daurica* (Coleoptera: Chrysomelidae). *Environ. Entomol.* 45, 1076–1080. doi:10.1093/ee/nvw074

Zhou, X. R., Shan, Y. M., Tan, Y., Zhang, Z. R., and Pang, B. P. (2019). Comparative analysis of transcriptome responses to cold stress in *Galeruca daurica* (Coleoptera: Chrysomelidae). *J. Insect Sci.* 19, 8–6. doi:10.1093/jisesa/iez109

Zuo, Y. Y., Ma, H. H., Lu, W. J., Wang, X. L., Wu, S. W., Nauen, R., et al. (2019). Identification of the ryanodine receptor mutation I4743M and its contribution to diamide insecticide resistance in *Spodoptera exigua* (Lepidoptera: Noctuidae). *Insect Sci.* 27, 791–800. doi:10.1111/1744-7917.12695

Zuo, Y. Y., Wang, H., Xu, Y., Huang, J., Wu, S., Wu, Y. D., et al. (2017). CRISPR/Cas9 mediated G4946E substitution in the ryanodine receptor of *Spodoptera exigua* confers high levels of resistance to diamide insecticides. *Insect Biochem. molec* 89, 79–85. doi:10.1016/j.ibmb.2017.09.005

frontiers | Frontiers in Physiology

TYPE Original Research
PUBLISHED 16 January 2023
DOI 10.3389/fphys.2023.1088712

OPEN ACCESS

EDITED BY
Asad Ali,
Abdul Wali Khan University, Pakistan

REVIEWED BY
Waqar Jaleel,
Central Cotton Research Institute (CCRI), Pakistan
Ran Wang,
Beijing Academy of Agriculture and Forestry Sciences, China

*CORRESPONDENCE
Atif Idrees,
atif_entomologist@giabr.gd.cn
Jun Li,
junl@giabr.gd.cn

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Invertebrate Physiology, a section of the journal Frontiers in Physiology

RECEIVED 03 November 2022
ACCEPTED 04 January 2023
PUBLISHED 16 January 2023

CITATION
Afza R, Afzal A, Riaz MA, Majeed MZ, Idrees A, Qadir ZA, Afzal M, Hassan B and Li J (2023), Sublethal and transgenerational effects of synthetic insecticides on the biological parameters and functional response of *Coccinella septempunctata* (Coleoptera: Coccinellidae) under laboratory conditions.
Front. Physiol. 14:1088712.
doi: 10.3389/fphys.2023.1088712

Sublethal and transgenerational effects of synthetic insecticides on the biological parameters and functional response of *Coccinella septempunctata* (Coleoptera: Coccinellidae) under laboratory conditions

Rahat Afza[1†], Ayesha Afzal[2,3†], Muhammad Asam Riaz[1], Muhammad Zeeshan Majeed[1], Atif Idrees[2,4*], Ziyad Abdul Qadir[5,6], Muhammad Afzal[1], Babar Hassan[7] and Jun Li[2*]

[1]Department of Entomology, College of Agriculture, University of Sargodha, Sargodha, Pakistan, [2]Guangdong Key Laboratory of Animal Conservation and Resource Utilization, Guangdong Public Laboratory of Wild Animal Conservation and Utilization, Institute of Zoology, Guangdong Academy of Sciences, Guangzhou, China, [3]Institute of Molecular Biology and Biotechnology, The University of Lahore, 1-Km Defense Road, Lahore, Pakistan, [4]Guizhou Provincial Key Laboratory for Agricultural Pest Management, Institute of Entomology, Ministry of Agriculture, Guizhou University, Guiyang, China, [5]Honeybee Research Institute, National Agricultural Research Centre, Park Road, Islamabad, Pakistan, [6]Department of Entomology and Wildlife Ecology, University of Delaware, Newark, DE, United States, [7]University of the Sunshine Coast, Maroochydore, QLD, Australia

Synthetic insecticides have been an inevitable part of plant protection throughout the world. Sublethal effects of these chemicals on beneficial insect species are one of the contemporary issues these days. Using the age-stage, two-sex life table model, this study evaluated the sublethal and transgenerational effects of six synthetic insecticides (imidacloprid, thiamethoxam, lambda-cyhalothrin, cypermethrin, chlorpyrifos and profenofos) commonly applied to winter vegetables, on the fitness and predation of the seven-spotted ladybeetle, *Coccinella septempunctata*, which is an efficient predator of aphids worldwide. According to results, all insecticides at their sublethal doses (LC_{30}) significantly suppressed the emergence of adults, adult weight, fertility and fecundity of the parental generation compared to control treatment. The larval stage was prolonged and oviposition, fecundity and total longevity of the adult beetles were decreased in unexposed progeny whose parents were exposed to sublethal doses of all insecticides. Moreover, the biological parameters of adults, including the intrinsic rate of increase (r), finite rate of increase (λ) and net reproductive rate (R_0) were significantly reduced when exposed to sublethal doses of insecticides. The predation rate of the F_1 generation adults was also decreased after exposure to the sublethal doses of insecticides. However, chlorpyrifos, profenofos, lambda-cyhalothrin and cypermethrin exhibited more deleterious effects on the fitness and population parameters of beetles than imidacloprid and thiamethoxam.

KEYWORDS

coccinellid beetles, seven-spotted lady beetle, synthetic insecticides, sublethal exposure, life parameters, functional response

1 Introduction

The ladybeetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) is a well-known predator of various species of agricultural insect pests worldwide including Pakistan (Hodek and Michaud 2013; Saljoqi and Ali 2013). It is a generalist predator often observed on arable crops, feeding on aphids, whiteflies, psyllids, mites, and scales that are destructive insect pests of several crops (Bianchi and Van der Werf 2003; Farooq and Tasawar 2008; Sarwar 2013; Afza et al., 2019; He et al., 2019; Jiang et al., 2019; Afza et al., 2021). This species is considered a useful biological control agent of brassica aphids because of its voracious appetite. It feeds on more than twelve aphid species and rapidly responds to aphid populations (Jiang et al., 2019).

The use of synthetic insecticides by farmers is the predominant practice to keep pest populations below economic injury level (Afza et al., 2019; Afza et al., 2021). Chemical insecticides are cost-effective, easy to use and adequate against target pests. However, their excessive usage may have various adverse effects on beneficial arthropods in agroecosystems. The predatory potential of *C. septempunctata* is negatively affected after feeding on contaminated prey and plant material during foraging. Both direct contact with spray droplets and residues of insecticides affect its predation (Desneux et al., 2007; Afza et al., 2019; Afza et al., 2021). The deleterious effects of insecticides on coccinellid beetles include acute toxicity and changes in physiological, biochemical and behavioural processes. A lethal dose may not represent the overall adverse effects of synthetic insecticides, as sublethal concentrations do also have effect on insect behaviour and physiology (Galvan et al., 2005; Desneux et al., 2007; Rahmani and Bandani 2016). Effects of sublethal doses on the physiology of insects range from immunological to biochemistry, neurophysiological, sex ratio, fecundity, longevity and weight. Behavioural effects include disturbed mobility, orientation, feeding behaviour and learning performance (Galvan et al., 2005; Desneux et al., 2007; Afza et al., 2021). Sublethal doses of synthetic insecticides also have deleterious effects on the functional response of predatory beetles and other biological control agents (He et al., 2012; Jiang et al., 2019).

Indigenous farmers in Pakistan use several insecticides to manage aphids and other pests on their crops. All these insecticides have different ranges of toxicity and adverse effects on *C. septempunctata* (Arshad et al., 2017; Afza et al., 2019; Afza et al., 2021). Although biological control is an integral part of integrated pest management (IPM) programs, chemical control is the primary and most effective method for combating pests. However, some of the synthetic insecticides are relatively safer to be used as components of IPM programs. Therefore, knowledge of the non-target effects of insecticides on the behavioural traits, growth and development of biological control agents, is essential for an effective incorporation of insecticides in successful pest management programs (Tillman and Mulrooney 2000; Youn et al., 2003; Galvan et al., 2005; Bozsik 2006; Stark et al., 2007; Tengfei et al., 2019).

Several studies have reported on the lethal and sublethal effects of pesticides on predatory coccinellid beetles, showing that pesticides are harmful to these natural enemies (Urbaneja et al., 2008; Cabral et al., 2011; He et al., 2012; He et al., 2019; Jiang et al., 2019). However, data on age-related life table analyses and demographic parameters of *C. septempunctata* to evaluate the effects of currently used insecticides is still lacking. No study has evaluated the sublethal impacts of formulated insecticides commonly used by farmers for the management of aphids in Pakistan. Hence, an overall risk assessment of the exposure of *C. septempunctata* to currently-used synthetic insecticides is necessary. Apropos of the context, this study was aimed to assess the sublethal effects of six commonly-used synesthetic insecticides at their lower concentrations (LC_{30}), on the fecundity, development and demographic parameters of *C. septempunctata* based on the life table theory. Moreover, the effects of their sublethal doses on the functional response of *C. septempunctata* were also assessed. The results of the current study can be useful for guiding assessment of the compatibility between the *C. septempunctata* and different insecticides in future IPM strategies for aphids. They would also contribute to the conservation of *C. septempunctata* in the field.

2 Materials and methods

2.1 Insect rearing

C. septempunctata adults were collected from canola (*Brassica napus* L.) fields managed without application of insecticides, at a farm area of the University of Sargodha, Pakistan (32°4′ N; 72°40′ E). Adult beetles were identified using identification keys described by (Poorani 2002; Rafi et al., 2005; Saeed et al., 2016) and were sorted into fifty couples (male and female). Then each pair of male and female coccinellid beetles was released into glass bowl (7.0 × 2.5 cm), which were then covered with muslin cloth and placed in an incubator (at 25 ± 2°C and 60 ± 5% R.H.), to obtain eggs for single cohort progeny. Collected adults and newly-hatched larvae of beetles were offered twigs and branches of canola infested by aphids (Brevicoryne brassicae L.) in a bowl. Aphids collected from the canola field were provided after every 12 h. For this purpose, the canola crop was sown in the campus and no insecticide was sprayed on the crop. Adult beetles were also offered a continuous supply of 10% glucose solution *via* a soaked cotton wool plug (Sarwar and Saqib 2010; Afza et al., 2019; Afza et al., 2021).

2.2 Selection and source of insecticides

A field survey was conducted in the brassica growing region of Punjab (Pakistan) for 2 years. One hundred farmers were asked about the insecticides sprayed for the control of aphids on brassica and other winter crops every year. Survey results revealed six insecticides which were commonly sprayed by the farmers to manage their crop pests. These included imidacloprid, thiamethoxam, lambda-cyhalothrin, cypermethrin, chlorpyrifos and profenofos. Hence, these six insecticides were selected for the current study. Trade names, type of formulations, percent active ingredients (a.i.) and sources of the tested insecticides are given in Supplementary Table S1.

2.3 Determination of sublethal concentration (LC_{30}) values

The experiment for the determination of lethal and sublethal concentration values was conducted following Afza et al. (2021). Based on mortality results obtained from preliminary assays

(0–90%), five to six concentrations of each insecticide were prepared by dissolving them in water. A sample of 25–30 fourth instar larvae of *C. septempunctata* were treated with the concentrations of each insecticide separately. The control involved treatment of larvae with water. The experiment was replicated five times. Larvae were placed on ice to reduce their mobility during the bioassays. Then, 1 µl of each insecticide at different concentration and water (for control) was applied separately on the mesonotum of fourth instar larvae using a micropipette.

Treated larvae were placed in Petri-plates (90 mm diameter) lined with moistened Whatman No. 1 filter paper discs. To prevent the larvae from escaping, the plates were covered with PVC film which had small holes for aeration and were placed in an incubator (at 25 ± 2°C and 60 ± 5% R.H). Aphids were provided *ad-libitum* daily. Larval mortality was recorded 24 h after treatment. All beetles used in this experiment were 12–15 weeks old. Probit analysis using the Polo Plus® software was performed to calculate the median lethal concentrations (LC_{50} and LC_{30}) values for each insecticide. The toxicity of sublethal dose (LC_{30}) was confirmed in a separate test against the fourth instar larval beetle.

2.4 Effects of LC_{30}s on parental generation (F0) of *C. septempunctata*

Three groups of freshly laid (0–6 h old) 100 eggs were placed on moistened Whatman No. 1 filter paper discs lined in Petri-plates (90 mm diameter). After inspection every 6 h, newly-hatched larvae were transferred to new Petri-plates and were fed on mixed instars of canola aphids (*Brevicoryne brassicae* L). All Petri-plates were incubated in an environmental chamber at 25 ± 2°C, 60 ± 5% R.H. and 14:10 h (L: D) photoperiod. When larvae reached the fourth instar (0–12 h old), sublethal concentrations (LC_{30}) of each insecticide was applied topically. The treatment method was similar as described above. Forty-five larvae (15 larvae per replicate) were treated in each treatment and the experiment was repeated thrice. For the control treatment, larvae were treated with water. Fourth instar larvae were chosen for this experiment because of their lower natural mortality, compared to that of the initial three instars. Larval mortality, percentage of pupae formed and adult emergence from pupae were recorded at every 12 h until the adult stage. Larvae found motionless were considered dead. Newly-emerged adults were weighed and fed with B. brassicae individuals and 10% glucose solution. Male and female beetles from the same treatment were paired the same or second day of emergence and were observed daily to record their survival rate, fecundity and egg hatchability, until all the individuals were dead. For the fecundity, fifteen couples were tested per insecticide treatment.

2.5 Effects of LC_{30}s on demographic growth parameters of F1 generation

A total 45–50 eggs (0–6 h old) from three to four couples of the parental generation, from the same treatment, were separated and kept in Petri-plates. Eggs were checked at 12 h intervals and newly-emerged larvae were transferred to new Petri-plates and fed on aphids. Each larva was considered as one replicate and its developmental time was monitored until pupation. Newly-emerged adults were sexed (12–15 couples) and their fecundity, survival rate and developmental time were recorded until the death of all individuals of the F_1 generation.

2.6 Effects of LC_{30}s on functional response of F1 generation

F1 adult beetles starved for 24 h, were confined on canola foliage in Petri-plates (150 mm diameter) lined with moistened Whatman No. 1 filter paper discs and were offered B. brassicae aphid individuals at five densities, i.e. 20, 80, 160, 320 and 640 aphid individuals, as a food source. These aphids were pre-exposed to sublethal doses (LC_{30}) of the insecticides. Water was used as control treatment. Twelve replications were carried out for each aphid density treatment. Individual coccinellids were offered insecticide-treated aphids. The data on aphid consumption by each beetle was recorded. One replicate under each density served as no-predator control, to determine the mortality of aphids after treating with insecticides. Experiments were conducted under controlled condition at 25 ± 2°C, 60 ± 5% R.H, and 14:10 h (L: D) photoperiod.

2.7 Statistical analysis

The data obtained from the toxicity bioassays were corrected using Abbott's formula before analysis. The LC_{50} and LC_{30} values were calculated for each insecticide by probit analysis using the Polo Plus® software (Finney 1971). The normality of the parental generation (F_0) data was assessed using the Kolmogorov-Smirnov test in SPSS® version 21.0 (SPSS Inc, Chicago, IL, United States) and data were analysed using one-way ANOVA in GraphPad Prism version 7.0 (GraphPad Software Inc, San Diego, CA, United States). Treatment means were compared by Tukey's honest significant difference (HSD) *post hoc* test. Life table data were analysed using age-stage, two-sex life table using Two Sex-MS Chart program (Chi 1988). Standard errors of the life table parameters were calculated *via* 100,000 bootstrap replicates to obtain stable S.E. estimates. Sigma Plot® version 12.0 (Systat Software, Inc, San Jose, CA, United States) was used to generate curves for all population parameters including age-stage specific survival rate (Sxj), age-stage life expectancy (exj), age-specific survival rate (lx), age-specific fecundity (mx), net maternity (lx*mx) and reproductive value (vxj). Moreover, a two-step approach recommended by Juliano (2001) was used to determine the type of functional response and its parameters. Further details are given in Supplementary Table S1 and Supplementary Table S2.

3 Results

3.1 Toxicity bioassay

The LC_{50} values of the six insecticides against *C. septempunctata* larvae were 256.6, 191.3, 122.3, 92.2, 153.1 and 235.9 mg a. i./ml for imidacloprid, thiamethoxam, profenofos, chlorpyrifos, lambda-

10.3389/fphys.2023.1088712

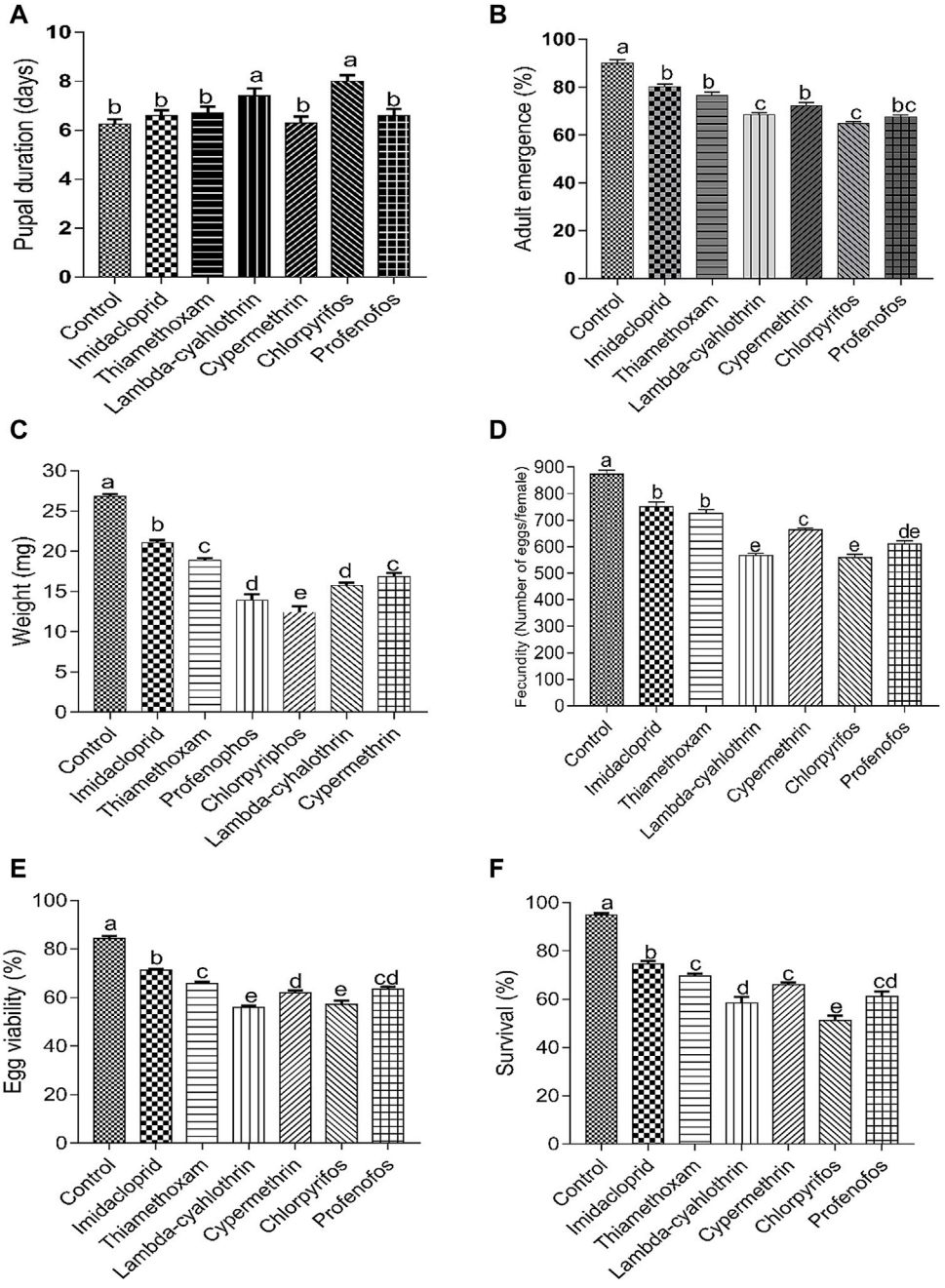


FIGURE 1
Effect of sublethal concentrations (LC_{30}) of synthetic insecticides on the pupal duration **(A)**, adult emergence **(B)**, weight of newly-emerged adults **(C)**, fecundity (eggs/female) **(D)** egg viability **(E)** and adult survival **(F)** of the F_0 generation of *Coccinella septempunctata*. Larvae of *Coccinella septempunctata* were exposed to sublethal concentrations of imidacloprid, thiamethoxam, profenofos, chlorpyrifos, lambda-cyhalothrin and cypermethrin. Treatment means were compared by Tukey's Honest Significant Difference (HSD) *post hoc* test. Means with the same letters are not significantly different at $p \leq 0.005$.

cyhalothrin and cypermethrin, respectively. The highest LC_{50} was recorded for imidacloprid, while the minimum LC_{50} was observed in chlorpyrifos. The organophosphate insecticides, profenofos and chlorpyrifos, exhibited comparatively lower LC_{50} values against the fourth instar larvae of *C. septempunctata* than that of the other insecticides. The LC_{30} values of these insecticides were determined as 158.7, 117.5, 75.5, 56.4, 95.9, and 110.4 mg a. i./ml for imidacloprid, thiamethoxam, profenofos, chlorpyrifos, lambda-cyhalothrin and cypermethrin, respectively.

3.2 Effects of LC_{30}s on parental (F0) *C. septempunctata* generation

The treatment of larvae with sublethal concentrations of the six insecticides had significant negative effects on the biological parameters of *C. septempunctata* compared to the control groups (Figure 1). Exposure to sublethal concentration of lambda-cyhalothrin, chlorpyrifos and profenofos, significantly increased the pupal duration of beetles (>7 days), compared to the other insecticides

10.3389/fphys.2023.1088712

TABLE 1 Means (days ±SE) developmental time of the different life stages of *Coccinella septempunctata* F1 generation.

Basic statistic	Control	Imidacloprid	Thiamethoxam	Lambda-cyhalothrin	Cypermethrin	Chlorpyrifos	Profenofos
Egg duration	3.20 ± 0.10a	3.20 ± 0.1a	3.20 ± 0.10a	3.79 ± 0.1a	3.29 ± 0.1a	3.29 ± 0.1a	3.29 ± 0.10a
Larvae 1 duration (d)	3.28 ± 0.09a	3.27 ± 0.09a	3.27 ± 0.09a	3.26 ± 0.09a	3.26 ± 0.09a	3.26 ± 0.09a	3.26 ± 0.09a
Larvae 2 duration (d)	3.30 ± 0.13c	3.39 ± 0.10b	3.40 ± 0.10b	3.55 ± 0.12a	3.48 ± 0.11b	3.45 ± 0.10b	3.45 ± 0.10b
Larvae 3 duration (d)	3.59 ± 0.15c	3.70 ± 0.16b	3.73 ± 0.15b	3.82 ± 0.17a	3.68 ± 0.19b	3.85 ± 0.17a	3.85 ± 0.14a
Larvae 4 duration (d)	4.0 ± 0.11c	4.17 ± 0.11b	4.18 ± 0.12b	4.27 ± 0.17a	4.27 ± 0.17a	4.20 ± 0.15ab	4.20 ± 0.15ab
Pupae duration (d)	6.36 ± 0.41b	6.42 ± 0.12ab	6.43 ± 0.12ab	6.63 ± 0.23ab	6.54 ± 0.22ab	6.63 ± 0.23ab	7.04 ± 0.21a
Adult duration (d)	55.48 ± 0.63a	53.98 ± 2.13b	52.22 ± 0.65b	45.58 ± 2.32d	46.25 ± 2.45c	47.96 ± 2.13c	49.88 ± 2.09bc
Total longevity (d)	67.77 ± 4.91a	64.8 ± 4.73ab	52.33 ± 4.76b	57.81 ± 4.58c	58.01 ± 4.67c	59.25 ± 4.80bc	61.04 ± 4.94bc

Means sharing same letters in rows are not significantly different from one another at $p > 0.05$.

and control (water-treated) insects (<6.8 days) (F = 6.22; $p < 0.0001$) (Figure 1A). The adult emergence rate of insecticide-treated *C. septempunctata* was also significantly affected compared to the control insects (F = 58.84; $p < 0.0001$). The sublethal doses of lambda-cyhalothrin, chlorpyrifos and profenofos significantly reduced the emergence rate compared to insects treated to cypermethrin, imidacloprid and thiamethoxam. A higher adult emergence rate was recorded for control (water treated) insects (Figure 1B). Similarly, the weight of newly-emerged beetles treated with insecticides was reduced compared to control groups (F = 5.09; $p = 0.001$). A significantly higher decrease in weight of adult beetles was observed after treatment with chlorpyrifos, followed by lambda-cyhalothrin and profenofos. Thiamethoxam and cypermethrin caused similar effects on the weight of beetles (Figure 1C). The fecundity rate of females was also significantly suppressed after treatment with sublethal concentrations of the six insecticides, compared to control treatment (F = 83.01; $p < 0.0001$). The most significant reduction in fecundity occurred after treatment with lambda-cyhalothrin, chlorpyrifos and profenofos followed by cypermethrin. Thiamethoxam and imidacloprid caused similar effects on the fecundity of beetles. A higher fecundity was observed in beetles treated with water (872.4 ± 15.75 eggs) (Figure 1D). Egg hatchability (%) (F = 103.40; $p < 0.0001$) and survival (%) (F = 80.50; $p < 0.0001$) of *C. septempunctata* treated with sublethal doses of six insecticides was also significantly reduced compared to control groups. A sublethal treatment of chlorpyrifos and lambda-cyhalothrin, significantly reduced the egg viability and survival of beetles compared to the rest of insecticides. However, egg hatchability and survival of water-treated beetles were substantially higher compared to insecticides-treated beetles (Figure 1E, F).

3.3 Effect of LC_{30}s on the demographic growth parameters of *C. septempunctata*

Results of the effect of sublethal doses of the insecticides on the developmental time of the F_1 generation are presented in Table 1. All sublethal insecticidal treatments did not significantly affect the egg hatching and first instar larval period of the beetles (egg hatching duration: F = 4.55; $p = 0.090$; first instar duration: F = 12.33; $p = 0.10$). The larval duration of the second instar of all insecticide-treated beetles significantly lengthened, compared to control treatment (F = 47.15; $p = 0.040$). Here, lambda-cyhalothrin had the most significant effect on this duration compared to other insecticidal treatments. Similarly, the period of the third instar larvae was significantly increased after treatment with lambda-cyhalothrin, chlorpyrifos, and profenofos compared to other insecticides. Overall, the third instar larval period of insecticide-treated beetles was substantially longer compared to that of water-treated beetles (F = 43.65; $p = 0.030$). The fourth instar period of beetles treated with lambda-cyhalothrin and cypermethrin was significantly increased compared to thiamethoxam and imidacloprid (F = 25.19; $p = 0.032$). Moreover, the pupal duration of beetles treated with profenofos significantly lengthened compared to water-treated beetles. Nevertheless, the pupal period of beetles treated with the other insecticides did not differ significantly from control treatment and with profenofos (F = 51.23; $p = 0.041$). Adult duration and total longevity of insecticide-treated beetles were reduced considerably compared to water-treated beetles (Adult duration: F = 157.11; $p = 0.002$; overall longevity: F = 412.13; $p = 0.001$) (Table 1).

Results of the effects of the six insecticides at LC_{30}, on the population parameters of the F_1 generation of *C. septempunctata* are presented in Table 2. Sublethal doses of all six insecticides significantly reduced the intrinsic rate of increase (r) and finite rate (λ) of the beetles. However, the finite rate of beetles treated with thiamethoxam and imidacloprid was similar to that of water-treated beetles. Sublethal treatments of chlorpyrifos, profenofos, and lambda-cyhalothrin significantly decreased the intrinsic rate of increase compared to the other three insecticides ($p < 0.05$). All insecticides at sublethal doses (LC_{30}) significantly reduced the net reproduction rate (R0) (F = 351.65; $p < 0.0001$) and increased the mean generation time (T) (F = 253.15; $p = 0.002$) compared to that of water-treated *C. septempunctata*. However, chlorpyrifos, profenofos, and lambda-cyhalothrin had significantly greater effects on R_0 and T, compared to the other insecticides (Table 2). Adult pre-oviposition period (APOP) (F = 51.20; $p = 0.001$) and total pre-oviposition period (TPOP) (F = 98.27; $p = 0.002$) were significantly prolonged in beetles treated with the six insecticides compared to those of water-treated beetles. Chlorpyrifos, profenofos, and lambda-cyhalothrin had greater effects on TPOP and APOP compared to those of the other three insecticides. Female fecundity was also reduced in beetles treated with insecticides compared to that of the control (water-treated)

TABLE 2 Population parameters (means ± SE) of *Coccinella septempunctata* F1 generation adults in response to sublethal exposure (LC_{30}) to six insecticides.

Basic statistic	Control	Imidacloprid	Thiamethoxam	Lambda-cyhalothrin	Cypermethrin	Chlorpyrifos	Profenofos
Intrinsic rate of increase (r) (d−1)	0.121 ± 0.005a	0.120 ± 0.007b	0.119 ± 0.006b	0.106 ± 0.006c	0.113 ± 0.006b	0.108 ± 0.006c	0.107 ± 0.006c
Finite rate (λ) (d−1)	1.128 ± 0.006a	1.126 ± 0.007ab	1.122 ± 0.007ab	1.112 ± 0.006c	1.120 ± 0.006b	1.114 ± 0.007c	1.114 ± 0.007c
Net reproduction rate (R0)	293.1 ± 45.69a	248.99 ± 64.96b	234.90 ± 58.53b	191.03 ± 49.17d	204.28 ± 50.42c	210.97 ± 55.34c	224.05 ± 58.76c
Mean generation time (T) (d)	45.86 ± 0.84c	47.80 ± 0.80b	47.94 ± 1.15b	49.17 ± 0.88a	48.66 ± 0.83ab	49.44 ± 0.60a	49.66 ± 0.63a
APOP (d)	9.50 ± 0.31d	10.49 ± 0.30bc	9.91 ± 0.79c	12.0 ± 0.52a	10.76 ± 0.43b	11.90 ± 0.28a	11.72 ± 0.32a
TPOP (d)	34.30 ± 0.82d	36.30 ± 0.80b	36.16 ± 1.03bc	37.58 ± 0.96a	35.92 ± 0.79c	37.45 ± 0.66a	37.72 ± 0.67a
Fecundity	879.3 ± 18.61a	746.9 ± 30.77b	641.09 ± 41.73c	454.54 ± 69.73e	452.50 ± 70.46e	545.08 ± 74.66d	579.25 ± 79.66d
Oviposition duration (d)	35.80 ± 1.30a	31.59 ± 1.01b	28.0 ± 1.61c	24.50 ± 2.85d	24.38 ± 3.16d	26.91 ± 2.58cd	28.60 ± 2.72bc

Means sharing same letters in rows are not significantly different from one another at $p > 0.05$. Adult Pre-Oviposition Period (APOP) of female is the interval from adult emergence to first oviposition and the Total Pre-Oviposition Period (TPOP) of female is the duration from egg to first oviposition.

beetles (F = 698.27; $p < 0.0001$) (Table 2). A higher reduction in eggs/female was recorded in beetles treated with sublethal doses of profenofos, chlorpyrifos, and lambda-cyhalothrin than that from the other insecticides. Oviposition durations of females were significantly higher in water-treated beetles, compared to insecticide-treated beetles (F = 173.27; $p < 0.0001$).

The age-stage specific survival rate (sxj) curves showed the variation in different developmental stages of the coccinellids. There were distinct overlaps between the control and insecticidal treatments (Figure 2). The survival rate of adult males and females was decreased after treatment with lambda-cyhalothrin and chlorpyrifos, compared to the control and that of the other insecticidal treatments. The larval duration after treatments with lambda-cyhalothrin, profenofos, and chlorpyrifos was significantly reduced compared to that of control and other insecticidal treatments. The exj was significantly reduced in beetles treated with sublethal doses of all insecticides except imidacloprid, compared to that of the control treatment (Figure 3). The lx, mx, and lxmx are presented in Figure 4. Age-stage specific survival rate was significantly reduced in beetles treated with chlorpyrifos and lambda-cyhalothrin compared to that of beetles treated with water and other insecticides. The maximal survival time for control treatment was 82 days, which was higher than that under insecticidal treatments. Similarly, compared to control treatment, the vxj of *C. septempunctata* was significantly reduced after treatment with sublethal doses of all insecticides except for imidacloprid and chlorpyrifos (Figure 5). A higher reduction in vxj was observed in beetles treated with a sublethal dose of lambda-cyhalothrin followed by thiamethoxam compared to that from the other insecticides.

3.4 *C. septempunctata* functional response after feeding on insecticide-treated aphids

The functional response of F_1 coccinellid adults to B. brassicae aphids fitted o the Holling's type II curves (Figure 6). Logistic regression analysis coefficients of the proportion of sublethal (LC_{30}) dose-treated aphids devoured by adult coccinellids are presented in Supplementary Table S2. Values of the parameters α, Th and T/Th derived from all treatments are presented in Table 3. The instantaneous attacking rate (α) derived from control treatment remained significantly higher (0.075) compared to that from insecticidal treatments. Significantly minimum attack rate was observed in beetles which fed on aphids treated with lambda-cyhalothrin (0.001), followed by chlorpyrifos (0.011) and thiamethoxam (0.010). The maximum handling time (Th) (0.069) was recorded for beetles which fed on aphids treated with chlorpyrifos, followed by lambda-cyhalothrin (0.051) and profenofos (0.050). The handling time of prey by beetles treated with imidacloprid, thiamethoxam and cypermethrin did not differ significantly. The minimum handling (0.017) time was recorded under control treatment.

4 Discussion

Insecticides are mainly used as a foliar spray on canola and other winter crops. Therefore, predators of insect pests, including coccinellid beetles are likely to be exposed to insecticides while foraging on the treated plants and pests. Exposure to pesticides may cause various adverse effects on the life-history traits and feeding behaviour of coccinellid beetles. In the current study, the sublethal effect of six insecticides belonging to three chemical classes was presented with experimental evidences. We found that the sublethal doses (LC_{30}) of the six insecticides adversely affected the biological parameters of *C. septempunctata* (F_0 generation). Lambda-cyhalothrin and chlorpyrifos increased the pupal duration compared to that from the other insecticides. All the tested insecticides reduced the adult emergence, fecundity rates, weight of adults, and survival compared to the control treatment. These results are similar to those from other studies which showed that most of the tested insecticides impaired the reproduction and survival of *C. septempunctata* and other predators (Yu et al., 2014; Skouras et al., 2017; Kang et al., 2018; Jiang et al., 2019; Noelia et al., 2022).

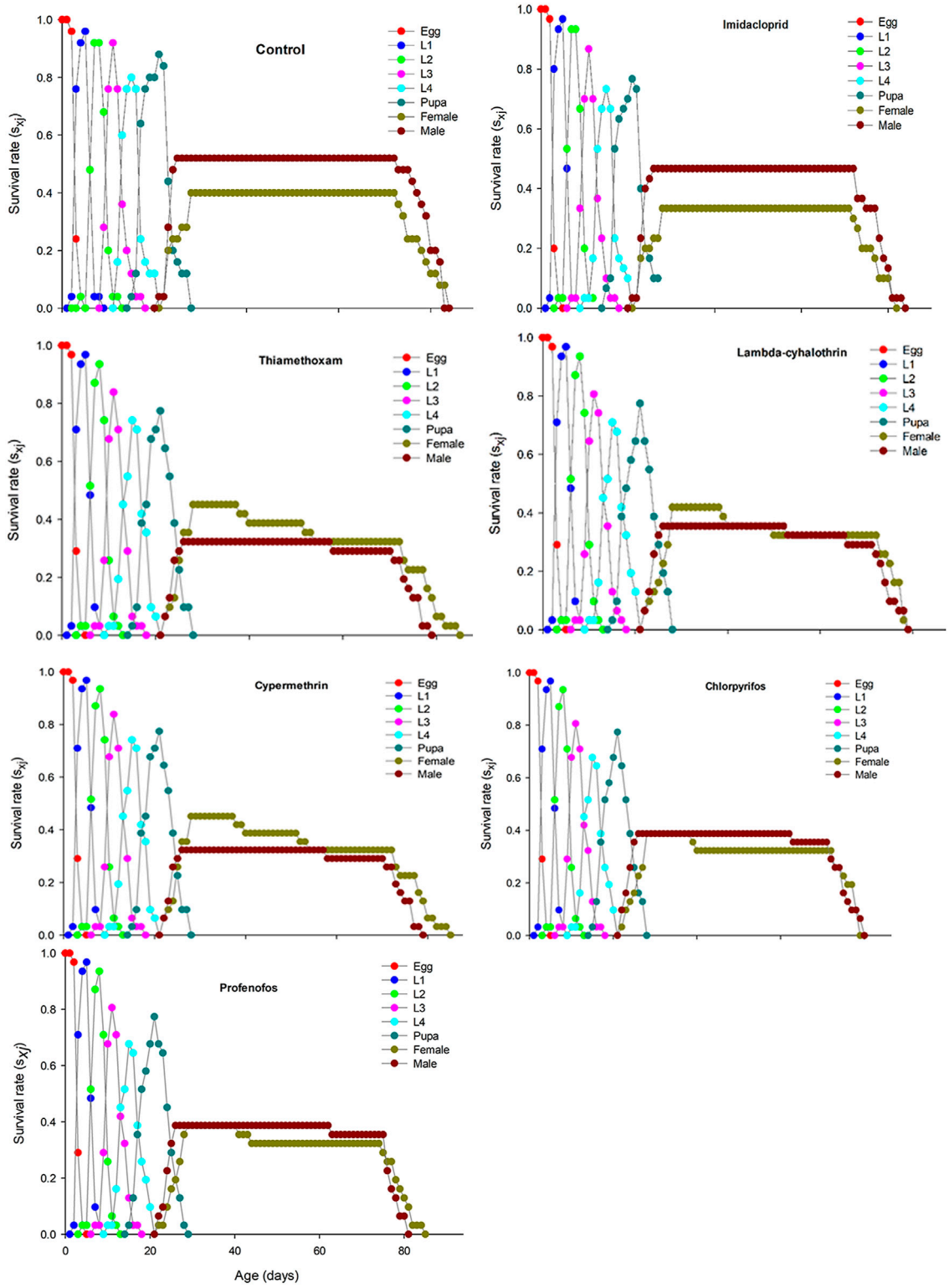


FIGURE 2
Age-stage specific survival (*sxj*) of *Coccinella septempunctata* (F_1) treated with water (control) and sublethal concentrations (LC_{30}) of synthetic insecticides.

Transgenerational impacts of the six insecticides on the predatory beetle, *C. septempunctata*, were studied by age-stage, two-sex life table model analyses. Results showed that the second, third, fourth instar and pupation stages of *C. septempunctata* in the F_1 generation were prolonged when their parental generations (F_0) were exposed to sublethal doses of all insecticides. This prolongation in the larval and pupal development may be due to physiological effects such as reduced food assimilation or stunted development at the cost of

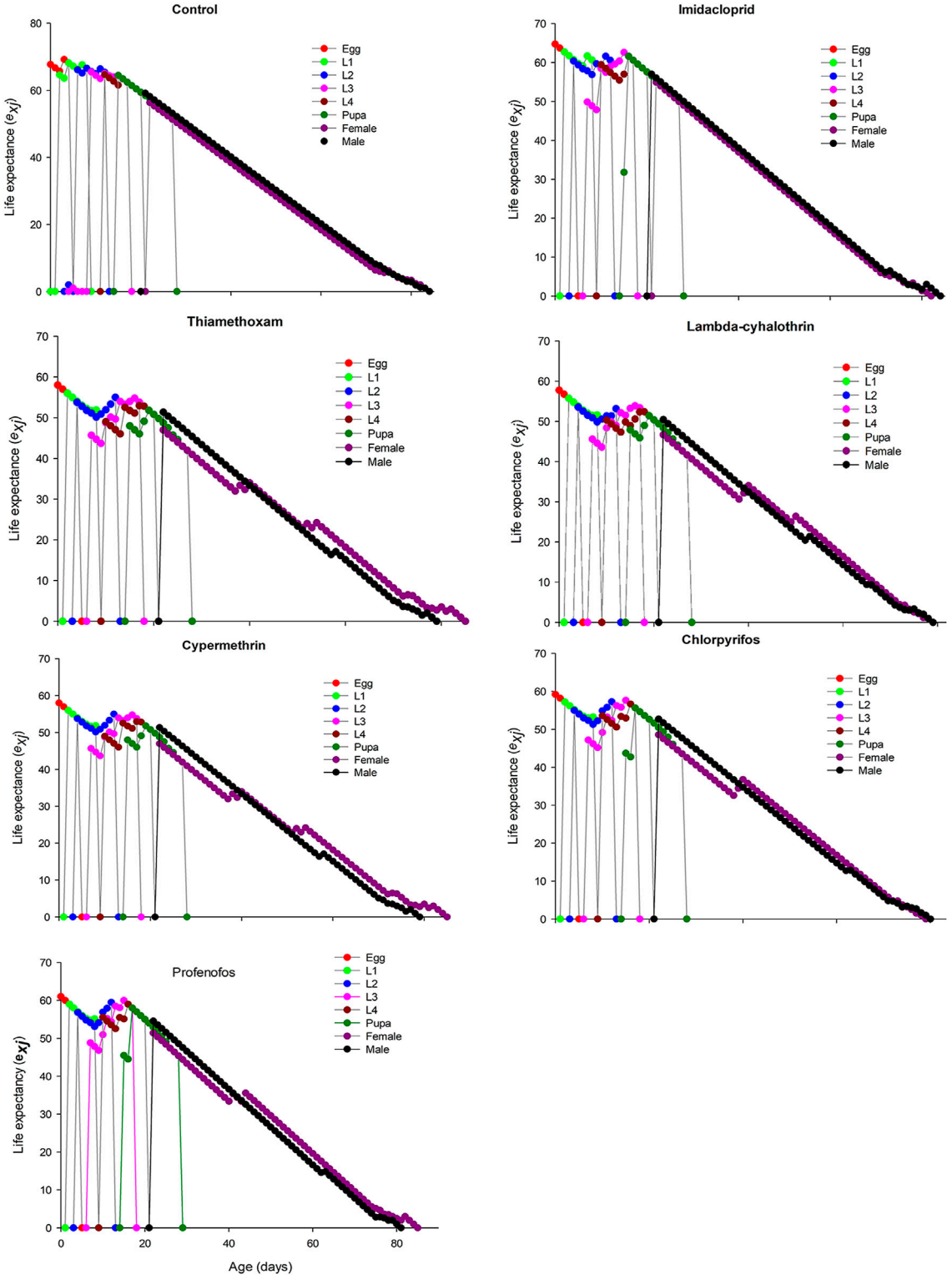


FIGURE 3
Age-stage specific life expectancy (*exj*) of *Coccinella septempunctata* (F_1) treated with water (control) and sublethal concentration (LC_{30}) of synthetic insecticides.

detoxification mechanism (Skouras et al., 2017). However, treatment with a sublethal dose of lambda-cyhalothrin had higher significant effects on the second instar than the other insecticides. Similarly, the sublethal dose of lambda-cyhalothrin, chlorpyrifos and profenofos prolonged the third instar duration compared to the other three insecticides. All insecticides reduced the adult duration compared to the control treatment, and beetles treated with a sublethal dose of lambda-cyhalothrin had the least adult duration. The total longevity of

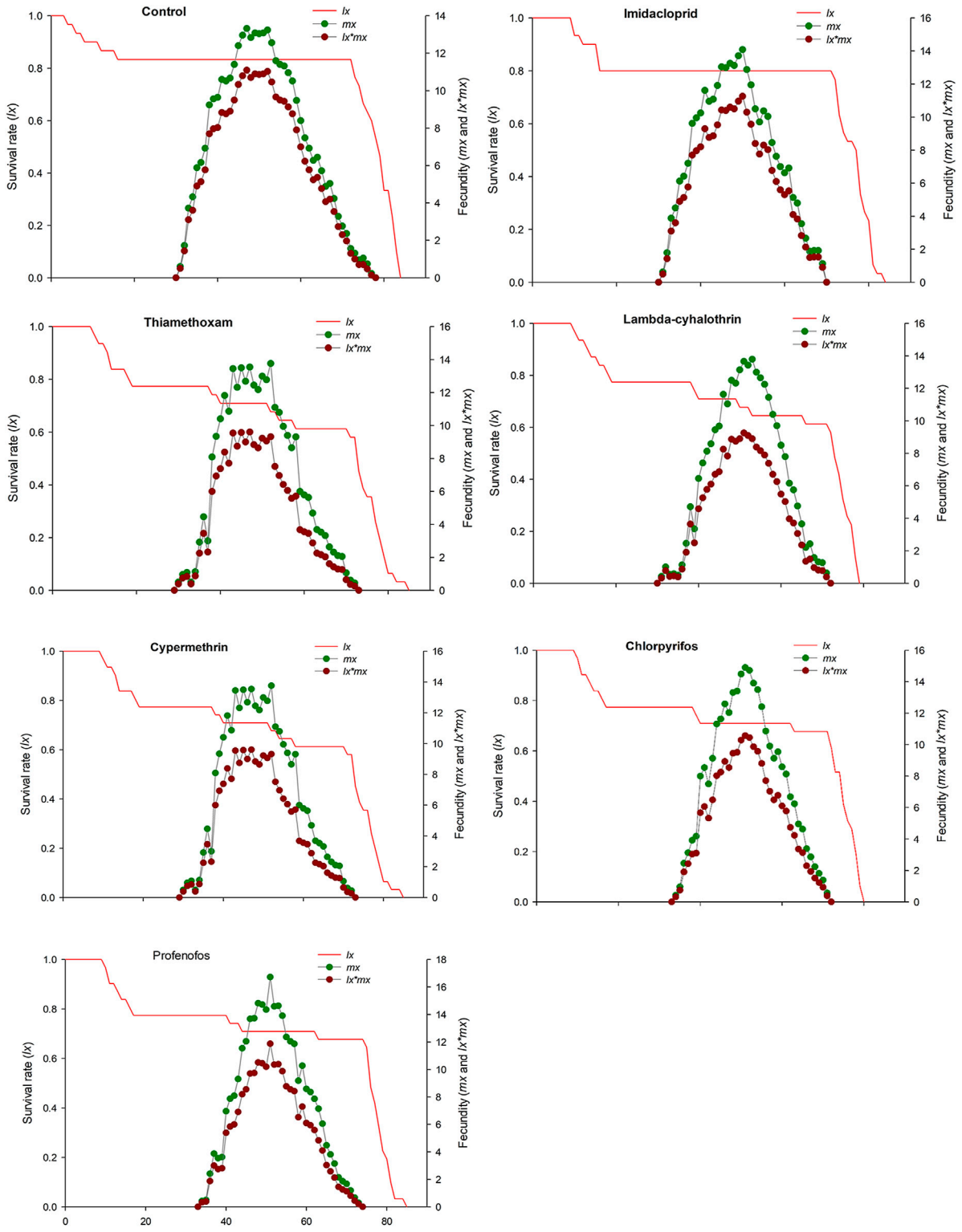


FIGURE 4
Age-stage specific survival rate (*lx*), age-specific fecundity (*mx*) and net maternity (*lx* × *mx*) of *C. septempunctata* (F_1) treated with water (control) and sublethal concentration (LC_{30}) of synthetic insecticides.

beetles treated with the sublethal dose was significantly reduced compared to that under control treatment. The differences between the sublethal effects of insecticides on insect depend on the variations in the structure and efficacy of insecticides (Alkassab and Kirchner 2016; Skouras et al., 2017; Dai et al., 2019; Tan et al., 2021).

The sublethal doses of all insecticides adversely influenced the reproductive performance of the F_1 generation. Reductions in the

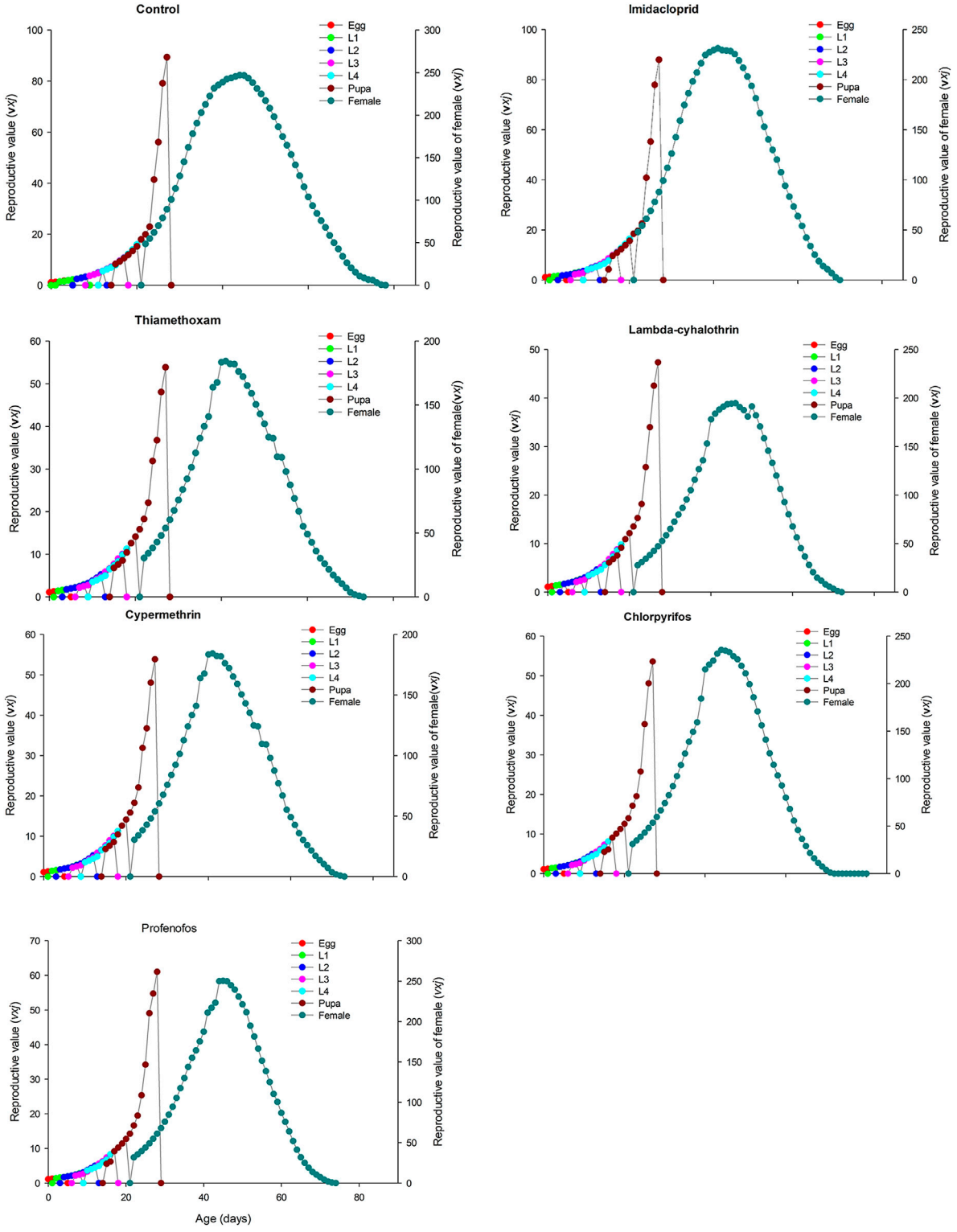


FIGURE 5
Age-stage specific reproductive value (*vxj*) of *Coccinella septempunctata* (F_1) treated with water (control) and sublethal concentration (LC_{30}) of synthetic insecticides.

fecundity of females exposed to insecticides may result from both physiological and behavioural effects (Desneux et al., 2007; Wu et al., 2022). The duration of the F1 generation's pre-oviposition period (APOP and TPOP) and the oviposition period changed significantly in beetles treated with lambda-cyhalothrin, chlorpyrifos, and profenofos (Desneux et al., 2004; De Castro et al., 2013). Moreover, there was a significant reduction in the number of offspring (F_2) from parent individuals exposed to

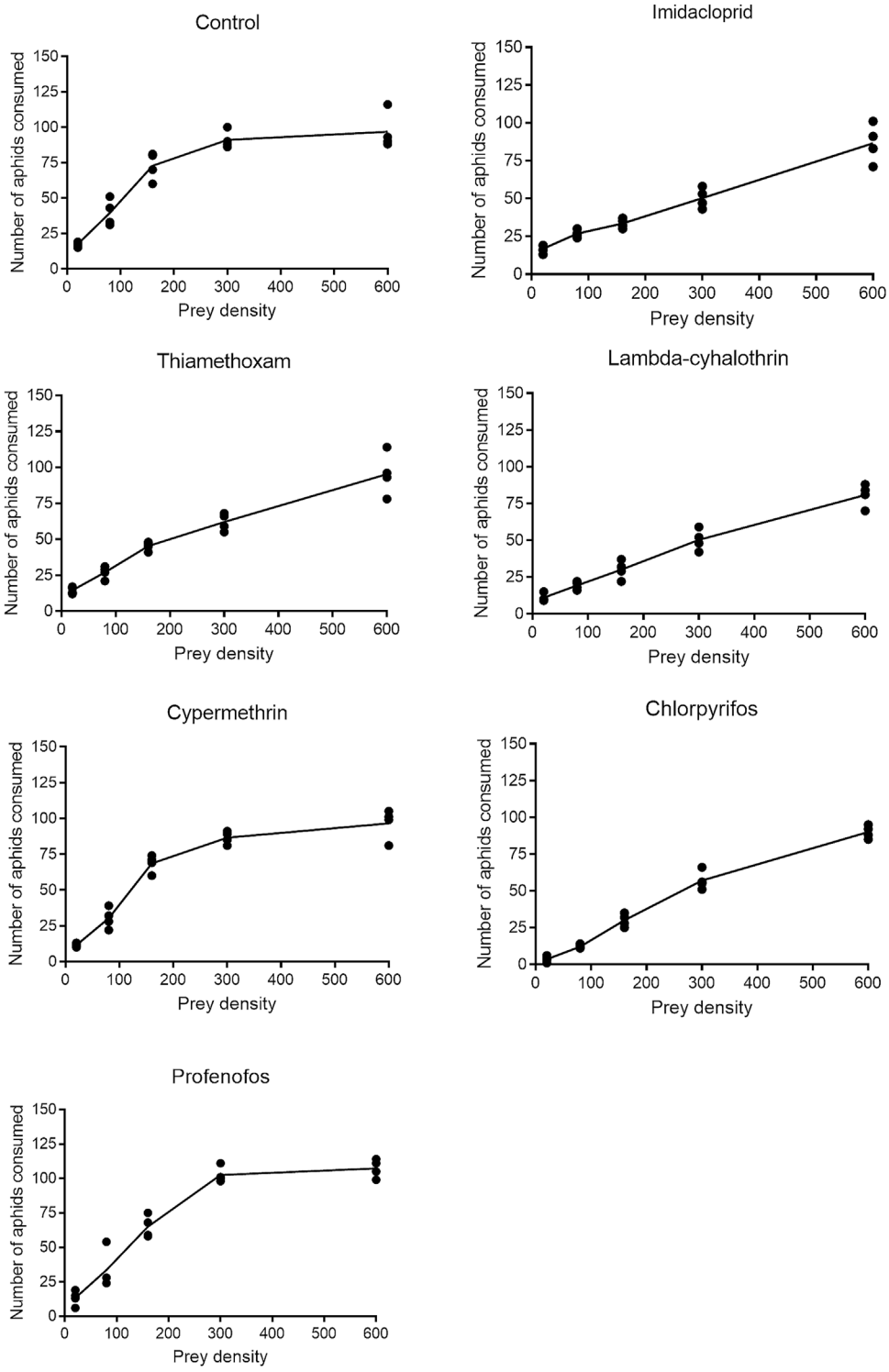


FIGURE 6
Functional responses of *Coccinella septempunctata* adults exposed to sublethal doses of synthetic insecticides and control.

sublethal doses of all insecticides (Fernandes et al., 2010). These insecticides might have disrupted the very precise coordination of the nervous and hormonal systems in the insects, resulting in a breakdown in behavioural and physiological events related to oviposition (Desneux et al., 2007).

Population parameters such as intrinsic (r) and finite (λ) rates of increase, net reproductive rate (R_0) and mean generation time (T) can be useful to better understand the population dynamics of insects (Liu et al., 2017; Zheng et al., 2017; Negi et al., 2018; Jiang et al., 2019). Results showed that the treatment of F_0 with sublethal doses of all

TABLE 3 Parameters for functional response model for adult *Coccinella septempunctata* exposed to sublethal concentrations (LC_{30}) of six insecticides.

Treatment	A	Th	T/Th	R2
Control	0.075a	0.017d	135.5	0.98
Imidacloprid	0.020c	0.033c	72.72	0.96
Thiamethoxam	0.010cd	0.038c	63.15	0.92
Profenofos	0.021c	0.050b	82.75	0.89
Chlorpyriphos	0.011cd	0.069a	48.01	0.96
Lambda-cyhalothrin	0.001e	0.051b	77.41	0.99
Cypermethrin	0.031bc	0.044c	71.56	0.85

a–attack rate; Th–handling time; T/Th, maximum attack rate; Means sharing same letters in rows are not significantly different from one another at $p > 0.05$.

insecticides significantly reduced the r, λ, and R_0 parameters of the F1 generation.

However, the sublethal doses of lambda-cyhalothrin, chlorpyrifos, and profenofos significantly reduced r and λ than sublethal doses of imidacloprid and thiamethoxam. In general, the fluctuations in demographic features demonstrated that sublethal concentrations pyrethroid and organophosphate insecticides had the greatest effects on the reproduction and survival of *C. septempunctata* transgenerationally than the two neonicotinoids.

Functional response is generally determined to assess the feeding efficiency of predatory species to agricultural pests (Juliano 2001; Xue et al., 2009; He et al., 2012; Sarkar et al., 2022). Sublethal exposure to all insecticides impaired the functional responses and prolonged the handling time of aphid by the predator beetle. This agrees with studies of He et al. (2012) and Yao et al. (2015) which showed that thiamethoxam and imidacloprid prolonged the handling time of *Bemisia tabaci* eggs by the predatory coccinellid beetle, *Serangium japonicum* at sublethal doses. However, a maximum handling time was observed when beetles were offered aphids treated with chlorpyrifos, followed by profenofos (GholamzadehChitgar et al., 2014).

Overall, sublethal doses of all the tested insecticides impaired the fitness and predation efficacy of *C. septempunctata* individuals up to two successive generations. However, the effects of the pyrethroids and organophosphates were greater, compared to neonicotinoids. This agrees with previous studies (Ahmad et al., 2011). Organophosphates and pyrethroids have low selectivity to insect predators and parasitoids than neonicotinoids (Galvan et al., 2002; Fernandes et al., 2010). This greater negative effects of the two insecticide groups, may be associated with their pro-insecticide activities. Moreover, organophosphates and pyrethroids have lipophilic characteristic and can be adsorbed in the beetle cuticle very easily when topically applied (GholamzadehChitgar et al., 2014; Costa 2015). This may be responsible for thegreater negative effects they have on insects.

5 Conclusion

Our laboratory experiments demonstrated that all the tested insecticides had adverse effects on the fitness and predation efficacy of *C. septempunctata* individuals. However, among these, the organophosphates and pyrethroids had greater negative effects on the population parameters of beetles compared to the neonicotinoids. Therefore, these neonicotinoids have some potential which can enable their integration into IPM programs. Additionally, we also evaluated the demographic and transgenerational effects mediated by exposure to sublethal doses of these insecticides. Future work will focus on the mechanisms underlying these adverse effects of the sublethal doses of the insecticides on *C. septempunctata*.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author contributions

All authors listed have made considerable direct or indirect and intellectual contribution to the work and have read and agreed to the manuscript.

Funding

This research work was financially supported by the GDAS Special Project of Science and Technology Development (No. 2020GDASYL-20200301003), GDAS Action Capital Project to build a comprehensive industrial technology innovation Center (No. 2022GDASZH-2022010106). Higher Education Commission of Pakistan (HEC) (Project No. NRPU-11326).

Acknowledgments

The authors are thankful to Hsin Chi (Laboratory of Theoretical and Applied Ecology, Department of Entomology, National Chung Hsing University, Taiwan) for providing the age-stage, two-sex life-table program.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2023.1088712/full#supplementary-material

References

Afza, R., Afzal, M., Majeed, M. Z., and Riaz, M. A. (2019). Effect of intra-guild predation and sub lethal concentrations of insecticides on the predation of coccinellids. *Pak. J. Zool.* 51, 611–617. doi:10.17582/journal.pjz/2019.51.2.611.617

Afza, R., Riaz, M. A., Afzal, M., and Majeed, M. Z. (2021). Adverse effect of sublethal concentrations of insecticides on the biological parameters and functional response of predatory beetle *Coccinella septempunctata* (Coleoptera: Coccinellidae) of Brassica aphid. *Sarh. J. Agric.* 37, 226–234. doi:10.17582/journal.sja/2021/37.1.226.234

Ahmad, M., Rafiq, M., Arif, M. I., and Sayyed, A. H. (2011). Toxicity of some commonly used insecticides against *Coccinella undecimpunctata* (Coleoptera: Coccinellidae). *Pak. J. Zool.* 43, 1161–1165.

Alkassab, A. T., and Kirchner, W. H. (2016). Impacts of chronic sublethal exposure to clothianidin on winter honeybees. *Ecotoxico. L.* 25, 1000–1010. doi:10.1007/s10646-016-1657-3

Arshad, M., Khan, H. A. A., Hafeez, F., Sherazi, R., and Iqbal, N. (2017). Predatory potential of *Coccinella septempunctata* L. Against four aphid species. *Pak. J. Zool.* 49, 623–627. doi:10.17582/journal.pjz/2017.49.2.623.627

Bianchi, F. J., and Van Der Werf, W. (2003). The effect of the area and configuration of hibernation sites on the control of aphids by *Coccinella septempunctata* (Coleoptera: Coccinellidae) in agricultural landscapes: A simulation study. *Environ. Entomol.* 32, 1290–1304. doi:10.1603/0046-225X-32.6.1290

Bozsik, A. (2006). Susceptibility of adult *Coccinella septempunctata* (Coleoptera: Coccinellidae) to insecticides with different modes of action. *Pest. Manage. Sci.* 62, 651–654. doi:10.1002/ps.1221

Cabral, S., Soares, A. O., and Garcia, P. (2011). Voracity of *Coccinella undecimpunctata*: Effects of insecticides when foraging in a prey/plant system. *J. Pest. Sci.* 84, 373–379. doi:10.1007/s10340-011-0373-2

Chi, H. (1988). Life-table analysis incorporating both sexes and variable development rates among individuals. *Environ. Entomol.* 17, 26–34. doi:10.1093/ee/17.1.26

Costa, L. G. (2015). "The neurotoxicity of organochlorine and pyrethroid pesticides," in *Handbook of clinical neurology*. Editors L. Marcello and L. B. Margit (Amsterdam, Netherlands: Elsevier), 135–148. doi:10.1016/B978-0-444-62627-1.00009-3

Dai, P., Jack, C. J., Mortensen, A. N., Bustamante, T. A., Bloomquist, J. R., and Ellis, J. D. (2019). Chronic toxicity of clothianidin, imidacloprid, chlorpyrifos, and dimethoate to *Apis mellifera L.* Larvae reared *in vitro*. *Pest. Manage. Sci.* 75, 29–36. doi:10.1002/ps.5124

De Castro, A., Corrêa, A., Legaspi, J., Guedes, R., Serrão, J., and Zanuncio, J. (2013). Survival and behavior of the insecticide-exposed predators *podisus nigrispinus* and *supputius cincticeps* (heteroptera: Pentatomidae). *Chemosphere* 93, 1043–1050. doi:10.1016/j.chemosphere.2013.05.075

Desneux, N., Decourtye, A., and Delpuech, J. M. (2007). The sublethal effects of pesticides on beneficial arthropods. *Annu. Rev. Entomol.* 52, 81–106. doi:10.1146/annurev.ento.52.110405.091440

Desneux, N., Pham-Delègue, M. H., and Kaiser, L. (2004). Effects of sub-lethal and lethal doses of lambda-cyhalothrin on oviposition experience and host-searching behaviour of a parasitic wasp. *Aphidius Ervi. Pest. Manage. Sci.* 60, 381–389. doi:10.1002/ps.822

Farooq, A., and Tasawar, Z. (2008). Evaluation of integrated management of aphid pests, *brevicoryne brassicae* and *lipaphis erysimi* on canola crop in southern Punjab, Pakistan. *Pak. J. Zool.* 40, 13–17.

Fernandes, F. L., Bacci, L., and Fernandes, M. S. (2010). Impact and selectivity of insecticides to predators and parasitoids. *Entomo. Bras.* 3, 1–10. doi:10.12741/ebrasilis.v3i1.52

Finney, D. J. (1971). *Probit analysis*. London: Cambridge University Press, 333.

Galvan, T., Koch, R. L., and Hutchison, W. D. (2005). Effects of spinosad and indoxacarb on survival, development, and reproduction of the multicolored asian lady beetle (Coleoptera: Coccinellidae). *Biol. Control.* 34, 108–114. doi:10.1016/j.biocontrol.2005.04.005

Galvan, T. L., Picanço, M. C., Bacci, L., Pereira, E. J. G., and Crespo, A. L. B. (2002). Seletividade de oito inseticidas a predadores de lagartas em citros. *Pesqui. Agropecu. Bras.* 37, 117–122. doi:10.1590/S0100-204X2002000200001

Gholamzadehchitgar, M., Hajizadeh, J., Ghadamyari, M., Karimi-Malati, A., and Hoda, H. (2014). Sublethal effects of diazinon, fenitrothion and chlorpyrifos on the functional response of predatory bug, *andrallus spinidens* fabricius (Hemiptera: Pentatomidae) in the laboratory conditions. *J. King. Saud. Univ-Sci.* 26, 113–118. doi:10.1016/j.jksus.2013.09.001

He, F., Sun, S., Tan, H., Sun, X., Shang, D., Yao, C., et al. (2019). Compatibility of chlorantraniliprole with the generalist predator *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) based toxicity, life-cycle development and population parameters in laboratory microcosms. *Chemosphere* 225, 182–190. doi:10.1016/j.chemosphere.2019.03.025

He, Y., Zhao, J., Zheng, Y., Desneux, N., and Wu, K. (2012). Lethal effect of imidacloprid on the coccinellid predator *Serangium japonicum* and sublethal effects on predator voracity and on functional response to the whitefly *Bemisia tabaci*. *Ecotoxicol* 21, 1291–1300. doi:10.1007/s10646-012-0883-6

Hodek, I., and Michaud, J. (2013). Why is *Coccinella septempunctata* so successful? (a point-of-view). *Eur. J. Entomol.* 105, 1–12. doi:10.14411/eje.2008.001

Jiang, J., Zhang, Z., Yu, X., Yu, C., Liu, F., and Mu, W. (2019). Sublethal and transgenerational effects of thiamethoxam on the demographic fitness and predation performance of the seven-spot ladybeetle *Coccinella septempunctata* L.(Coleoptera: Coccinellidae). *Chemosphere* 216, 168–178. doi:10.1016/j.chemosphere.2018.10.126

Juliano, S. A. (2001). Nonlinear curve fitting: Predation and functional response curves. *Des. Anal. Ecol. Exp.* 2, 178–196.

Kang, Z. W., Liu, F. H., Pang, R. P., Tian, H. G., and Liu, T. X. (2018). Effect of sublethal doses of imidacloprid on the biological performance of aphid endoparasitoid *aphidius gifuensis* (hymenoptera: Aphidiidae) and influence on its related gene expression. *Front. Physiol.* 9, 1729. doi:10.3389/fphys.2018.01729

Liu, J., Huang, W., Chi, H., Wang, C., Hua, H., and Wu, G. (2017). Effects of elevated CO_2 on the fitness and potential population damage of *helicoverpa armigera* based on two-sex life table. *Sci. Rep.* 7, 1119–1213. doi:10.1038/s41598-017-01257-7

Negi, S., Sharma, P. L., Sharma, K. C., and Verma, S. C. (2018). Effect of host plants on developmental and population parameters of invasive leafminer, *tuta absoluta* (meyrick) (Lepidoptera: Gelechiidae). *Phytoparasitica* 46, 213–221. doi:10.1007/s12600-018-0661-y

Noelia, F. M., Scorsetti, A. C., Minardi, G., and Schneider, M. I. (2022). Toxicity assessment of two IGR insecticides on eggs and larvae of the ladybird Eriopis connexa (Coleoptera: Coccinellidae). *Pest Manage.Sci.* doi:10.1002/ps.7293

Poorani, J. (2002). An annotated checklist of the Coccinellidae (Coleoptera) (excluding epilachninae) of the Indian subregion. *Orient. Insects.* 36, 307–383. doi:10.1080/00305316.2002.10417335

Rafi, M., Irshad, M., and Inayatullah, M. (2005). *National insect museum and insect pest informatics, IPM programme*. Islamabad, Pakistan: Rohani Art Press.Predatory Ladybird Beetles of Pakistan.

Rahmani, S., and Bandani, A. R. (2016). Pirimicarb, an aphid selective insecticide, adversely affects demographic parameters of the aphid predator *Hippodamia variegata* (goeze)(Coleoptera: Coccinellidae). *J. Plant. Prot. Res.* 56, 353–363. doi:10.1515/jppr-2016-0048

Saeed, K., Khattak, M. N. K., Khan, F., Naz, F., and Akhtar, N. (2016). Morphological characteristics of ladybird beetles (Coccinellidae: Coleoptera) of district buner, khyber pakhtunkhwa, Pakistan. *Pak. J. Zool.* 48, 1367–1372. doi:10.4172/2161-0983.1000187

Saljoqi, A. U. R., and Ali, S. (2013). Population dynamics of *Aphis gossypii* (glover) and its associated natural enemies in different okra varieties. *Pak. J. Zool.* 45, 1197–1205.

Sarkar, S. C., Milroy, S. P., and Xu, W. (2022). Potential of variegated lady beetle *Hippodamia variegata* in management of invasive tomato potato psyllid *Bactericera cockerelli*. *Pest Manage.Sci.* doi:10.1002/ps.7247

Sarwar, M., and Saqib, S. M. (2010). Rearing of predatory seven spotted ladybird beetle *Coccinella septempunctata* L.(Coleoptera: Coccinellidae) on natural and artificial diets under laboratory conditions. *Pak. J. Zool.* 42, 47–51.

Sarwar, M. (2013). Studies on incidence of insect pests (aphids) and their natural enemies in canola *Brassica napus* L.(Brassicaceae) crop ecosystem. *Int. J. Sci. Res. Environ. Sci.* 1, 78–84. doi:10.12983/ijsres-2013-p078-084

Skouras, P. J., Stathas, G. J., Voudouris, C. C., Darras, A. I., Tsitsipis, J. A., and Margaritopoulos, J. T. (2017). Effect of synthetic insecticides on the larvae of *Coccinella septempunctata* from Greek populations. *Phytoparasitica* 45, 165–173. doi:10.1007/s12600-017-0577-y

Stark, J. D., Vargas, R., and Banks, J. E. (2007). Incorporating ecologically relevant measures of pesticide effect for estimating the compatibility of pesticides and biocontrol agents. *J. Econ. Entomol.* 100, 1027–1032. doi:10.1603/0022-0493(2007)100[1027:iermop]2.0.co;2

Tan, Y., Jia, B., Foster, S. P., Homem, R. A., Williamson, M. S., Han, H. B., et al. (2021). Sublethal and transgenerational effects of lambda-cyhalothrin on the mirid bugs Lygus pratensis Linnaeus and Polymerus cognatus Fieber. *Crop Prot.* 139, 105354. doi:10.1016/j.cropro.2020.105354

Tengfei, L., Yao, W., Lixia, Z., Yongyu, X., Zhengqun, Z., and Wei, M. (2019). Sublethal effects of four insecticides on the seven-spotted lady beetle (Coleoptera: Coccinellidae). *J. Econ. Entomol.* 112, 2177–2185. doi:10.1093/jee/toz146

Tillman, P., and Mulrooney, J. (2000). Effect of selected insecticides on the natural enemies *coleomegilla maculata* and *Hippodamia convergens* (Coleoptera: Coccinellidae), *geocoris punctipes* (Hemiptera: Lygaeidae), and *bracon mellitor, cardiochiles nigriceps*, and *cotesia marginiventris* (hymenoptera: Braconidae) in cotton. *J. Econ. Entomol.* 93, 1638–1643. doi:10.1603/0022-0493-93.6.1638

Urbaneja, A., Pascual-Ruiz, S., Pina, T., Abad-Moyano, R., Vanaclocha, P., Montón, H., et al. (2008). Efficacy of five selected Acaricides against *tetranychus urticae* (Acari: Tetranychidae) and their side effects on relevant natural enemies occurring in citrus orchards. *Pest. Manage. Sci.* 64, 834–842. doi:10.1002/ps.1572

Wu, H. M., Feng, H. L., Wang, G. D., Zhang, L. L., Zulu, L., Liu, Y. H., et al. (2022). Sublethal effects of three insecticides on development and reproduction of spodoptera frugiperda (Lepidoptera: Noctuidae). *Agronomy* 12 (6), 1334. doi:10.3390/agronomy12061334

Xue, Y., Bahlai, C. A., Frewin, A., Sears, M., Schaafsma, A., and Hallett, R. H. (2009). Predation by *Coccinella septempunctata* and *Harmonia axyridis* (Coleoptera: Coccinellidae) on *Aphis glycines* (Homoptera: Aphididae). *Environ. Entomol.* 38, 708–714. doi:10.1603/022.038.0322

Yao, F. L., Zheng, Y., Zhao, J. W., Desneux, N., He, Y. X., and Weng, Q. Y. (2015). Lethal and sublethal effects of thiamethoxam on the whitefly predator *Serangium japonicum* (Coleoptera: Coccinellidae) through different exposure routes. *Chemosphere* 128, 49–55. doi:10.1016/j.chemosphere.2015.01.010

Youn, Y. N., Seo, M., Shin, J., Jang, C., and Yu, Y. (2003). Toxicity of greenhouse pesticides to multicolored asian lady beetles, *Harmonia axyridis* (Coleoptera: Coccinellidae). *Biol. L. Control.* 28, 164–170. doi:10.1016/S1049-9644(03)00098-7

Yu, C., Lin, R., Fu, M., Zhou, Y., Zong, F., Jiang, H., et al. (2014). Impact of imidacloprid on life-cycle development of *Coccinella septempunctata* in laboratory microcosms. *Ecotoxicol. Environ. Safe.* 110, 168–173. doi:10.1016/j.ecoenv.2014.08.022

Zheng, X. M., Tao, Y. L., Chi, H., Wan, F. H., and Chu, D. (2017). Adaptability of small Brown planthopper to four rice cultivars using life table and population projection method. *Sci. Rep.* 7, 42399. doi:10.1038/srep42399

frontiers | Frontiers in Physiology

TYPE Original Research
PUBLISHED 03 February 2023
DOI 10.3389/fphys.2023.1113464

OPEN ACCESS

EDITED BY
Ting Li,
Alabama State University, United States

REVIEWED BY
Jianhong Li,
Huazhong Agricultural University, China
Xinzheng Huang,
China Agricultural University, China

*CORRESPONDENCE
Yunhui Zhang,
yhzhang@ippcaas.cn

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Invertebrate Physiology, a section of the journal Frontiers in Physiology

RECEIVED 01 December 2022
ACCEPTED 25 January 2023
PUBLISHED 03 February 2023

CITATION
Li X, Li Y, Zhu X, Li X, Cheng D and Zhang Y (2023), Effects of imidacloprid-induced hormesis on the development and reproduction of the rose-grain aphid *Metopolophium dirhodum* (Hemiptera: Aphididae).
Front. Physiol. 14:1113464.
doi: 10.3389/fphys.2023.1113464

Effects of imidacloprid-induced hormesis on the development and reproduction of the rose-grain aphid *Metopolophium dirhodum* (Hemiptera: Aphididae)

Xinan Li[1,2†], Yaping Li[1†], Xun Zhu[1,3†], Xiangrui Li[1], Dengfa Cheng[1,3] and Yunhui Zhang[1,3*]

[1]State Key Laboratory for Biology of Plant Disease and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China, [2]School of Resource and Environmental Sciences, Henan Institute of Science and Technology, Xinxiang, China, [3]Scientific Observing and Experimental Station of Crop Pests in Guilin, Ministry of Agriculture, Guilin, China

Field populations of insect pests are affected by sub-lethal doses of insecticides, leading to hormesis. Imidacloprid is a neonicotinoid insecticide widely used to control various sucking insect pests, including aphids. In this study, the effects of sub-lethal concentrations of imidacloprid on the life table traits of the rose-grain aphid *Metopolophium dirhodum* (Walker) were evaluated on parental and first filial generations. The results showed that sub-lethal concentrations of imidacloprid significantly reduced the fecundity, adult longevity, and reproductive period of *M. dirhodum* in parental generation (F_0). However, the imidacloprid-induced hormetic effects on development and reproduction were detected in the F_1 generation. These hormetic effects were indicated by significantly higher adult longevity, fecundity, survival rate, intrinsic and finite rates of increase, and net reproductive rate of first filial generation (F_1) of *M. dirhodum*. Our finding indicated that the application of sub-lethal concentrations of imidacloprid inhibited parental generation (F_0), but it significantly stimulated the population growth of filial generation (F_1) in the *M. dirhodum*. The results support the inclusion of insecticides in integrated pest management programs for managing wheat aphids.

KEYWORDS

hormesis, imidacloprid, *Metopolophium dirhodum*, longevity, fecundity

Introduction

The insecticide concentration initially applied for managing insect pests degrades with the passage of time (Desneux and Fauvergue, 2005). The insect pests are eventually exposed to these time driven sub-lethal insecticide concentrations (Desneux et al., 2007). This exposure may affect their current and future generations. These sub-lethal concentrations induce physiological and behavioral changes in individuals that survive the initial pesticide exposure (Desneux et al., 2007). In some insects, sub-lethal pesticide exposure adversely affect survival, longevity, fecundity, developmental time, neurophysiological processes, biochemistry, immune capacity (Guo et al., 2013; Ceuppens et al., 2015; Ma et al., 2022), and even induce multiple behaviours (Samuelson et al., 2016). Sub-lethal insecticidal doses may also increase pest infestation by stimulating the growth and reproduction of arthropods (Cordeiro et al., 2013), including green peach aphid *Myzus persicae* (Tang et al., 2015; Tang et al., 2019), English grain aphid *Sitobion avenae*, bird

cherry-oat aphid *Rhopalosiphum padi* (Xin et al., 2019), and cotton aphid *Aphis gossypii* (Ullah et al., 2020).

The biphasic dose-responsive adaptive response (i.e., hormesis) is characterized by low-dose stimulations and high-dose inhibitions (Guedes and Cutler, 2014). Hormetic responses have been detected in various organisms, including insects exposed to insecticides (Calabrese and Baldwin, 2001). The general overcompensation for a disruption in homeostasis (e.g., toxicity) is one of the mechanisms underlying hormesis (Guedes and Cutler, 2014). Additionally, hormesis is an essential consideration when evaluating the impact of pesticides in insect pest management strategies as it may also lead to pest resurgence (Shi et al., 2011; Chen et al., 2015).

Wheat aphids are one of the most harmful insect pests, which adversely affects grain crop production, and are responsible for considerable economic losses (Chopa and Descamps, 2012). The rose-grain aphid, *Metopolophium dirhodum* Walker (Hemiptera: Aphididae) is a major wheat aphid species that substantially lowers the productivity of cultivated winter cereals (Cannon, 1986; Ma et al., 2003). It was first detected in the 1970s in crops cultivated in South America. Since then, it has spread to new areas worldwide as an important pest of wheat (Chopa and Descamps, 2012; Abdelaziz et al., 2018; Honek et al., 2018). The nymphs and adults of this aphid, feed on phloem fluids from wheat plants during the wheat seedling and jointing stages. Aphid feeding exacerbates nutrient deficiency in wheat plant, resulting in the low grain production. Additionally, the aphids secrete honeydew, which can cover the leaf surface, and then hinders plant respiration and photosynthesis, ultimately leading to low-quality wheat and yield losses up to 27%–30% (Cannon, 1986). Also, the rose-grain aphid may function as a vector for several plant viruses that can damage cereal crops, especially the barley yellow dwarf virus (*Luteovirus*) (Chopa and Descamps, 2012).

Synthetic insecticides have a key role in modern pest management. Imidacloprid is the first commercially available systemic neonicotinoid insecticide. Its belongs to IRAC class 4A (nicotinic acetylcholine receptor (nAChR) competitive modulators) and blocks the nicotinic acetylcholine receptors in the central and peripheral nervous systems of insects (Palumbo et al., 2001; Byrne et al., 2003; Fernandez et al., 2009). Because of its long-lasting efficacy against diverse homopterous insect pests and relatively low toxicity to non-target organisms, imidacloprid has been commonly used to control insect pests such as *M. persicae*, *Bemisia tabaci* and *Nilaparvata lugens* (Palumbo et al., 2001; Byrne et al., 2003; Liu and Han, 2006). It has also been used in seed treatments for the long-term control of residual wheat aphids (Ahmed et al., 2001). Imidacloprid-induced pest resurgence, including those due to hormesis, have been reported for many insect pests such as the *M. persicae* (Yu et al., 2010) and *A. gossypii* (Ullah et al., 2019). However, the possible effects of sub-lethal doses of imidacloprid on *M. dirhodum* remain relatively unknown. In the present study, the sub-lethal effects of imidacloprid on *M. dirhodum* were investigated based on two-sex life tables, with a focus on transgenerational effects.

Materials and methods

Aphid rearing

The *M. dirhodum* population used in this research was sampled in 2016 from wheat fields located at the Pest Sciences Observation and Testing station in Haidian district, Beijing, China. After field collection, the aphids were maintained on young winter wheat (*Triticum aestivum* Linnaeus) plants in a growth chamber set at 18°C ± 1°C and 60% ± 10% relative humidity, with a 16-h light/8-h dark photoperiod. The aphids were not exposed to any insecticides prior to this study.

Imidacloprid application

Imidacloprid (Gaucho® 600FS) (97.4%, technical grade) was obtained from Bayer Crop Science (Beijing, China). A stock solution was prepared in acetone (Beijing Chemical Works, China) and diluted with water containing 0.05% (w/v) Triton X-100 (Beijing Solarbio Science and Technology Co., Ltd., China) to concentrations appropriate for generating dose-response curves between 0% and 100% *M. dirhodum* mortality.

Bioassay

A wheat seedling dipping method was used in this study (Gong et al., 2020). Briefly, the imidacloprid stock solution was diluted with distilled water containing 0.05% (w/v) Triton-X to generate nine treatment solutions (i.e., 12.5, 25, 50, 200, 600, 1,200, 1,500, 1,800, and 2,000 mg/L). Wheat seedlings were dipped in the prepared imidacloprid solutions or distilled water containing 0.05% (w/v) Triton-X (control) for 10 s. The seedling roots were wrapped with moistened cotton. The seedlings were then air-dried at room temperature and then placed in 500-mL plastic plates, each containing approximately 10 *M. dirhodum* adults. The treated and control insects were incubated in a plant growth chamber maintained at 18°C ± 1°C and 60% ± 10% relative humidity, with a 16-h light/8-h dark photoperiod. Insect mortality was recorded after 24 h. If no more than two legs moved in response to a slight touch with a soft brush, the individual was considered dead. The imidacloprid treatments along with the control treatment were replicated ten times.

Life table analysis

A life table study was completed with 50, 100, and 200 mg/L imidacloprid solutions (prepared in distilled water) and water containing 0.05% (w/v) Triton X-100 as the control. Wheat seedlings were dipped in the imidacloprid or control solutions for 10 s. A cohort of 90 third instar nymphs was treated with imidacloprid as described in bioassay section. After 24 h, the surviving aphids were individually transferred to pots (10 × 10 × 10 cm) containing young winter wheat plants. The short-term (i.e., in the parental generation) sub-lethal effects of imidacloprid on the treated *M. dirhodum* (F_0) fecundity and survival were determined. The number of new born nymphs was recorded and the offspring were removed daily until death. The long-term (i.e., in the filial generation) sub-lethal effects of imidacloprid on *M. dirhodum* (F_1) life table parameters were also examined, including the developmental period, life span, survival rate and fecundity. Accordingly, the life table data of the filial generation were recorded. For each treated female, one

10.3389/fphys.2023.1113464

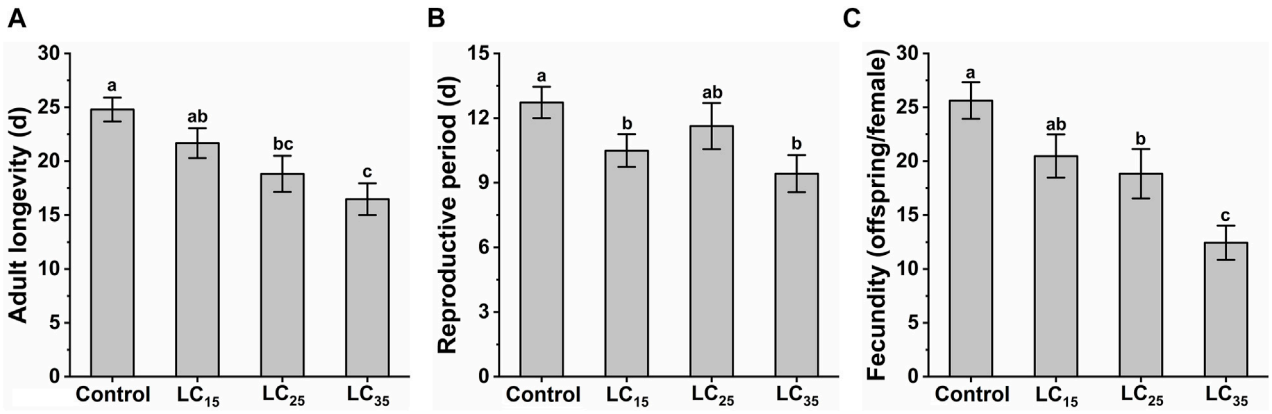


FIGURE 1
The adult longevity **(A)**, reproductive period **(B)** and fecundity **(C)** of *Metopolophium dirhodum* F_0 generation under control conditions, treated with 50 mg/L of imidacloprid (LC_{15}), 100 mg/L of imidacloprid (LC_{25}) and 200 mg/L of imidacloprid (LC_{35}).

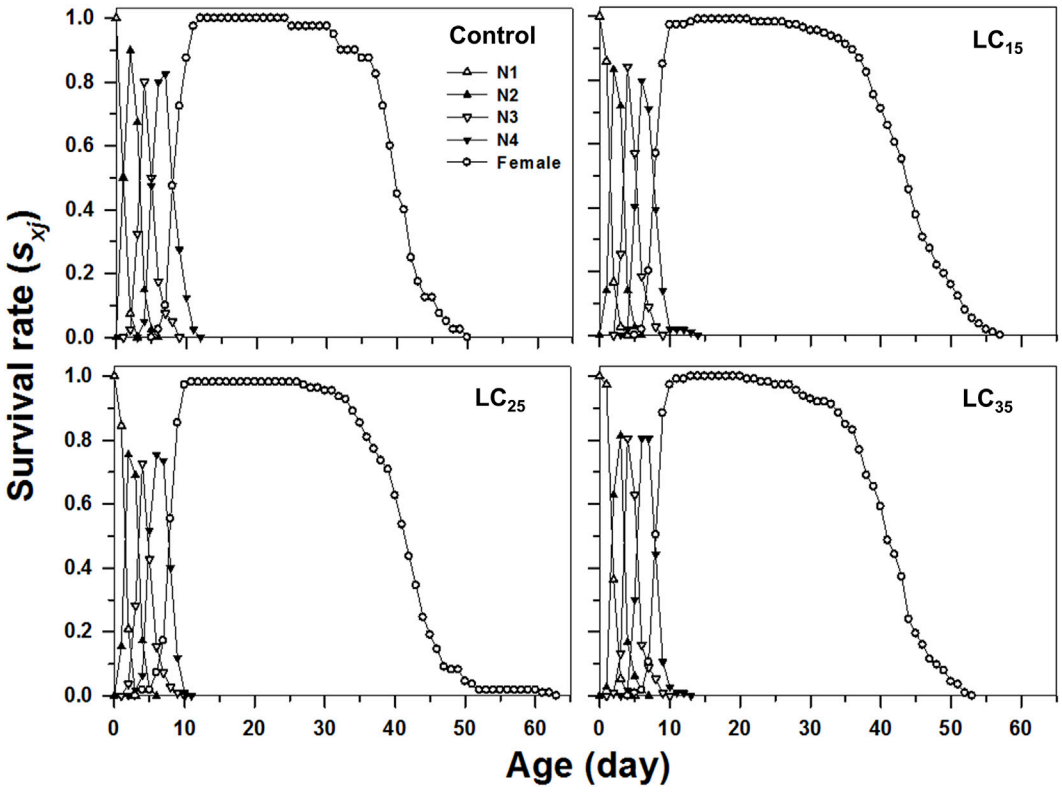


FIGURE 2
Age-stage-specifc survival rates (s_{xj}) of *Metopolophium dirhodum* individuals of the F_1 generation under control conditions, treated with 50 mg/L of imidacloprid (LC_{15}), 100 mg/L of imidacloprid (LC_{25}) and 200 mg/L of imidacloprid (LC_{35}).

or two new born nymphs produced overnight were transferred to a new wheat plant, i.e., 100–120 newborn nymphs (≤24-h old) were observed individually in each group. The number of new-born nymphs was recorded and the offspring were removed daily until death. In each experiment, the aphids were transferred to new plants every 3–4 days.

Data analyses

Corrected aphid mortality was calculated using Abbott's formula. The LC_{15}, LC_{25}, LC_{35}, were calculated with 95% confidence interval (95% CI), and slope were calculated by a probit analysis using the DPS software (version 7.05). The raw life history data for the *M. dirhodum* F_0 and F_1 were

10.3389/fphys.2023.1113464

TABLE 1 The sub-lethal effects of imidacloprid on developmental duration and fecundity of the F_1 generation of *Metopolophium dirhodum*.

Parameter[a]	Control	Imidacloprid leaf treatment (Mean ± SE)		
		LC_{15}	LC_{25}	LC_{35}
N1 (d)	1.58 ± 0.10 c	2.05 ± 0.06 b	2.05 ± 0.06 b	2.40 ± 0.06 a
N2 (d)	2.25 ± 0.10 a	1.86 ± 0.06 b	1.81 ± 0.06 b	1.72 ± 0.06 b
N3 (d)	1.95 ± 0.12 a	1.96 ± 0.08 a	1.77 ± 0.08 a	1.88 ± 0.07 a
N4 (d)	3.05 ± 0.13 a	2.54 ± 0.09 b	2.67 ± 0.09 b	2.52 ± 0.08 b
Pre-adult (d)	8.82 ± 0.20 a	8.41 ± 0.11 ab	8.29 ± 0.11 b	8.52 ± 0.10 ab
Adult longevity (d)	31.27 ± 0.75 c	35.42 ± 0.61 a	33.47 ± 0.56 b	32.47 ± 0.59 bc
Total longevity (d)	40.09 ± 0.73 b	43.51 ± 0.66 a	41.07 ± 0.72 b	40.99 ± 0.57 b
APRP (d)	1.55 ± 0.12 a	1.23 ± 0.12 ab	1.02 ± 0.08 bc	0.88 ± 0.07 c
TPRP (d)	10.37 ± 0.23 a	9.64 ± 0.17 b	9.31 ± 0.13 b	9.40 ± 0.10 b
Reproductive period (d)	17.35 ± 0.78 a	17.32 ± 0.59 a	18.66 ± 0.58 a	17.62 ± 0.56 a
Fecundity (nymphs per female)	40.39 ± 2.44 b	42.39 ± 1.95 ab	47.60 ± 2.05 a	45.68 ± 2.01 ab

[a]N1, first nymph stage; N2, second nymph stage; N3, third nymph stage; N4, fourth nymph stage; Pre-adult, complete nymph stage; APRP, adult pre-reproductive period; TPRP, total pre-reproductive period. Data in the table are represented as mean ± SE, estimated with bootstrapping (100,000). Different letters in the same row indicated significantly different ($p < 0.05$) by the paired bootstrap test.

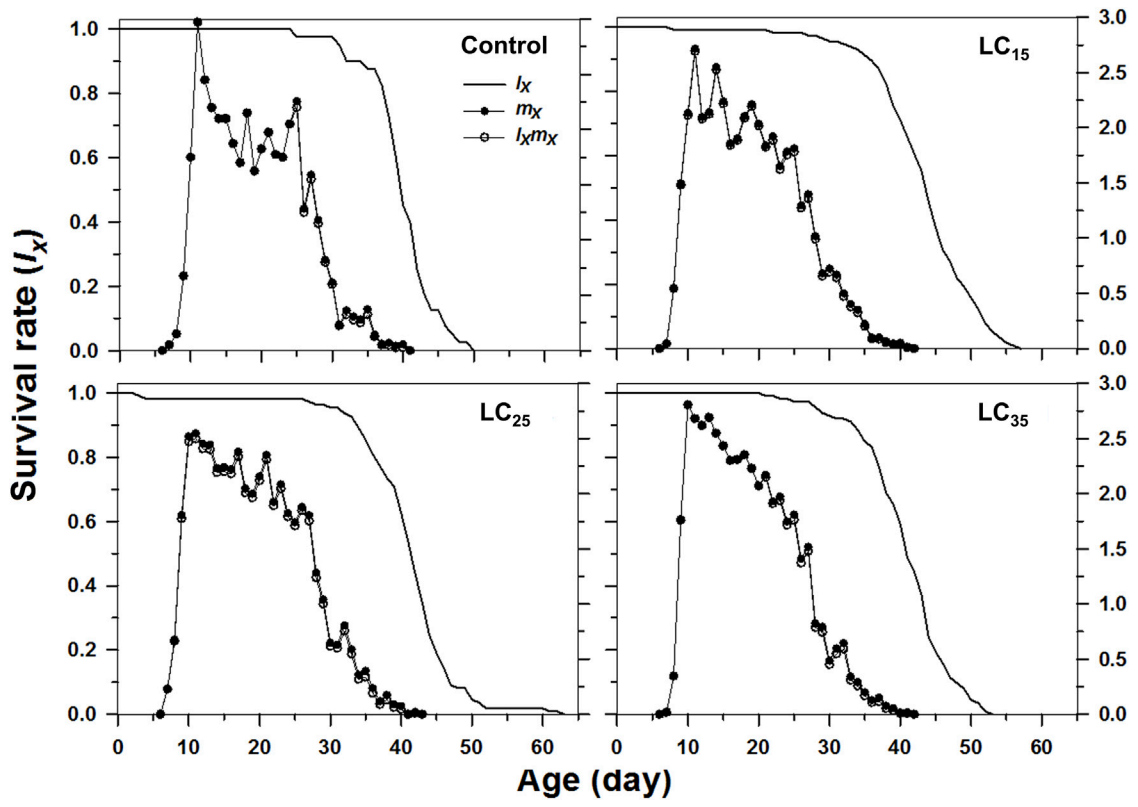


FIGURE 3
Age-specifc survival rates (l_x), age-specifc fecundity (m_x), and net maternity (l_xm_x) of *Metopolophium dirhodum* individuals of the F1 generation under control conditions, treated with 50 mg/L of imidacloprid (LC_{15}), 100 mg/L of imidacloprid (LC_{25}) and 200 mg/L of imidacloprid (LC_{35}).

evaluated with the TWOSEX-MSChart program (Chi, 2022b), which is based on an age-stage, two-sex life table (Chi, 1988). A bootstrap test with a sample size of 100,000 was completed to detect differences among the means and standard errors of the populations and minimize the variation in the results (Efron and Tibshirani, 1993). To estimate the total population growth, the analysis of the initial *M. dirhodum* population (50 newborn

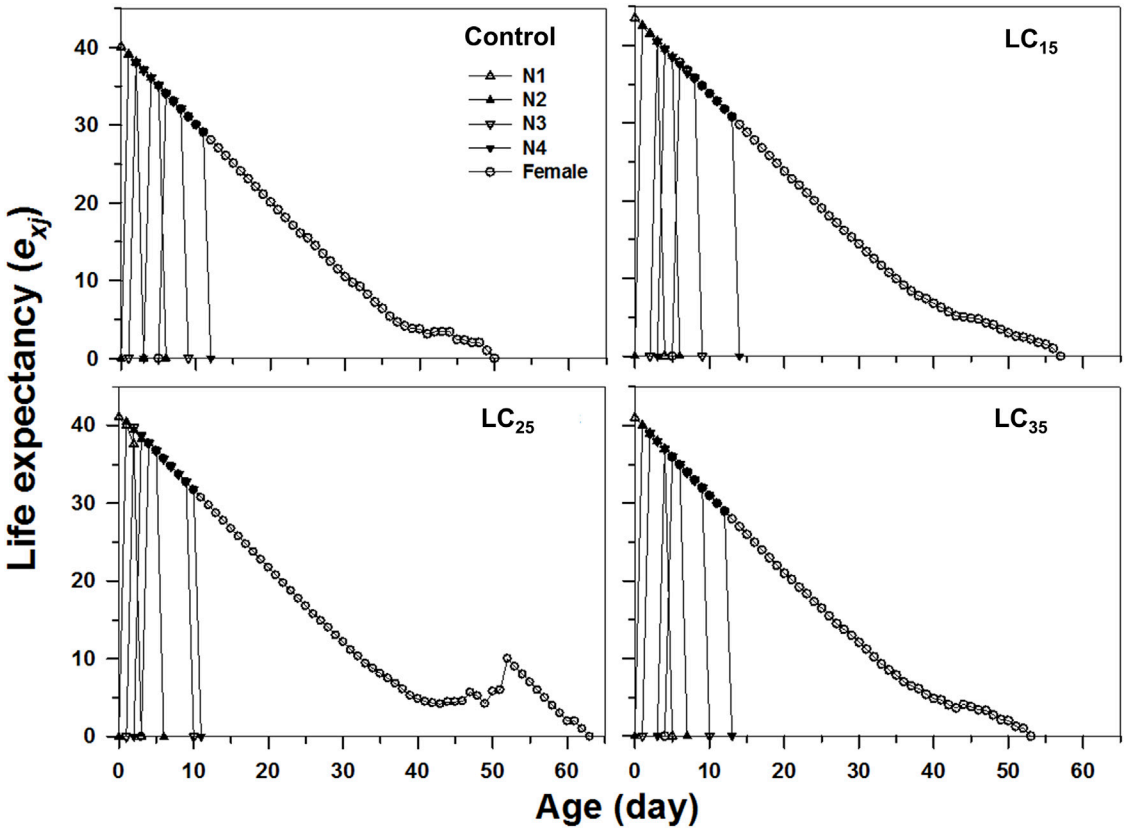


FIGURE 4
Age-stage-specifc life expectancy (e_{xj}) of *Metopolophium dirhodum* individuals of the F1 generation under control conditions, treated with 50 mg/L of imidacloprid (LC_{15}), 100 mg/L of imidacloprid (LC_{25}) and 200 mg/L of imidacloprid (LC_{35}).

TABLE 2 Sub-lethal effects of imidacloprid on population parameters of the F_1 generation of *Metopolophium dirhodum*.

Parameter[a]	Control	Imidacloprid leaf treatment (Mean ± SE)		
		LC_{15}	LC_{25}	LC_{35}
Intrinsic rate of increase/*r*	0.2243 ± 0.0051 b	0.2347 ± 0.0036 ab	0.2433 ± 0.0041 a	0.2435 ± 0.0032 a
Finite rate of increase/λ	1.2514 ± 0.0064 b	1.2646 ± 0.0045 ab	1.2755 ± 0.0052 a	1.2757 ± 0.0041 a
Net reproductive rate/R_0	40.40 ± 2.43 b	42.02 ± 1.97 ab	46.73 ± 2.10 a	45.68 ± 2.01 ab
Mean generation time/*T*	16.48 ± 0.25 a	15.92 ± 0.17 ab	15.80 ± 0.19 b	15.69 ± 0.14 b

[a]Data in the table are represented as mean ± SE, estimated with bootstrapping (100,000). Different letters in the same row indicated significantly different ($p < 0.05$) by the paired bootstrap test.

nymphs) was projected to 60 days based on the above data with the TIMING-MSChart program (Chi, 2022a).

Results

The sub-lethal concentrations of imidacloprid against *M. dirhodum*

The toxicity of imidacloprid to the *M. dirhodum* adults was determined, the results show that the estimated LC_{15}, LC_{25}, LC_{35}, and LC_{50} values were 53.936 mg/L (95% CI: 22.867–94.934 mg/L), 114.734 mg/L (95% CI: 59.716–182.705 mg/L), 209.699 mg/L (95% CI: 124.736–317.737 mg/L), and 380 mg/L (95% CI: 274–852 mg/L), respectively. Finally, 50, 100, and 200 mg/L imidacloprid were used as the sub-lethal concentrations (i.e., LC_{15}, LC_{25}, and LC_{35}, respectively) in subsequent experiments. The effects of these concentrations on third instar nymphs were evaluated. At 24 h after the 50, 100, and 200 mg/L imidacloprid treatments, the aphid mortality rates were 15% ± 0.925%, 24% ± 0.845%, and 35% ± 1.352%, respectively.

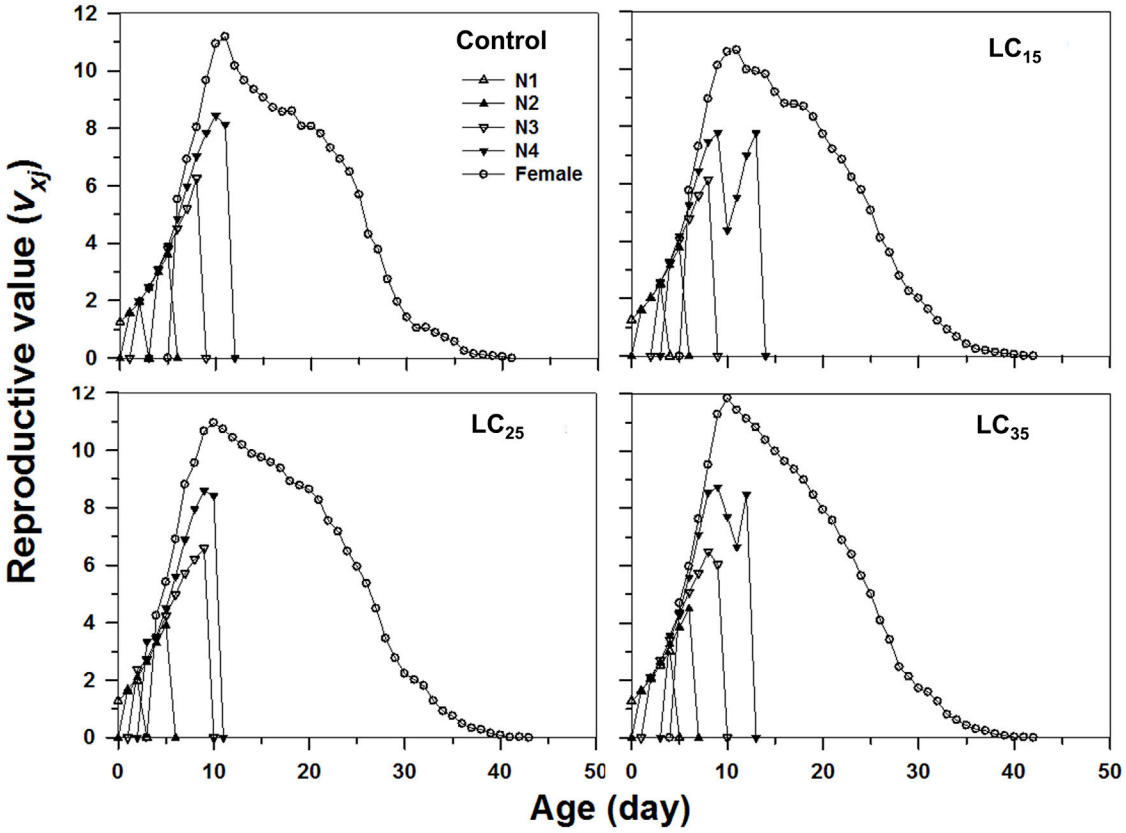


FIGURE 5
Age-stage-specifc reproductive value (v_{xj}) of *Metopolophium dirhodum* individuals of the F1 generation under control conditions, treated with 50 mg/L of imidacloprid (LC_{15}), 100 mg/L of imidacloprid (LC_{25}) and 200 mg/L of imidacloprid (LC_{35}).

The sub-lethal effects of imidacloprid on *M. dirhodum* parental generation (F_0)

The *M. dirhodum* third instar nymphs were treated with three sub-lethal concentrations of imidacloprid (50, 100, and 200 mg/L). The effects of different sub-lethal doses of imidacloprid on adult longevity, reproductive period and fecundity of *M. dirhodum* F_0 are shown in Figure 1. The results show that the sub-lethal dose of imidacloprid had a significant negative effect on the adult longevity, reproductive period and fecundity of *M. dirhodum* F_0, which showed a trend of significant decrease with the increase of the concentrations of imidacloprid.

Transgenerational sub-lethal effects of imidacloprid on the developmental duration, longevity and fecundity of the first filial generation (F_1)

The developmental duration, longevity and fecundity for the *M. dirhodum* F_1 whose parents were treated with three sub-lethal concentrations of imidacloprid are presented in Table 1. The filial *M. dirhodum* aphids (first to fourth instars) developed rapidly following all treatments. Moreover, except for the third instar nymphs, the development was significantly negatively (first instars nymphs) and positively (second and fourth instar nymphs) affected by the three sub-lethal concentrations of imidacloprid. The pre-adult stage duration of F_1 individuals tended to decrease, which was in contrast to the increase in the average adult stage duration following the imidacloprid treatments (relative to the effects of the control solution). The adult and total longevity of the F_1 individuals was highest in response to the 50 mg/L imidacloprid treatment (LC_{15}). The adult pre-reproductive period (APRP) and total pre-reproductive period (TPRP) was significantly reduced by the imidacloprid treatments, but the reproductive period was not significantly different between imidacloprid and the control treatments. Also, the fecundity of the F_1 individuals was highest in response to the 100 mg/L imidacloprid treatment (LC_{25}).

Transgenerational sub-lethal effects of the imidacloprid on survival rates, life expectancy and reproductive value of the *M. dirhodum* F_1 generation

The curves of age-stage specific survival rates (s_{xj}) reflect the high age-stage-specific survival rates for the females following all treatments, however, the imidacloprid-treated *M. dirhodum* F_1 generation had a higher survival rate than that of the control in later stages (Figure 2). The age-specific survival rate (l_x), the age-specific fecundity (m_x), and the age-specific maternity (l_xm_x) reflected the effects of the increasing imidacloprid concentrations on the *M. dirhodum* population over time, which indicate that these parameters

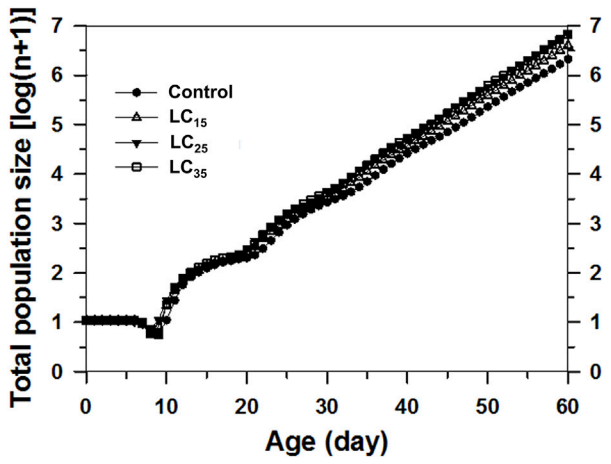


FIGURE 6
Comparison of population projections for *Metopolophium dirhodum* whose parental females treated with three concentrations of imidacloprid or as the control, based on the age-stage, two-sex life table. The regression formulas describe the linear population growth of each cohort from day 40 onwards as the population approached the stable age-stage distribution.

were higher in different doses of imidacloprid treated groups of aphids middle and later stage (Figure 3).

The age-stage specific life expectancy (e_{xj}) refers to the predicted survival time of an individual at age x and stage j. Compared with control, the F_1 individuals produced by F_0 imidacloprid-treated had a higher life expectancy (Figure 4). Moreover, newborn *M. dirhodum* nymphs were expected to live for 57, 63, and 53 days following the 50, 100, and 200 mg/L imidacloprid treatments, respectively, for only 50 days in response to the control treatment (Figure 4). An analysis of the *M. dirhodum* age-stage-specific reproductive rate (v_{xj}) following each imidacloprid treatment revealed that it was highest for LC_{35} (day 10) than for the control (day 12), as well as for LC_{15} and LC_{25} (day 11) (Figure 5).

The effects of the imidacloprid on population parameters and total population size of the *M. dirhodum* F_1 generation

Population life-table parameters of the control and imidacloprid-treated *M. dirhodum* F_1 generation are listed in Table 2. The data presented in this table shows that the imidacloprid treatments decreased the mean generation time (T) of *M. dirhodum* F_1 generation. However, increases in imidacloprid concentrations significantly increased the net reproductive rate (R_0), intrinsic rate of increase (r) and finite rate of increase (λ), indicated that all imidacloprid treatments induced a rapid *M. dirhodum* population growth.

The projected population was analysed based on the age-stage, two-sex life table and the data for F_1 (Figure 6). The control population on day 60 was expected to reach approximately 2.47 million aphids. In contrast, the 50 (LC_{15}), 100 (LC_{25}), and 200 (LC_{35}) mg/L imidacloprid treatments on day 60 were predicted to result in 4.72, 7.81, and 7.80 million aphids, respectively. Forty days later, the *M. dirhodum* population growth curves calculated on a logarithmic scale were nearly linear, implying that *M. dirhodum* populations were approaching the stable age-stage distribution. Such linear population increases are indicated by the slopes of the regression lines, which are equal to $\log(\lambda)$ for each cohort. These results suggest that an exposure to sub-lethal concentrations of imidacloprid induced *M. dirhodum* population growth.

Discussion

Imidacloprid is a typical neonicotinoid insecticide widely used to control various sucking pests, including aphids (Palumbo et al., 2001; Byrne et al., 2003; Fernandez et al., 2009) Target and non-target arthropods are often exposed to low insecticide concentrations in treated fields. The potential sub-lethal effects of insecticides should be evaluated while developing effective integrated pest management programs (Desneux et al., 2007; Guedes et al., 2016). Assessment of sub-lethal insecticidal effects on target insects is critical for enhancing the pesticide efficiency (Liang et al., 2012; Xiao et al., 2015). The primary objective of the current study was to clarify the sub-lethal effects of imidacloprid on the development of the *M. dirhodum* population.

Insecticide-induced hormesis may result in pest resurgence and/or secondary pest outbreaks, which may necessitate additional pesticide applications and a steady accumulation of potentially harmful chemicals in the field (Cordeiro et al., 2013; Guedes et al., 2016). Hormesis due to insecticide applications has been observed in other aphid species, including *M. persicae* treated with low imidacloprid concentrations (Christopher Cutler et al., 2009; Murali-Mohan et al., 2013) and *A. gossypii* exposed to sulfoxaflor, pirimicarb, and flonicamid (Koo et al., 2015; Xiao et al., 2015; Chen et al., 2016).

Earlier investigations indicated that treatments with sub-lethal doses of thiamethoxam adversely affect *Hippodamia variegate* and *Bradysia odoriphaga* population growth (Rahmani and Bandani, 2013; Zhang et al., 2014). In the current study, the sub-lethal dose of imidacloprid had also a significant adverse effect on *M. dirhodum* F_0 generation, indicating that the stimulatory (hormetic) effects were not observed in F_0 individuals treated with low concentrations of imidacloprid. This is consistent with the reported lack of hormetic effects in the F_0 generations of *A. gossypii* exposed to a sub-lethal concentration of afidopyropen and sulfoxaflor (Chen et al., 2016; Ma et al., 2022) and an imidacloprid-resistant *A. gossypii* strain treated with a low lethal concentration of imidacloprid (Shi et al., 2011). Similar effects were also observed in *M. persicae*, *B. brassicae*, *B. tabaci*, and *Apolygus lucorum* (Meyer-Dür) treated with sub-lethal doses of imidacloprid (Lashkari et al., 2007; Wang et al., 2008; Tan et al., 2012; He et al., 2013).

In this study, assessing the transgenerational effects of imidacloprid treatments on *M. dirhodum*, we observed that an exposure to sub-lethal concentrations of imidacloprid in the F_0 generation (i.e., parent generation) significantly stimulated the population growth of the F_1 generation, the analysed life table parameters (r, λ, and R_0), fecundity, longevity, and survival of the F_1 generation were positively affected by the sub-lethal concentrations of imidacloprid treatments. Additionally, the population prediction results show that an exposure to sub-lethal concentrations of imidacloprid induced *M. dirhodum* population growth. Similar effects on population growth were observed in an earlier study on sweetpotato whitefly *B. tabaci*, which proved that imidacloprid

increases the gross reproduction rate, but does not significantly affect the mean generation time (Esmaeily et al., 2014). Another study involving field experiments in Australian revealed that the egg production and population development of the Australian predatory mite *Amblyseius victoriensis* (Womersley) significantly increase in response to systemic spray treatments of imidacloprid (James, 1997). Other investigations confirmed that a low dose of imidacloprid shortens the mean generation time of cabbage aphid *Brevicoryne brassicae* (Lashkari et al., 2007), whereas a sub-lethal imidacloprid concentration significantly extends the mean generation time of *B. tabaci*, while also increasing fecundity and egg production (Sohrabi et al., 2011). Furthermore, the systemic application of imidacloprid to control psyllids on pear trees reportedly increase the fecundity of mite populations (James and Price, 2002).

The stimulated reproduction of insects exposed to low lethal concentration of insecticides is due to hormesis (Guedes and Cutler, 2014). The imidacloprid or one of its metabolites might have altered aphid physiology, with potential consequences for reproduction and population growth. There are reports describing the increased fecundity in *Tetranychus urticae*, *A. victoriensis*, and *Tryporyza incertulas* due to low imidacloprid concentrations (James and Price, 2002; Wang et al., 2005). Additionally, the exposure of *M. persicae* to sub-lethal doses of imidacloprid was observed to result in a hormetic effect on fecundity in the F_2 generation (Christopher Cutler et al., 2009). In this study, the hormetic effects of imidacloprid on the life table parameters of *M. dirhodum* were detected in F_1 generation, and fecundity was highest for LC_{25} and the adult longevity was longest for LC_{15}. When the insecticide concentration is too low, the overcorrection is not triggered or it is not discernible (Stebbing, 2003). These results imply that the development of hormetic effects may be influenced by time and the imidacloprid concentration, and the complex mechanism underlying the imidacloprid dose effect on the occurrence of hormesis.

In conclusion, our findings indicated that the application of sub-lethal concentrations of imidacloprid has inhibitory effects on the parental generation (F_0), but has stimulatory effects on the first filial generation (F_1). Considering all of the arthropod biological processes affected by pesticides, it is possible that hormetic responses are induced in arthropods exposed to these chemicals (Kendig et al., 2010; Liang et al., 2012). Future research should examine the effects of various low lethal and sub-lethal insecticide concentrations to comprehensively characterize the putative hormetic responses of wheat aphid pests to neonicotinoid insecticides. The results of this research may be relevant for optimizing integrated pest management strategies involving neonicotinoid insecticides.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

YZ and DC conceived and designed the research. XnL and YL conducted the experiments. XZ analysed the data. XnL and XZ wrote the manuscript. XiL and DC revised the manuscript. All authors have read and approved the manuscript.

Funding

This study was supported by the China Agriculture Research System of MOF and MARA (CARS-03).

Acknowledgments

We thank Liwen Bianji, Edanz Editing China (www.liwenbianji.cn/ac) for editing the English text of a draft of this manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Abdelaziz, O., Senoussi, M. M., Oufroukh, A., Birgücü, A. K., Karaca, İ., Kouadri, F., et al. (2018). Pathogenicity of three entomopathogenic fungi, to the aphid species, *Metopolophium dirhodum* (Walker) (Hemiptera: Aphididae), and their Alkaline protease activities. *Egypt. J. Biol. Pest Co.* 28, 24. doi:10.1186/s41938-018-0030-7

Ahmed, N. E., Kanan, H. O., Inanaga, S., Ma, Y. Q., and Sugimoto, Y. (2001). Impact of pesticide seed treatments on aphid control and yield of wheat in the Sudan. *Crop Prot.* 20, 929–934. doi:10.1016/S0261-2194(01)00047-3

Byrne, F. J., Castle, S., Prabhaker, N., and Toscano, N. C. (2003). Biochemical study of resistance to imidacloprid in B biotype *Bemisia tabaci* from Guatemala. *Pest Manag. Sci.* 59, 347–352. doi:10.1002/ps.649

Calabrese, E. J., and Baldwin, L. A. (2001). Hormesis: A generalizable and unifying hypothesis. *Crit. Rev. Toxicol.* 31, 353–424. doi:10.1080/20014091111730

Cannon, R. J. C. (1986). Summer populations of the cereal aphid Metopolophium dirhodum (walker) on winter wheat: Three contrasting years. *J. Appl. Ecol.* 23, 101–114. doi:10.2307/2403084

Ceuppens, B., Eeraerts, M., Vleugels, T., Cnops, G., Roldan-Ruiz, I., and Smagghe, G. (2015). Effects of dietary lambda-cyhalothrin exposure on bumblebee survival, reproduction, and foraging behavior in laboratory and greenhouse. *J. Pest Sci.* 88, 777–783. doi:10.1007/s10340-015-0676-9

Chen, X., Ma, K., Li, F., Liang, P., Liu, Y., Guo, T., et al. (2016). Sublethal and transgenerational effects of sulfoxaflor on the biological traits of the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae). *Ecotoxicology* 25, 1841–1848. doi:10.1007/s10646-016-1732-9

Chen, Z., Qu, Y., Xiao, D., Song, L., Zhang, S., Gao, X., et al. (2015). Lethal and social-mediated effects of ten insecticides on the subterranean termite *Reticulitermes speratus*. *J. Pest Sci.* 88, 741–751. doi:10.1007/s10340-015-0656-0

Chi, H. (1988). Life-table analysis incorporating both sexes and variable development rates among individuals. *Environ. Entomol.* 17, 26–34. doi:10.1093/ee/17.1.26

Chi, H. (2022a). *TIMING-MSChart-exe.rar*. Available at: http://140.120.197.173/Ecology/prod102.htm.

Chi, H. (2022b). *TWOSEX-MSChart-exe-B100000*. Available at: http://140.120.197.173/Ecology/prod102. htm.

Chopa, C. S., and Descamps, L. R. (2012). Composition and biological activity of essential oils against *Metopolophium dirhodum* (Hemiptera: Aphididae) cereal crop pest. *Pest Manag. Sci.* 68, 1492–1500. doi:10.1002/ps.3334

Christopher Cutler, G., Ramanaidu, K., Astatkie, T., and Isman, M. B. (2009). Green peach aphid, *Myzus persicae* (Hemiptera: Aphididae), reproduction during exposure to sublethal concentrations of imidacloprid and azadirachtin. *Pest Manag. Sci.* 65, 205–209. doi:10.1002/ps.1669

Cordeiro, E. M., de Moura, I. L., Fadini, M. A., and Guedes, R. N. (2013). Beyond selectivity: Are behavioral avoidance and hormesis likely causes of pyrethroid-induced outbreaks of the southern red mite *Oligonychus ilicis*? *Chemosphere* 93, 1111–1116. doi:10.1016/j.chemosphere.2013.06.030

Desneux, N., Decourtye, A., and Delpuech, J. M. (2007). The sublethal effects of pesticides on beneficial arthropods. *Annu. Rev. Entomol.* 52, 81–106. doi:10.1146/annurev.ento.52.110405.091440

Desneux, N., Fauvergue, X., Dechaume-Moncharmont, F. X., Kerhoas, L., Ballanger, Y., and Kaiser, L. (2005). Diaeretiella rapae limits *Myzus persicae* populations after applications of deltamethrin in oilseed rape. *J. Econ. Entomol.* 98, 9–17. doi:10.1093/jee/98.1.9

Efron, B., and Tibshirani, R. J. (1993). *Monographs on Statistics and Applied Probability*. London: Chapman & Hall, 436. No. 57.An introduction to the bootstrap

Esmaeily, S., Samih, M. A., Zarabi, M., and Jafarbeigi, F. (2014). Sublethal effects of some synthetic and botanical insecticides on *Bemisia tabaci* (Hemiptera: Aleyrodidae). *J. Plant Prot. Res.* 54, 171–178. doi:10.2478/jppr-2014-0027

Fernandez, E., Gravalos, C., Haro, P. J., Cifuentes, D., and Bielza, P. (2009). Insecticide resistance status of *Bemisia tabaci* Q-biotype in south-eastern Spain. *Pest Manag. Sci.* 65, 885–891. doi:10.1002/ps.1769

Gong, P., Chen, D., Wang, C., Li, M., Li, X., Zhang, Y., et al. (2020). Susceptibility of four species of aphids in wheat to seven insecticides and its relationship to detoxifying enzymes. *Front. Physiol.* 11, 623612. doi:10.3389/fphys.2020.623612

Guedes, R. N., and Cutler, G. C. (2014). Insecticide-induced hormesis and arthropod pest management. *Pest Manag. Sci.* 70, 690–697. doi:10.1002/ps.3669

Guedes, R. N., Smagghe, G., Stark, J. D., and Desneux, N. (2016). Pesticide-induced stress in arthropod pests for optimized integrated pest management programs. *Annu. Rev. Entomol.* 61, 43–62. doi:10.1146/annurev-ento-010715-023646

Guo, L., Desneux, N., Sonoda, S., Liang, P., Han, P., and Gao, X.-W. (2013). Sublethal and transgenerational effects of chlorantraniliprole on biological traits of the diamondback moth, Plutella xylostella L. *Plutella xylostella L. Crop Prot.* 48, 29–34. doi:10.1016/j.cropro.2013.02.009

He, Y., Zhao, J., Zheng, Y., Weng, Q., Biondi, A., Desneux, N., et al. (2013). Assessment of potential sublethal effects of various insecticides on key biological traits of the tobacco whitefly, *Bemisia tabaci*. *Int. J. Biol. Sci.* 9, 246–255. doi:10.7150/ijbs.5762

Honek, A., Martinkova, Z., Saska, P., and Dixon, A. F. G. (2018). Aphids (Homoptera: Aphididae) on winter wheat: Predicting maximum abundance of *Metopolophium dirhodum*. *J. Econ. Entomol.* 111, 1751–1759. doi:10.1093/jee/toy157

James, D. G. (1997). Imidacloprid increases egg production in *Amblyseius victoriensis* (Acari: Phytoseiidae). *Exp. Appl. Acarol.* 21, 75–82. doi:10.1023/A:1018493409832

James, D. G., and Price, T. S. (2002). Fecundity in two spotted spider mite (Acari: Tetranychidae) is increased by direct and systemic exposure to imidacloprid. *J. Econ. Entomol.* 95, 729–732. doi:10.1603/0022-0493-95.4.729

Kendig, E. L., Le, H. H., and Belcher, S. M. (2010). Defining hormesis: Evaluation of a complex concentration response phenomenon. *Int. J. Toxicol.* 29, 235–246. doi:10.1177/1091581810363012

Koo, H. N., Lee, S. W., Yun, S. w., Kim, H. K., and Kim, G. H. (2015). Feeding response of the cotton aphid, *Aphis gossypii,* to sublethal rates of flonicamid and imidacloprid. *Entomol. Exp. Appl.* 154, 110–119. doi:10.1111/eea.12260

Lashkari, M. R., Sahragard, A., and Ghadamyari, M. (2007). Sublethal effects of imidacloprid and pymetrozine on population growth parameters of cabbage aphid, *Brevicoryne brassicae* on rapeseed, *Brassica napus* L. *Insect Sci.* 14, 207–212. doi:10.1111/j.1744-7917.2007.00145.x

Liang, P., Tian, Y.-A., Biondi, A., Desneux, N., and Gao, X.-W. (2012). Short-term and transgenerational effects of the neonicotinoid nitenpyram on susceptibility to insecticides in two whitefly species. *Ecotoxicology* 21, 1889–1898. doi:10.1007/s10646-012-0922-3

Liu, Z., and Han, Z. (2006). Fitness costs of laboratory-selected imidacloprid resistance in the Brown planthopper, Nilaparvata lugens Stål. *Nil. lugens Stål. Pest Manag. Sci.* 62, 279–282. doi:10.1002/ps.1169

Ma, C., Hau, B., and Poehling, H. (2003). Effects of pattern and timing of high temperature exposure on reproduction of the rose grain aphid, Metopolophium dirhodum. *Metopolophium Dirhodum. Entomol. Exp. Appl.* 110, 65–71. doi:10.1111/j.0013-8703.2004.00123.x

Ma, K., Tang, Q., Liang, P., Li, J., and Gao, X. (2022). A sublethal concentration of afidopyropen suppresses the population growth of the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae). *J. Integr. Agr.* 21, 2055–2064. doi:10.1016/s2095-3119(21)63714-0

Murali-Mohan, A., Christopher, C. G., Scott-Dupree, C. D., Sibley, P. K., and Guy, S. (2013). Transgenerational shifts in reproduction hormesis in green peach aphid exposed to low concentrations of imidacloprid. *PLoS ONE* 8, e74532. doi:10.1371/journal.pone.0074532

Palumbo, J. C., Horowitz, A. R., and Prabhaker, N. (2001). Insecticidal control and resistance management for *Bemisia tabaci*. *Crop Prot.* 20, 739–765. doi:10.1016/S0261-2194(01)00117-X

Rahmani, S., and Bandani, A. R. (2013). Sublethal concentrations of thiamethoxam adversely affect life table parameters of the aphid predator, *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae). *Crop Prot.* 54, 168–175. doi:10.1016/j.cropro.2013.08.002

Samuelson, E. E., Chen-Wishart, Z. P., Gill, R. J., and Leadbeater, E. (2016). Effect of acute pesticide exposure on bee spatial working memory using an analogue of the radial-arm maze. *Sci. Rep.* 6, 38957. doi:10.1038/srep38957

Shi, X., Jiang, L., Wang, H., Qiao, K., Wang, D., and Wang, K. (2011). Toxicities and sublethal effects of seven neonicotinoid insecticides on survival, growth and reproduction of imidacloprid-resistant cotton aphid, *Aphis gossypii*. *Pest Manag. Sci.* 67, 1528–1533. doi:10.1002/ps.2207

Sohrabi, F., Shishehbor, P., Saber, M., and Mosaddegh, M. S. (2011). Lethal and sublethal effects of buprofezin and imidacloprid on *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Crop Prot.* 30, 1190–1195. doi:10.1016/j.cropro.2011.05.004

Stebbing, A. R. D. (2003). A mechanism for hormesis-a problem in the wrong discipline. *Crit. Rev. Toxicol.* 33, 463–467. doi:10.1080/713611038

Tan, Y., Biondi, A., Desneux, N., and Gao, X.-W. (2012). Assessment of physiological sublethal effects of imidacloprid on the mirid bug *Apolygus lucorum* (Meyer-Dür). *Ecotoxicology* 21, 1989–1997. doi:10.1007/s10646-012-0933-0

Tang, Q., Ma, K., Chi, H., Hou, Y., and Gao, X. (2019). Transgenerational hormetic effects of sublethal dose of flupyradifurone on the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). *PLoS ONE* 14, e0208058. doi:10.1371/journal.pone.0208058

Tang, Q., Xiang, M., Hu, H., An, C., and Gao, X. (2015). Evaluation of sublethal effects of sulfoxaflor on the green peach aphid (Hemiptera: Aphididae) using life table parameters. *J. Econ. Entomol.* 108, 2720–2728. doi:10.1093/jee/tov221

Ullah, F., Gul, H., Desneux, N., Gao, X., and Song, D. (2019). Imidacloprid-induced hormesis effects on demographic traits of the melon aphid, *Aphis gossypii*. *Entomol. Gen.* 39, 325–337. doi:10.1127/entomologia/2019/0892

Ullah, F., Gul, H., Tariq, K., Desneux, N., Gao, X., and Song, D. (2020). Thiamethoxam induces transgenerational hormesis effects and alteration of genes expression in *Aphis gossypii*. *Pestic. Biochem. Physiol.* 165, 104557. doi:10.1016/j.pestbp.2020.104557

Wang, A., Wu, J., Yu, Y., Liu, J., Yue, J., and Wang, M. (2005). Selective insecticide-induced stimulation on fecundity and biochemical changes in *Tryporyza incertulas* (Lepidoptera: Pyralidae). *J. Econ. Entomol.* 98, 1144–1149. doi:10.1603/0022-0493-98.4.1144

Wang, X. Y., Yang, Z. Q., Shen, Z. R., Lu, J., and Xu, W. B. (2008). Sublethal effects of selected insecticides on fecundity and wing dimorphism of green peach aphid (Hom., Aphididae). *J. Appl. Entomol.* 132, 135–142. doi:10.1111/j.1439-0418.2007.01225.x

Xiao, D., Yang, T., Desneux, N., Han, P., and Gao, X. (2015). Assessment of sublethal and transgenerational effects of pirimicarb on the wheat aphids *Rhopalosiphum padi* and *Sitobion avenae*. *PLoS ONE* 10, e0128936. doi:10.1371/journal.pone.0128936

Xin, J. J., Yu, W. X., Yi, X. Q., Gao, J. P., Gao, X. W., and Zeng, X. P. (2019). Sublethal effects of sulfoxaflor on the fitness of two species of wheat aphids, Sitobion avenae (F.) and Rhopalosiphum padi (L.). *J. Integr. Agr.* 18, 1613–1623. doi:10.1016/s2095-3119(18)62094-5

Yu, Y., Shen, G., Zhu, H., and Lu, Y. (2010). Imidacloprid-induced hormesis on the fecundity and juvenile hormone levels of the green peach aphid *Myzus persicae* (Sulzer). *Pestic. Biochem. Physiol.* 98, 238–242. doi:10.1016/j.pestbp.2010.06.013

Zhang, J., Yuan, F., Liu, J., Chen, H., and Zhang, R. (2010). Sublethal effects of nitenpyram on life-table parameters and wing formation of *Nilaparvata lugens* (stål) (Homoptera: Delphacidae). *Appl. Entomol. Zool.* 45, 569–574. doi:10.1303/aez.2010.569

Zhang, P., Liu, F., Mu, W., Wang, Q., Li, H., and Chen, C. (2014). Life table study of the effects of sublethal concentrations of thiamethoxam on *Bradysia odoriphaga* Yang and Zhang. *Pestic. Biochem. Physiol.* 111, 31–37. doi:10.1016/j.pestbp.2014.04.003

frontiers | Frontiers in Physiology

TYPE Correction
PUBLISHED 06 June 2023
DOI 10.3389/fphys.2023.1186884

OPEN ACCESS

EDITED BY
Ting Li,
Alabama State University, United States

REVIEWED BY
Jianhong Li,
Huazhong Agricultural University, China
Xinzheng Huang,
China Agricultural University, China

*CORRESPONDENCE
Yunhui Zhang,
yhzhang@ippcaas.cn

†These authors have contributed equally to this work

RECEIVED 15 March 2023
ACCEPTED 22 May 2023
PUBLISHED 06 June 2023

CITATION
Li X, Li Y, Zhu X, Li X, Cheng D and Zhang Y (2023), Corrigendum: Effects of imidacloprid-induced hormesis on the development and reproduction of the rose-grain aphid *Metopolophium dirhodum* (Hemiptera: aphididae). *Front. Physiol.* 14:1186884. doi: 10.3389/fphys.2023.1186884

Corrigendum: Effects of imidacloprid-induced hormesis on the development and reproduction of the rose-grain aphid *Metopolophium dirhodum* (Hemiptera: aphididae)

Xinan Li[1,2†], Yaping Li[1†], Xun Zhu[1,3†], Xiangrui Li[1], Dengfa Cheng[1,3] and Yunhui Zhang[1,3*]

[1]State Key Laboratory for Biology of Plant Disease and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China, [2]School of Resource and Environmental Sciences, Henan Institute of Science and Technology, Xinxiang, China, [3]Scientific Observing and Experimental Station of Crop Pests in Guilin, Ministry of Agriculture, Guilin, China

KEYWORDS

hormesis, imidacloprid, *Metopolophium dirhodum*, longevity, fecundity

A Corrigendum on
Effects of imidacloprid-induced hormesis on the development and reproduction of the rose-grain aphid *Metopolophium dirhodum* (Hemiptera: Aphididae)

by Li X, Li Y, Zhu X, Li X, Cheng D and Zhang Y (2023). Front. Physiol. 14:1113464. doi: 10.3389/fphys.2023.1113464

In the published article, there was an error in the note for Table 1 as published. The word "paired" was missing from the following sentence: "Different letters in the same row indicated significantly different ($p < 0.05$) by the bootstrap test." The corrected table note appears below.

In the published article, there was an error in the note for Table 2 as published. The word "paired" was missing from the following sentence: "Different letters in the same row indicated significantly different ($p < 0.05$) by the bootstrap test." The corrected table note appears below.

In the published article, there was an error in Table 2 as published. Some of the data in Table 2 should be kept 4 digits behind the decimal point**.** The corrected Table 2 and its caption appear below.

In the published article, there was an error. Some corrections have been made to **Results**, *Transgenerational sub-lethal effects of the imidacloprid on survival rates, life expectancy and reproductive value of the M. dirhodum F_1 generation*, paragraph two. This sentence previously stated:

"The age-stage specific life expectancy (e_{xj}) refers to the predicted survival of an individual at age x and stage j at a later age x. Compared with control, the F_1 individuals produced by F0 imidacloprid-treated had a higher life expectancy (Figure 4). Moreover, newborn *M. dirhodum* nymphs were expected to live for 56, 62, and 52 days

TABLE 1 The sub-lethal effects of imidacloprid on developmental duration and fecundity of the F_1 generation of *Metopolophium dirhodum*.

Parameter[a]	Control	Imidacloprid leaf treatment (Mean ± SE)		
		LC_{15}	LC_{25}	LC_{35}
N1 (d)	1.58 ± 0.10 c	2.05 ± 0.06 b	2.05 ± 0.06 b	2.40 ± 0.06 a
N2 (d)	2.25 ± 0.10 a	1.86 ± 0.06 b	1.81 ± 0.06 b	1.72 ± 0.06 b
N3 (d)	1.95 ± 0.12 a	1.96 ± 0.08 a	1.77 ± 0.08 a	1.88 ± 0.07 a
N4 (d)	3.05 ± 0.13 a	2.54 ± 0.09 b	2.67 ± 0.09 b	2.52 ± 0.08 b
Pre-adult (d)	8.82 ± 0.20 a	8.41 ± 0.11 ab	8.29 ± 0.11 b	8.52 ± 0.10 ab
Adult longevity (d)	31.27 ± 0.75 c	35.42 ± 0.61 a	33.47 ± 0.56 b	32.47 ± 0.59 bc
Total longevity (d)	40.09 ± 0.73 b	43.51 ± 0.66 a	41.07 ± 0.72 b	40.99 ± 0.57 b
APRP (d)	1.55 ± 0.12 a	1.23 ± 0.12 ab	1.02 ± 0.08 bc	0.88 ± 0.07 c
TPRP (d)	10.37 ± 0.23 a	9.64 ± 0.17 b	9.31 ± 0.13 b	9.40 ± 0.10 b
Reproductive period (d)	17.35 ± 0.78 a	17.32 ± 0.59 a	18.66 ± 0.58 a	17.62 ± 0.56 a
Fecundity (nymphs per female)	40.39 ± 2.44 b	42.39 ± 1.95 ab	47.60 ± 2.05 a	45.68 ± 2.01 ab

[a]N1, first nymph stage; N2, second nymph stage; N3, third nymph stage; N4, fourth nymph stage; Pre-adult, complete nymph stage; APRP, adult pre-reproductive period; TPRP, total pre-reproductive period. Data in the table are represented as mean ± SE, estimated with bootstrapping (100,000). Different letters in the same row indicated significantly different ($p < 0.05$) by the paired bootstrap test.

TABLE 2 Sub-lethal effects of imidacloprid on population parameters of the F_1 generation of *Metopolophium dirhodum*.

Parameter[a]	Control	Imidacloprid leaf treatment (Mean ± SE)		
		LC_{15}	LC_{25}	LC_{35}
Intrinsic rate of increase/r	0.2243 ± 0.0051 b	0.2347 ± 0.0036 ab	0.2433 ± 0.0041 a	0.2435 ± 0.0032 a
Finite rate of increase/λ	1.2514 ± 0.0064 b	1.2646 ± 0.0045 ab	1.2755 ± 0.0052 a	1.2757 ± 0.0041 a
Net reproductive rate/R_0	40.40 ± 2.43 b	42.02 ± 1.97 ab	46.73 ± 2.10 a	45.68 ± 2.01 ab
Mean generation time/T	16.48 ± 0.25 a	15.92 ± 0.17 ab	15.80 ± 0.19 b	15.69 ± 0.14 b

[a]Data in the table are represented as mean ± SE, estimated with bootstrapping (100,000). Different letters in the same row indicated significantly different ($p < 0.05$) by the paired bootstrap test.

following the 50, 100, and 200 mg/L imidacloprid treatments, respectively, for only 49 days in response to the control treatment (Figure 4)."

In the corrected sentence, "survival" was changed to "survival time" due to a missing word, "at a later age x" was deleted as these were superfluous words, and "F0" was corrected to "F_0" since "0" should be subscripted. Additionally, "56, 62, and 52 days" was changed to "57, 63, and 53 days" and "49 days" was changed to "50 days". The first day that the life expectancy value in Figure 4 is 0 should be added, so the life expectancy value for each treatment should be added by 1 day.

The corrected sentence appears below:

"The age-stage specific life expectancy (e_{xj}) refers to the predicted survival time of an individual at age x and stage j. Compared with control, the F_1 individuals produced by F_0 imidacloprid-treated had a higher life expectancy (Figure 4). Moreover, newborn *M. dirhodum* nymphs were expected to live for 57, 63, and 53 days following the 50, 100, and 200 mg/L imidacloprid treatments, respectively, for only 50 days in response to the control treatment (Figure 4)."

The authors apologize for these errors and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

Publisher's note

frontiers | Frontiers in Physiology

TYPE Original Research
PUBLISHED 08 March 2023
DOI 10.3389/fphys.2023.1107434

OPEN ACCESS

EDITED BY
Asad Ali,
Abdul Wali Khan University, Pakistan

REVIEWED BY
Asem Saad Saad Elabasy,
Agricultural Research Center, Egypt
Faisal Hayat,
University of Florida, United States

*CORRESPONDENCE
Jun Li,
junl@giabr.gd.cn

SPECIALTY SECTION
This article was submitted to Invertebrate Physiology, a section of the journal Frontiers in Physiology

RECEIVED 24 November 2022
ACCEPTED 08 February 2023
PUBLISHED 08 March 2023

CITATION
Idrees A, Afzal A, Qadir ZA and Li J (2023), Virulence of entomopathogenic fungi against fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) under laboratory conditions.
Front. Physiol. 14:1107434.
doi: 10.3389/fphys.2023.1107434

Virulence of entomopathogenic fungi against fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) under laboratory conditions

Atif Idrees[1,2], Ayesha Afzal[1,3], Ziyad Abdul Qadir[4,5] and Jun Li[1]*

[1]Guangdong Key Laboratory of Animal Conservation and Resource Utilization, Guangdong Public Laboratory of Wild Animal Conservation and Utilization, Institute of Zoology, Guangdong Academy of Sciences, Guangzhou, China, [2]Guizhou Provincial Key Laboratory for Agricultural Pest Management of the Mountainous Region, Scientific Observing and Experimental Station of Crop Pest in Guiyang, Institute of Entomology, Ministry of Agriculture, Guizhou University, Guiyang, China, [3]Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan, [4]Honeybee Research Institute, National Agricultural Research Centre, Islamabad, Pakistan, [5]Department of Entomology and Wildlife Ecology, University of Delaware, Newark, DE, United States

Maize is an essential crop of China. The recent invasion of *Spodoptera frugiperda*, also known as fall armyworm (FAW), poses a danger to the country's ability to maintain a sustainable level of productivity from this core crop. Entomopathogenic fungi (EPF) *Metarhizium anisopliae* MA, *Penicillium citrinum* CTD-28 and CTD-2, *Cladosporium* sp. BM-8, *Aspergillus* sp. SE-25 and SE-5, *Metarhizium sp.* CA-7, and *Syncephalastrum racemosum* SR-23 were tested to determine their effectiveness in causing mortality in second instars, eggs, and neonate larvae. *Metarhizium anisopliae* MA, *P. citrinum* CTD-28, and *Cladosporium* sp. BM-8 caused the highest levels of egg mortality, with 86.0, 75.3, and 70.0%, respectively, followed by *Penicillium* sp. CTD-2 (60.0%). Additionally, *M. anisopliae* MA caused the highest neonatal mortality of 57.1%, followed by P. *citrinum* CTD-28 (40.7%). In addition, *M. anisopliae* MA, *P. citrinum* CTD-28, and *Penicillium* sp. CTD-2 decreased the feeding efficacy of second instar larvae of FAW by 77.8, 75.0, and 68.1%, respectively, followed by *Cladosporium* sp. BM-8 (59.7%). It is possible that EPF will play an important role as microbial agents against FAW after further research is conducted on the effectiveness of these EPF in the field.

KEYWORDS

fall army worm, egg mortality, neonate mortality, feeding performance, entomopathogenic fungi

1 Introduction

China's reliance on maize production is evidenced by the fact that the nation now ranks among the world's top net importers of grain (Tong, 2000). China is the world's second-largest producer of corn, behind only the United States. The fall armyworm (FAW), also known as *Spodoptera frugiperda*, J. E. Smith, 1797 (Lepidoptera: Noctuidae), is regarded as a serious and devastating pest of maize. Because of this, maize production continues to be at risk. The American continent, especially its tropical and subtropical parts, is the origin of this pest (Sparks, 1979); it has recently attacked several countries and its presence has had a

profoundly negative influence on world food security in all of the locations that it has colonized. FAW was firstly seen in Nigeria and Ghana (Goergen et al., 2016), but it quickly migrates in other African countries (Stokstad, 2017). In 2018, FAW was reported in the state of Karnataka, which is located in the southern portion of India (Sharanabasappa et al., 2018). By the end of 2018, its occurrence was also documented in nations located in Southeast Asia, including Thailand, Bangladesh, and Myanmar (Guo et al., 2018).

The corn strain of FAW was firstly observed in China in December 2018 (Zhang et al., 2019; Sun et al., 2021). Major provinces like Yunnan, were severely attacked by this invasive pest (Zhang et al., 2021), which damages important staple and economical crops but preferably feeds on corn and sorghum in China (Montezano et al., 2018). Significant damage was observed in cornfields of Yunnan during the initial attack of FAW (Yang et al., 2019). The damage losses to the maize crop were up to 22, 67, 32% and 47% in Ghana, Zambia, Ethiopia, and Kenya, respectively (Day et al., 2017; Kumela et al., 2019). Hence, this invasive pest is responsible for causing financial losses of up to 4.66 billion USD dollars in Africa (Rwomushana et al., 2018). FAW causes reduction in yield of 78, 80% and 90% of peanut, barley, and wheat crops, respectively (He et al., 2020; Yang et al., 2020). FAW also causes damage to tobacco crops if its population peaks (Xu et al., 2019).

Recently, the development of biopesticides, including plant extracts and novel insecticides is the serious concern of researchers for reducing crop damage and maximizing agricultural yield (Cook et al., 2004; Idrees et al., 2016; Idrees et al., 2017; Luo et al., 2018; Qadir et al., 2021; Ahmed et al., 2022; Liu et al., 2022; Idrees et al., 2022b). However, regular use of pesticides to control FAW have become a regular practice which is harmful to the ecosystem and natural enemies (Gu et al., 2018; Cai et al., 2017).

The application of entomopathogenic fungi (EPF) is considered to be one of the most common strategies for the control of FAW (Idrees et al., 2021; Idrees et al., 2022a). Because EPF and synthetic insecticides have distinct modes of action, therefore, EPF do not act as fast as synthetic insecticides to kill insect pests (Hajek, 1989; Fargues et al., 1994). EPF can cause infection when spores come in contact with the arthropod host. Fungal spores germinate and breach the insect cuticle through enzymatic degradation and mechanical pressure to gain entry into the insect body under an ideal condition. The EPF have fast multiplication after invading the insect tissues, and emerge from the dead insect to produce more fungal spores (Dara, 2017; Altinok et al., 2019; Ebani and Mancianti, 2021). However, it is worth noting that these EPF serve to minimize crop damage by causing host pest infection, which leads to a decrease in feeding, egg laying, development, and mating and disturbs the physiological function of pests (Thomas et al., 1997). Isolates of EPF exhibited considerable mortality of eggs and neonate larvae of FAW (Akutse et al., 2019) and significantly reduced the feeding efficacy of larvae (Qadir et al., 2021; Idrees et al., 2022a).

A few studies have been performed on the effectiveness of native EPF for the management of FAW in China. Therefore, the purpose of the present research was to evaluate the virulence of EPF against immature stages (eggs, neonate larvae and pupae) as well as on the feeding performance of FAW larvae to develop microbial-based biopesticides at the commercial level against FAW.

2 Materials and methods

2.1 Insect rearing

Eggs of FAW were collected from an established colony in the laboratory. The eggs were kept in a ventilated rectangular plastic box ($28\ cm^3 \times 17\ cm^3 \times 18\ cm^3$). The neonate larvae were fed with fresh maize insecticides free leaves. The first$^-$ to third$^-$instar larvae were kept in a rectangular plastic box ($28\ cm^3 \times 17\ cm^3 \times 18\ cm^3$), while fourth$^-$ to sixth-instar larvae were separately placed in six-well plates to prevent cannibalism until pupation. The new emerging adults were placed in cylindrical glasses. A paper towel was used to cover the top portion of the adult glasses, and sterile cotton balls were placed inside a plastic bottle lid soaked with a 10% concentration of honey. The larvae were kept at 25°C ± 2°C, with a photoperiod of 12: 12 (dark: light) and 65% ± 5% relative humidity (RH). Fifty laboratory-reared generations of larvae were used in the present study.

2.2 Entomopathogenic fungal Isolates

The EPF, *Metarhizium anisopliae* MA, *Penicillium citrinum* CTD-28 and CTD-2, *Cladosporium* sp. BM-8, *Aspergillus* sp. SE-25 and SE-5, *Metarhizium* sp. CA-7, and *Syncephalastrum racemosum* SR-23 were evaluated against immature stages and feeding efficacy of FAW. Detailed information about EPF species is described in Table 1. EPF species were obtained from laboratory collection at IZ-GDAS.

The fungal isolates were cultured by spreading a small portion of it on Sabouraud dextrose agar media (inoculation) in Petri dished (90 mm in diameter). The Petri dishes were incubated in dark incubator for 2–3 weeks. Fungal conidia were harvested from 2- to 3-week-old sporulated cultures and suspended in 10 mL of distilled water with 0.05% Tween-80 in universal bottles containing glass beads containing six to nine beads (3 mm in diameter) for each bottle. The fungal conidial suspensions were vortexed for 5 minutes at approximately 700 rpm to break up the conidial clumps and verify that the suspension was homogenous. Three conidial concentrations (1×10^6, 1×10^7, and 1×10^8 conidia/mL) were adjusted using hemocytometer before the bioassay.

The viability tests of fungal isolates were further conducted prior to starting the bioassay (Opisa et al., 2018). The eight fungal isolates showed ≥90% germination rates (Supplementary Table S1).

2.3 Efficacy of fungal Isolates on eggs and neonate larvae of FAW

FAW eggs that were one to 2 days old were taken from adult cylindrical glass. Under a light microscope, 50 eggs were divided using a camel hairbrush. A volume of 10 mL of each concentration was sprayed on a batch of 50 eggs using a manually atomized spray bottle (20 mL). A sterile paper towel was placed at the bottom of

TABLE 1 Information about the fungal isolates evaluated in the present study for the management of fall armyworm.

Fungal species	Isolates	Host or source of origin	Site of origin (Country)	Year of isolation
Metarhizium anisopliae	MA	*Spodoptera frugiperda* (Lepidoptera: Noctuidae)	Guangzhou, Guangdong	2019
Penicillium citrinum	CTD-28	*Spodoptera frugiperda* (Lepidoptera: Noctuidae)	Guangzhou, Guangdong	2019
Penicillium sp	CTD-2	*Spodoptera frugiperda* (Lepidoptera: Noctuidae)	Guangzhou, Guangdong	2019
Cladosporium sp	BM-8	*Spodoptera frugiperda* (Lepidoptera: Noctuidae)	Guangzhou, Guangdong	2019
Aspergillus versicolor	SE-25	*Trachymela sloanei* (Lepidoptera: Noctuidae)	Shenzhen, Guangdong	2019
Aspergillus sp	SE-5	*Trachymela sloanei* (Lepidoptera: Noctuidae)	Shenzhen, Guangdong	2019
Metarhizium sp	CA-7	*Trachymela sloanei* (Lepidoptera: Noctuidae)	Shenzhen, Guangdong	2019
Syncephalastrum racemosum	SR-13	*Trachymela sloanei* (Lepidoptera: Noctuidae)	Shenzhen, Guangdong	2019

rectangular box to absorb the extra spore suspension. Sterilized distilled water with 0.05% Tween-80 was considered as a control. The eggs were then air-dried for an hour in a laminar flow hood after exposure to the treatment. The eggs were then put into Petri dishes and incubated at room temperature (25°C ± 2°C) with 65% ± 5% relative humidity (RH). Egg mortality were measured 7 days post treatment. Neonates larvae that emerged from the treated eggs were kept in a perforated rectangular plastic box coated with wet filter paper. Fresh maize leaves were provided to the neonate's larvae daily. The data of neonate larvae were measured daily up to 14 days post treatment. The whole bioassays were repeated twice and completely randomized design (CRD) was used with three replications for each treatment.

The mortality of the neonate larvae was observed daily for 7 days. The cumulative mortality of both neonates and larvae of FAW was calculated by counting the dead eggs and neonates' larvae of FAW divided by a total number of eggs at 14 days post-treatment (Idrees et al., 2022a). The cadavers were examined to see whether mycosis was present by the approach of (Aktuse et al., 2019). The dead cadavers were surface sterilized with alcohol (70%) and rinsed three times in a distilled water. The surface-sterilized dead cadavers were kept in Petri dishes containing sterile filter paper. The mortality due to target EPF was tested by observing hyphae and conidia on the body of dead cadavers.

2.4 Efficacy of fungal Isolates on second Instar larvae of FAW

For laboratory bioassays involving EPF evaluations against lepidopterous pests, early second^{-} or third-instar larvae are usually used because these larvae are easy to handle or manipulate during experimentation and are more susceptible to different insecticidal treatments than the other larval instars. In fact, first instar larvae are delicate and soft and are vulnerable to mechanical damage while manipulating or handling, while later (fourth - sixth) instar larvae are somewhat resistant and do not respond well to treatments. Therefore, only early second instar larvae were used in this study.

A group of 30 s instar larvae were transferred on fresh maize leaves unexposed to insecticides in a rectangular plastic box ($28\ cm^3 \times 17\ cm^3 \times 18\ cm^3$) covered with perforated lid. The bioassays were laid out according to CRD with three replications for each treatment. Afterwards, 10 mL of each concentration was sprayed on the larvae in each rectangular plastic container using an atomized manual spray bottle (20 mL). Each concentration was sprayed on every second instar larva to ensure that none escaped from the fungal spore suspension by hiding beneath the leaves' surfaces. A sterile paper towel was put underneath the leaves to absorb the extra fungal suspension. The control larvae were treated with distilled water (0.05% Tween-80). Fresh maize leaves were given daily, and the treated larvae were kept at a temperature of 25°C ± 2°C. Larval mortality was recorded daily for up to 7 days. Mycosis was performed for the dead cadavers by the approach of Akutse et al. (2019).

2.5 Efficacy of fungal Isolates on the feeding performance of second Instar larvae of FAW

Using a hand-held atomizer spray bottle (20 mL), 15 FAW second-instar larvae were exposed to 10 mL of an EPF at three different concentrations (Fargues and Maniania, 1992). In a square plastic container, we inserted 12 g of fresh maize leaves. Sterilized distilled water with 0.05% Tween-80 was used to treat the control. The second instar larvae were weighed before and after being given a diet of fresh maize leaves. For this study, we calculated the feeding performance of the larvae in terms of feeding % by dividing the total weight of fresh maize leaf surface in Gram fed to the larvae by the total weight of leaves not eaten by the larvae and then multiplying that number by 100. The feeding performance was observed 24 and 48 h post treatment. The experiment was laid out according to CRD with three replications.

2.6 Efficacy of EPF on pupae of FAW

Using manual atomizer spray bottles (20 mL), 10 mL of each concentration of the investigated fungal isolates were applied to the pupae of FAW. Each treatment's 15 pupae were put inside a $28\ cm^3 \times 17\ cm^3 \times 18\ cm^3$ rectangular plastic box. Sterilized distilled water containing 0.05% Tween-80 was used to treat the

10.3389/fphys.2023.1107434

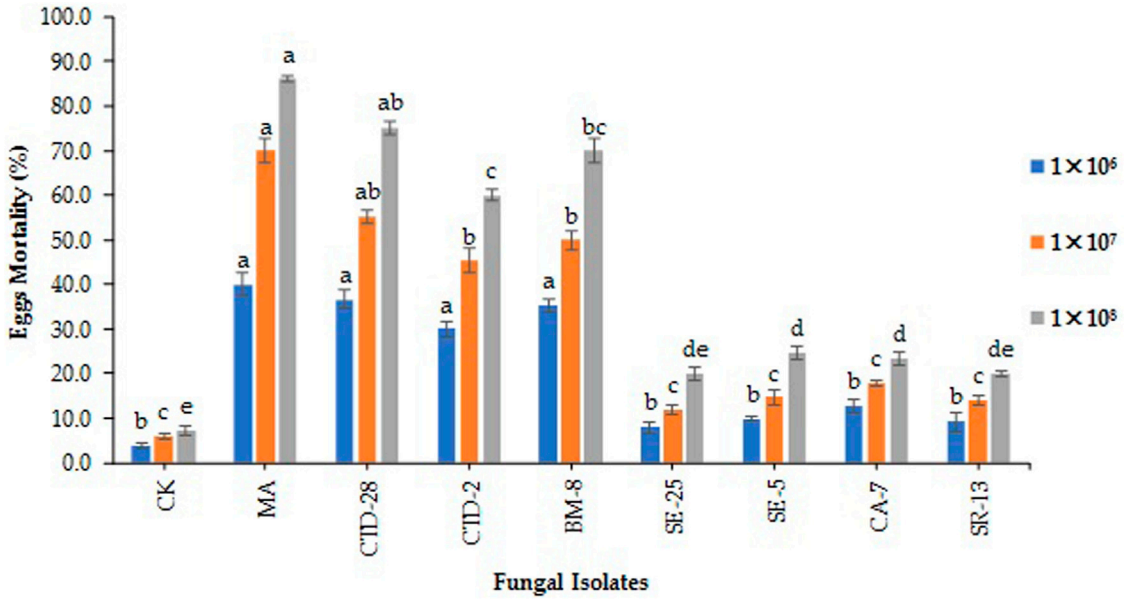


FIGURE 1
Fungal isolates induced fall armyworm's egg mortality treated with different concentrations at 7 days post treatment. Error bars denote the mean ± standard error at the 95% confidence interval. Means followed by the same letters are not significantly different by Tukey's test at $p < 0.05$.

control. Pupal mortality was observed for 15 days (Liu et al., 2022). The pupa did not turn black or emerge, or not show any movement upon touch considered as dead. The experiment was laid out according to CRD with three replications.

2.7 Statistical analysis

The Shapiro–Wilk test was used to analyze the normality of all of the stages before they were subjected to a one-way analysis of variance (ANOVA) using Tukey's highly significant difference (HSD) *post hoc* test at a 95% level of significance (Shapiro and Wilk, 1965). In addition to graphical depiction, Statistix® Version 8.1 was used to statistically evaluate the data (Analytical Software, Tallahassee, FL). Factorial ANOVA was used to analyze the interaction of the various factors, including the fungal conidial concentrations, treatments and immature stages, and this was followed by Tukey's highly significant difference (HSD) *post hoc* test. SPSS (version 22.0) was used to perform for the data analysis.

3 Results

3.1 Effect of fungal Isolates on Eggs of FAW

The results revealed that the isolate of *M. anisopliae* MA caused 40% egg mortality, followed by the isolates of *P. citrinum* CTD-28 and *Cladosporium* sp. BM-8 which caused 36.7% and 35.3% egg mortality, respectively, treated with 1×10^6 conidia/mL at 7 days post treatment ($F_{8,\ 18} = 19.3$; $p = 0.0000$). The isolate of *Penicillium* sp. CTD-2 induced 30.0% egg mortality compared to the control with 4.0% (Figure 1; Supplementary Table S2). The isolate of *M. anisopliae* MA caused the highest egg mortality of 70%, followed by the isolates of *P. citrinum* CTD-28 and *Cladosporium* sp. BM-8 which caused 55.3% and 50.0% egg mortality, respectively, treated with 1×10^7 conidia/mL ($F_{8,\ 18} = 44.1$; $p = 0.0000$). The isolate of *Penicillium* sp. CTD-2 induced 45.3% egg mortality compared to 4.0% in the control (Figure 1; Supplementary Table S3). The isolates of *M. anisopliae* MA and *P. citrinum* CTD-28 caused 86.0% and 75.3% egg mortality, respectively, followed by *Cladosporium* sp. BM-8 and *Penicillium* sp. CTD-2 isolates which caused 70.0% and 60.0% egg mortality, respectively, treated with 1×10^8 conidia/mL ($F_{8,\ 18} = 99.1$; $p = 0.0000$). The lowest egg mortality (24.7%) caused by the isolate of *Aspergillus* sp. SE-5 over the control with 7.3% (Figure 1; Supplementary Table S4).

3.2 Effect of fungal Isolates against neonate larvae of FAW

The results revealed that the *M. anisopliae* MA isolate caused 23.3% neonate larvae mortality, followed by *Cladosporium* sp. BM-8 and *Penicillium* sp. CTD-2 isolates with 19.6% and 17.1% treated with 1×10^6 conidia/mL at 7 days post treatment ($F_{8,\ 18} = 4.65$; $p = 0.0032$) (Figure 2; Supplementary Table S2) respectively, over 3.0% in the control. *Metarhizium anisopliae* MA and *Cladosporium* sp. BM-8 isolates caused neonate larvae mortality rates of 35.6% and 30.7%, respectively, followed by *Penicillium* sp. CTD-2 and *P. citrinum* CTD-28 isolates with 23.2% and 20.9%, respectively, over the control with 1.4% treated with 1×10^7 conidia/mL ($F_{8,\ 18} = 4.24$; $p = 0.0,052$) (Figure 2; Supplementary Table S3). *Metarhizium anisopliae* MA and *P. citrinum* CTD-28 isolates caused neonate

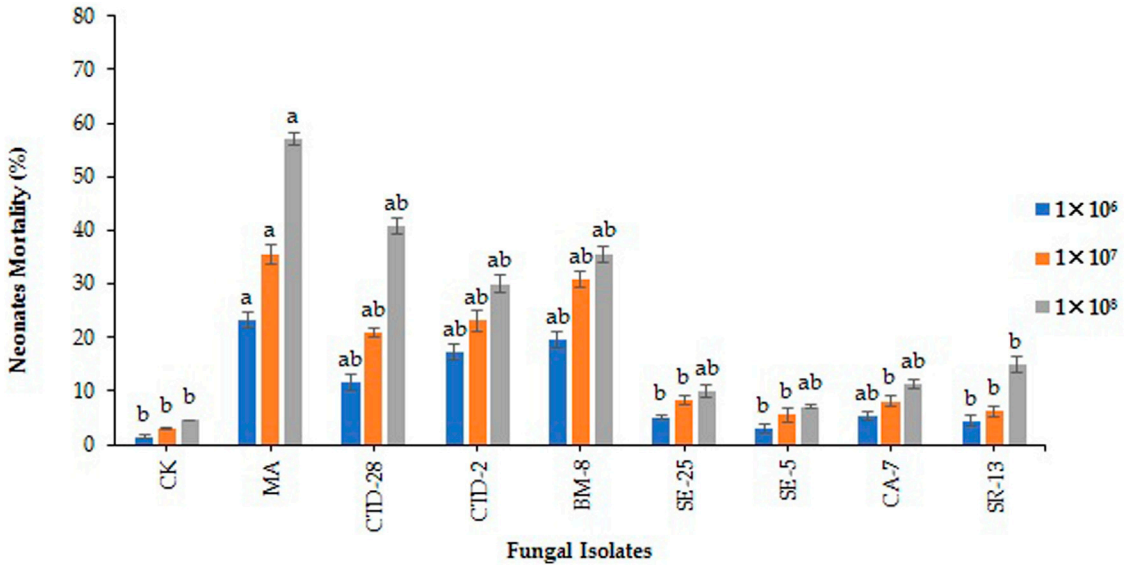


FIGURE 2
Fungal isolates induced fall armyworm's neonate larvae mortality when treated with different concentrations at 7 days post treatment. Error bars denote the mean ± standard error at the 95% confidence interval. Means followed by the same letters are not significantly different by Tukey's test at $p < 0.05$.

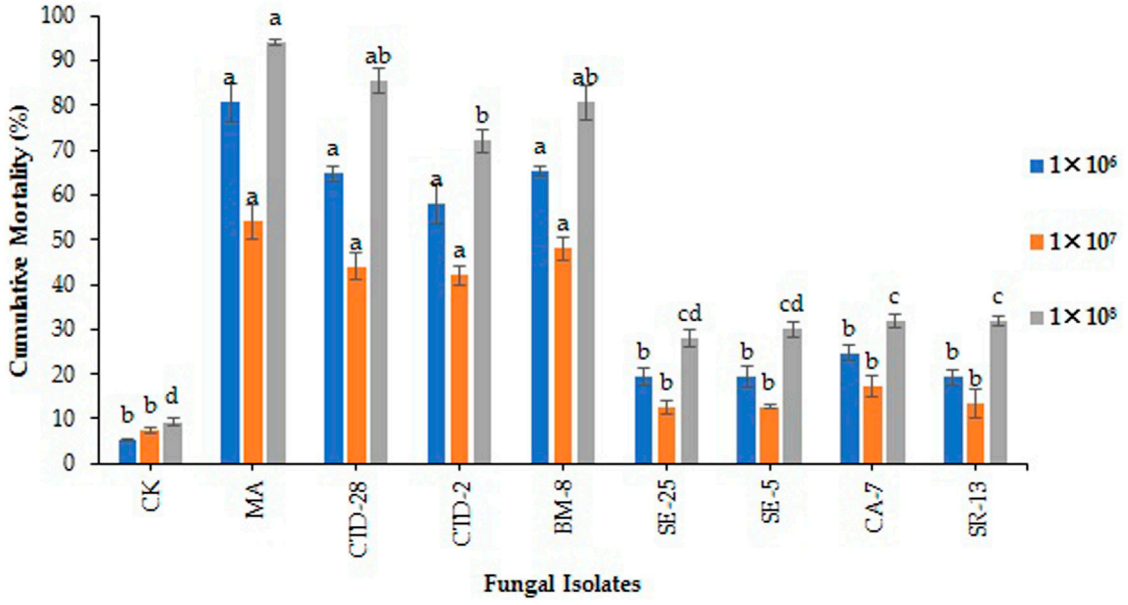


FIGURE 3
Effects of entomopathogenic fungal isolates on cumulative mortality to the eggs and neonates of fall armyworm treated with different concentrations at 14 days post treatment. Error bars denote the mean ± standard error at the 95% confidence interval. Means followed by the same letters are not significantly different by Tukey's test at $p < 0.05$.

mortality rates of 57.1% and 40.7%, respectively, followed by *Cladosporium* sp. BM-8 and *Penicillium* sp. CTD-2 isolates with 35.6% and 30.0%, respectively, over the control with 4.5% treated with 1×10^8 conidia/mL ($F_{8,\ 18} = 3.03$; $p = 0.0241$) (Figure 2; Supplementary Table S4). Furthermore, only 5%–10% of insect cadavers showed mycosis.

3.3 Effect of fungal Isolates on the cumulative mortality of eggs and neonate larvae of FAW

The results showed that *M. anisopliae* MA and *Cladosporium* sp. BM-8 isolates caused cumulative mortality of 54.0% and 48.0%,

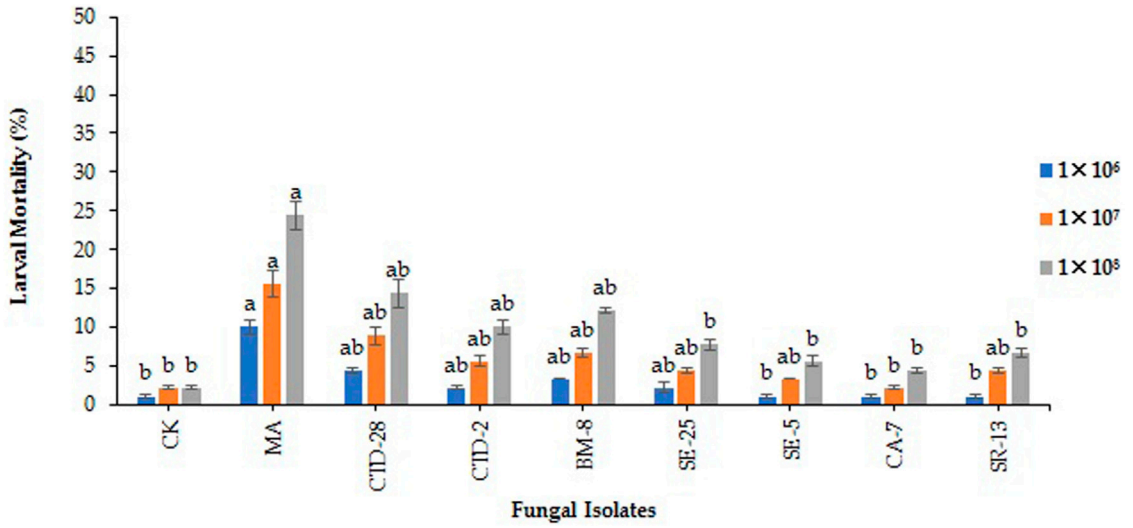


FIGURE 4
Effects of entomopathogenic fungal isolates on larval mortality of fall armyworm treated with different concentrations at 7 days post treatment. Error bars denote the mean ± standard error at the 95% confidence interval. Means followed by the same letters are not significantly different by Tukey's test at $p < 0.05$.

respectively, followed by *P. citrinum* CTD-28 and *Penicillium* sp. CTD-2 isolates, which caused 44.0% and 42.0% cumulative mortality to the eggs and neonates, respectively, compared to the control with 7.3%, respectively, over the control with 3.0% after treated with 1×10^6 conidia/mL at14 days post treatment ($F_{8, 18} = 4.7$; $p = 0.0000$) (Figure 3; Supplementary Table S2). *Metarhizium anisopliae* MA, *Cladosporium* sp. BM-8 and *P. citrinum* CTD-28 isolates caused cumulative mortalities of 80.7%, 65.3%, and 64.7%, respectively, followed by *Penicillium* sp. CTD-2 with 58.0% over the control (5.3%) treated with 1×10^7 conidia/mL ($F_{8, 18} = 27.4$; $p = 0.0000$) (Figure 3; Supplementary Table S3). *Metarhizium anisopliae* MA, *P. citrinum* CTD-28 and *Cladosporium* sp. BM-8 isolates revealed a significant effect by causing cumulative mortality of 94.0%, 85.3%, and 80.7%, respectively, followed by *Penicillium* sp. CTD-2 with 72.0% over the control (9.3%) treated with 1×10^8 conidia/mL ($F_{8, 18} = 52.5$; $p = 0.0000$) (Figure 3; Supplementary Table S4).

3.4 Effect of EPF on second instar of FAW larvae

The results revealed that among all tested EPF, only *Metarhizium anisopilae* MA isolates caused 10.0% larval mortality of FAW over the control (1.1%) treated with 1×10^6 conidia/mL at 7 days post treatment ($F_{8, 18} = 3.21$; $p = 0.0191$). There was no significant difference observed by the isolates of *M. anisopilae* MA and *P. citrinum* CTD-28 over the control which caused 15.6% and 8.9% larval mortality treated with 1×10^7 conidia/mL ($F_{8, 18} = 2.49$; $p = 0.0514$). The isolate of *M. anisopilae* MA caused 24.4% larval mortality, followed by *P. citrinum* CTD-28 with 14.4% over the control (2.21%) treated with 1×10^8 conidia/mL ($F_{8, 18} = 4.02$; $p = 0.0068$). Furthermore, mycosis was observed only in 5% of the dead larvae with effective strains (Figure 4; Supplementary Table S5).

3.5 Effect of EPF on the feeding performance of second instar larvae of FAW

The isolates of *M. anisopliae* MA and *P. citrinum* CTD-28 were found to be effective by reducing 52.8% and 47.2%, feeding performance of FAW larvae, respectively, followed by *Penicillium* sp. CTD-2 and *Cladosporium* sp. BM-8 isolates, which reduced by 44.4% and 40.3% feeding performance, respectively, over the control (4.2%) after treated with 1×10^6 conidia/mL at 48 h post treatment ($F_{8, 18} = 29.5$; $p = 0.0000$). *Metarhizium anisopliae* MA and *P. citrinum* CTD-28 isolates significantly reduced the feeding performance by 65.3% and 61.1%, respectively, followed by *Penicillium* sp. CTD-2 and *Cladosporium* sp. BM-8 isolates, which reduced the feeding performance of FAW larvae by 52.8% and 50.0%, respectively, compared to the control (4.2%) after treated with 1×10^7 conidia/mL ($F_{8, 18} = 17.2$; $p = 0.0000$). *Metarhizium anisopliae* MA and *P. citrinum* CTD-28 isolates outperformed all the other EPF by reducing feeding performance of 77.8% and 75.0%, respectively, followed by 68.1% and 59.7% with *Penicillium* sp. CTD-2 and *Cladosporium* sp. BM-8 isolates, respectively, over the control (4.2%) after treated with 1×10^8 conidia/mL ($F_{8, 18} = 38.5$; $p = 0.0000$) (Figure 5; Supplementary Table S6).

3.6 Effect of EPF against pupae of FAW

The results indicate that all the tested EPF isolates did not show significant pupal mortality of FAW compared to the control after treated with 1×10^6 conidia/mL at 15 days posttreatment ($F_{8, 18} = 1.19$; $p = 0.3591$). *M. anisopilae* MA isolate caused pupal mortality of 13.3%, followed by the *P. citrinum* CTD-28 isolate with 10.0% over the control (0.0%) when treated with 1×10^7 conidia/mL ($F_{8, 18} = 3.65$; $p = 0.0107$). *M. anisopilae* MA and *P. citrinum* CTD-28 isolates caused 23.3% and 20.0% pupal mortality, respectively, followed by *Penicillium* sp. CTD-2 and *Cladosporium* sp. BM-8 isolates with 13.3% and 10.0%

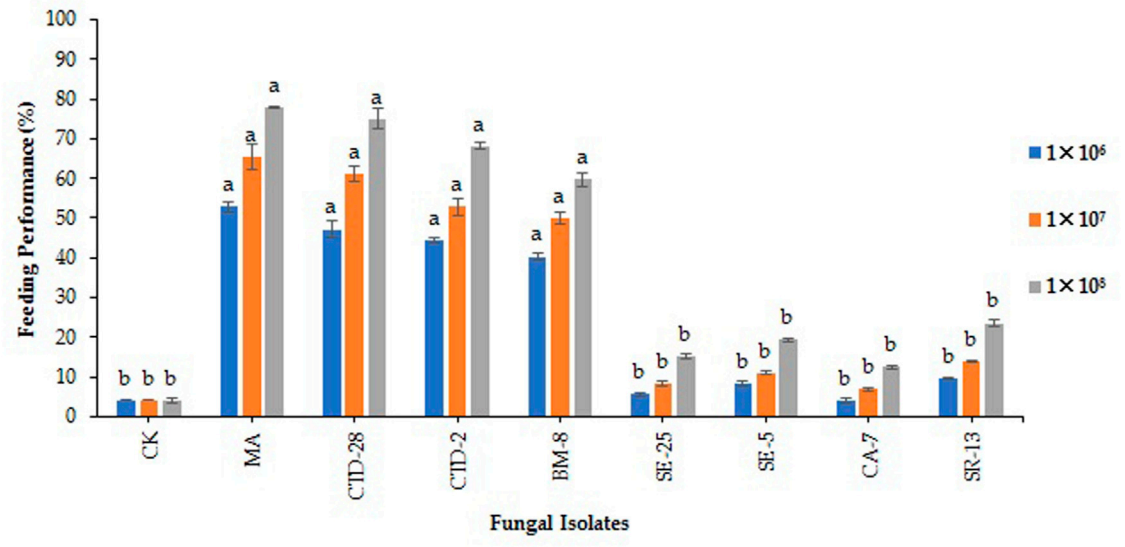


FIGURE 5
Effects of entomopathogenic fungal isolates on the feeding performance of second instar larvae of fall armyworm treated with different concentrations at 48 h post treatment. Error bars denote the mean ± standard error at the 95% confidence interval. Means followed by the same letters are not significantly different by Tukey's test at $p < 0.05$.

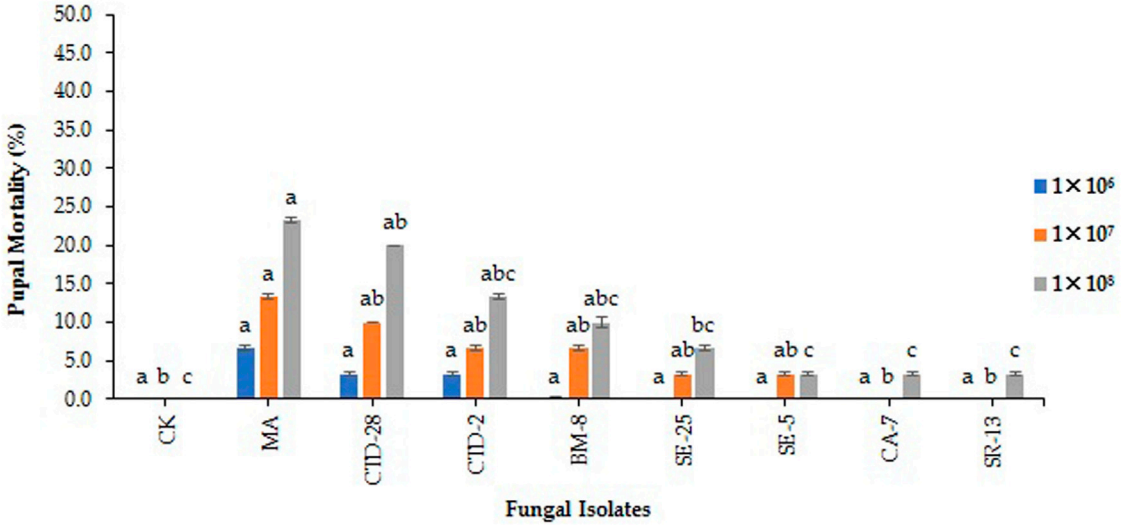


FIGURE 6
Effects of EPF on pupal mortality of fall armyworm treated with different concentrations at 14 days post treatment. Error bars denote the mean ± standard error at the 95% confidence interval. Means followed by the same letters are not significantly different by Tukey's test at $p < 0.05$.

compared to the control (0.0%) treated with 1× 10^8 conidia/mL ($F_{8, 18} = 5.94$; $p = 0.0008$) (Figure 6; Supplementary Table S7).

3.7 Factorial analysis of variance

Factorial analysis of variance revealed a significant effect of the conidial concentrations ($F_{2, 192} = 67.62$; $p = 0.0000$), the treatments ($F_{7, 192} = 59.46$; $p = 0.0000$), the life stages ($F_{3, 192} = 191.46$; $p = 0.0000$), and the significant interaction was observed between concentration and treatment ($F_{14, 192} = 3.18$; $p = 0.0000$), the concentration and life stages ($F_{6, 192} = 5.65$; $p = 0.0000$), the treatment and life stages ($F_{21, 192} = 9.41$; $p = 0.0000$), while non-significant interaction was found among concentrations, treatments and life stages ($F_{42, 192} = 0.40$; $p = 0.9997$) (Table 2; Supplementary Table S8).

10.3389/fphys.2023.1107434

TABLE 2 Mortality (Means ± SEs) of immature stages of fall armyworm treated with different concentrations of isolates of entomopathogenic fungi.

			Percent mortality ±means standard error			
Concentrations	Fungal Species	Isolates	Eggs[A]	Neonates[B]	Larvae[C]	Pupae[C]
1×10^6	*M. anisopliae*	MA[A]	40.0 ± 2.5b	23.3 ± 1.5a	10.0 ± 1.0 a	6.7 ± 0.3 a
Conidia/mL[C]	*P. citrinum*	CTD-28[B]	36.7 ± 2.0a	11.6 ± 1.5ab	8.9 ± 1.2 a	3.3 ± 0.3 a
	Penicillium sp	CTD-2[B]	30.0 ± 1.7a	17.1 ± 1.5ab	7.8 ± 0.9 a	3.3 ± 0.3 a
	Cladosporium sp	BM-8[B]	35.3 ± 1.5a	19.6 ± 1.5ab	7.8 ± 0.7a	3.3 ± 0.3 a
	A. versicolor	SE-25[C]	8.0 ± 1.2a	5.1 ± 0.3b	4.4 ± 0.9 a	0.0 ± 0.0 a
	Aspergillus sp	SE-5[C]	10.0 ± 0.6b	3.0 ± 0.9b	4.4 ± 0.3 a	3.3 ± 0.3 a
	Metarhizium sp	CA-7[C]	12.7 ± 1.5b	5.3 ± 0.9ab	1.1 ± 0.3 a	0.0 ± 0.0 a
	S. racemosum	SR-13 [C]	9.3 ± 2.2b	4.4 ± 1.0b	1.1 ± 0.3 a	0.0 ± 0.0 a
1×10^7	*M. anisopliae*	MA[A]	70.0 ± 2.9a	35.6 ± 1.8a	10.0 ± 1.0 a	6.7 ± 0.3 a
Conidia/mL[B]	*P. citrinum*	CTD-28[B]	55.3 ± 1.5ab	20.9 ± 0.9ab	8.9 ± 1.2 a	3.3 ± 0.3 a
	Penicillium sp	CTD-2[B]	45.3 ± 2.8b	23.2 ± 2.0ab	7.8 ± 0.9 a	3.3 ± 0.3 a
	Cladosporium sp	BM-8[B]	50.0 ± 2.1b	30.7 ± 1.5ab	7.8 ± 0.7a	3.3 ± 0.3 a
	A. versicolor	SE-25[C]	12.0 ± 1.2c	8.3 ± 0.9b	4.4 ± 0.9 a	0.0 ± 0.0 a
	Aspergillus sp	SE-5[C]	14.7 ± 1.5c	5.5 ± 1.2b	4.4 ± 0.3 a	3.3 ± 0.3 a
	Metarhizium sp	CA-7[C]	18.0 ± 0.6c	8.1 ± 0.9b	1.1 ± 0.3 a	0.0 ± 0.0 a
	S. racemosum	SR-13[C]	14.0 ± 1.0c	6.2 ± 0.9b	1.1 ± 0.3 a	0.0 ± 0.0 a
1×10^8	*M. anisopliae*	MA[A]	86.0 ± 0.6a	57.1 ± 1.2a	10.0 ± 1.0 a	6.7 ± 0.3 a
Conidia/mL[A]	*P. citrinum*	CTD-28[B]	75.3 ± 1.5ab	40.7 ± 1.5ab	8.9 ± 1.2 a	3.3 ± 0.3 a
	Penicillium sp	CTD-2[B]	60.0 ± 1.2c	30.0 ± 1.7ab	7.8 ± 0.9 a	3.3 ± 0.3 a
	Cladosporium sp	BM-8[B]	70.0 ± 2.9bc	35.6 ± 1.25ab	7.8 ± 0.7a	3.3 ± 0.3 a
	A. versicolor	SE-25[C]	20.0 ± 1.5de	10.0 ± 1.2ab	4.4 ± 0.9 a	0.0 ± 0.0 a
	Aspergillus sp	SE-5[C]	24.7 ± 1.5d	7.1 ± 0.3ab	4.4 ± 0.3 a	3.3 ± 0.3 a
	Metarhizium sp	CA-7[C]	23.3 ± 1.5d	11.3 ± 0.9ab	1.1 ± 0.3 a	0.0 ± 0.0 a
	S. racemosum	SR-13[C]	20.0 ± 0.6de	15.0 ± 1.5b	1.1 ± 0.3 a	0.0 ± 0.0 a

4 Discussion

There are a variety of microbial pathogens that have been associated with FAW, including fungi, bacteria and viruses (Gardner et al., 1984), but only a few pathogens among them are responsible for causing infection of the pests (Polanczyk et al., 2000; Garcia et al., 2008; Negrisoli et al., 2010b; Negrisoli et al., 2010a; Behle and Popham, 2012; Salvadori et al., 2012; Gómez et al., 2013). FAW nuclear polyhedrosis virus (NPV) is reported to be one of the most important pathogens inducing significant mortality to the pest. Thus, any entomopathogen that is able to cause infection to pests before it reaches its destructive stage might play a key role in the management of insect pests. Hence, the present study focused on screening selected EPF species for the control of FAW by causing infection at any susceptible stage of life.

The eggs are the most susceptible to microbial infection and require maximum nutrients for their development (Tillman, 2010); therefore, eggs are the sensitive stage by pathogenic microorganisms (Kellner, 2002; Trougakos and Margaritis, 2002). The results of the present study revealed that FAW eggs were the most vulnerable to the tested fungal isolates. Our results are supported by previous studies where isolates of *M. anisopliae* and *Cladosporium tenuissimum* showed 96.5% and 55.6% FAW egg mortality (Akutse et al., 2019; Idrees et al., 2021). The isolate of *Cladosporium sp.* was significantly virulent against *Helicoverpa armigera* egg mortality (Bahar et al., 2011). The findings of our study are in line with those of Idrees et al. (Idrees et al., 2021), who observed that isolates of *Aspergillus* sp. did not induce FAW egg mortality. The highest egg mortality of *Spodoptera litura* was observed by Anand and Tiwary (2009), when treated with isolates of *Aspergillus* sp. Similar to our results, the highest egg mortality of FAW was observed with the isolates of *P. citrinum* and *Pteroptyx bearni* (Foo et al., 2017; Idrees et al., 2021). Histopathological research proved that fungal spores can successfully penetrate eggs and cause fungal infections (Pires et al., 2009; Zhang et al., 2014).

The results of our research are supported by previous findings where isolates of *M. anisopliae* were significantly effective in inducing FAW neonate mortality (Akutse et al., 2019), while the isolates of *Aspergillus* sp, *C. tenuissimum* and *P. citrimum* did not induce significant mortality against neonates of FAW (Idress et al., 2021). The isolates of *P. citrinum* and *C. tenuissimum* caused significant cumulative mortality to the eggs and neonates of FAW in the present research. The isolates of *Cladosporium aphidis* were significantly effective in causing cumulative mortality of aphid species (Gui et al., 2005). Although all host stages are not equally susceptible to pathogen infection, EPF have the potential to cause infection of insect pests at any stage of their life (Opisa et al., 2018).

The tested EPF were not effective against second instar larvae of FAW. The isolates of *Aspergillus* sp. did not cause significant larval mortality in second instar larvae of FAW and *Chilo suppressalis* (Idrees et al., 2021; Shahriari et al., 2021). The isolate of *P. citrinum* was found to be the most effective against second instar larvae of *S. litura* but ineffective in causing larval mortality of FAW (Herlinda et al., 2020). The isolates of *P. citrinum* were found to be associated with mosquito larvae (Da et al., 2009) and caused significant larval mortality against *Culex quinquefasciatus* (Maketon et al., 2014). Interestingly, it was observed that some of the fungal isolates showed significant effectiveness against early instar larvae, while less virulent to mature larvae; for example, the isolate of *Cladosporium* sp. caused significant mortality of early instar larvae compared with matured larvae of *H. armigera* (Bahar et al., 2011). Fewer species in the *Cladosporium* genus were found to be virulent against aphids and whiteflies (Abdel-Baky and Abdel-Salam, 2003; Gui et al., 2005). The isolates of *M. anisopliae* did not show significant mortality against second instar larvae of FAW (Akutse et al., 2019). The effectiveness level of each fungal isolate varies for causing infection against FAW (Fargues and Maniania, 1992). Therefore, this situation provides a basis for further studies to reveal the mechanisms of higher resistance in FAW larvae to the tested fungal isolates in this study. The high resistance of the mature larvae against fungal infection may be due to the larval integument that does not permit effective penetration of the fungal spore (Bosa et al., 2004). There are minimal chances of fungal infection when the inoculum is lost due to molting, even though molting does not constantly result in the prevention of fungal infection (Meekes, 2001). The low susceptibility of larvae in the present study could be a feature of the tested fungal isolates and might be attributed to genetic diversity or perhaps phenotypic differences between the populations of FAW (Monnerat et al., 2006).

The production of toxic substances by EPF inside the host body leads to mechanical disruption in insect structural integrity, which ultimately reduces the feeding performance of insect pests (Tefera and Pringle, 2003). A previous study reported a significant feeding reduction in the feeding performance of insect pests treated with different fungal isolates (Fargues et al., 1994; Ekesi and Maniania, 2000; Hussain et al., 2009; Migiro et al., 2011; Idrees et al., 2021; Idrees et al., 2022a). The reduction in feeding performance by larvae treated with fungal isolates is one of the key factors in host mortality, indicating virulence of fungal isolates, and requires further investigation to assess the level of pathogenicity or antifeedant effects (Ondiaka et al., 2008).

The previous research finding concluded that EPF did not infect to all the stages of lepidopteran pests equally and in most of the research finds have been observed that EPF have to potential to infect the earliest stages of pest effective compared with later stages (Idrees et al., 2022b). It might be the reason that the resistance developed in later mature stages of pest. The fungal isolates did not show a significant effect in causing pupal mortality of FAW in our research. The previous findings are consistent with our study results, where fungal isolates did not cause significant pupal mortality of *S. litura* within 14 days post treatment (Anand et al., 2009; Asi et al., 2013). The different life stages of any insect do not response to any pathogen stress the same way. So, it is 'very normal' to test EPF on various life stages in order to understand which life stage is most susceptible, or either way, the most resistant to the said fungal isolate. As this would inform users the most appropriate stage of the insect to target for effective biological control programs as in our previous study concluded that eggs are the most susceptible stage for target as compared to the other life stages of FAW (Aktuse et al., 2019; Idrees et al., 2021).

5 Conclusion

The *M. anisopliae*, *P. citrinum*, *Penicillium* sp. and *Cladosporium* sp. have potential to infect the immature stages and feeding performance of FAW. Therefore, these EPF could be considered for the development of microbial pesticides against FAW. Further studies are needed to insight the key toxins which are responsible for affecting the physiological function of FAW and slowing down the feeding performance.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

All authors listed have made considerable direct or indirect and intellectual contribution to the work and have read and agreed to the manuscript.

Funding

This research work was financially supported by the Key-Area Research and Development Program of Guangdong Province (No. 2020B020223004), GDAS Special Project of Science and Technology Development (Nos. 2020GDASYL-20200301003 and 2020GDASYL-20200104025), GDAS Action Capital Project to build a comprehensive industrial technology innovation Center (No. 2022GDASZH-2022010106).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2023.1107434/full#supplementary-material

References

Abdel-Baky, N. F., and Abdel-Salam, A. H. (2003). Natural incidence of Cladosporium spp. as a bio-control agent against whiteflies and aphids in Egypt. *J. Appl. Entomol.* 127, 228–235. doi:10.1046/j.1439-0418.2003.00662.x

Ahmed, K. S., Idrees, A., Majeed, M. Z., Majeed, M. I., Shehzad, M. Z., Ullah, M. I., et al. (2022). Synergized toxicity of promising plant extracts and synthetic chemicals against fall armyworm *Spodoptera frugiperda* (JE smith) (Lepidoptera: Noctuidae) in Pakistan. *Agronomy* 12, 1289. doi:10.3390/agronomy12061289

Akutse, K. S., Kimemia, J. W., Ekesi, S., Khamis, F. M., Ombura, O. L., and Subramanian, S. (2019). Ovicidal effects of entomopathogenic fungal isolates on the invasive Fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *J. Appl. Entomol.* 143, 626–634. doi:10.1111/jen.12634

Altinok, H. H., Mahmut, A. A., and Abdurrahman, S. K. (2019). Modes of action of entomopathogenic fungi. *Curr. Trends Nat. Sci.* 8, 117–124.

Anand, R., Prasad, B., and Tiwary, B. N. (2009). Relative susceptibility of Spodoptera litura pupae to selected entomopathogenic fungi. *BioControl* 54, 85–92. doi:10.1007/S10526-008-9157-X

Anand, R., and Tiwary, B. N. (2009). Pathogenicity of entomopathogenic fungi to eggs and larvae of Spodoptera litura, the common cutworm. *Biocontrol Sci. Technol.* 19, 919–929. doi:10.1080/09583150903205069

Asi, M. R., Bashir, M. H., Afzal, M., Zia, K., and Akram, M. (2013). Potential of entomopathogenic fungi for biocontrol of Spodoptera litura fabricius (Lepidoptera: Noctuidae). *J. Anim. Plant Sci.* 23, 913–918.

Bahar, M. H., Backhouse, D., Gregg, P. C., and Mensah, R. (2011). Efficacy of a Cladosporium sp. fungus against Helicoverpa armigera (Lepidoptera: Noctuidae), other insect pests and beneficial insects of cotton. *Biocontrol Sci. Technol.* 21, 1387–1397. doi:10.1080/09583157.2011.622036

Behle, R. W., and Popham, H. J. (2012). Laboratory and field evaluations of the efficacy of a fast-killing baculovirus isolate from *Spodoptera frugiperda*. *J. Invertebr. Pathol.* 109, 194–200. doi:10.1016/j.jip.2011.11.002

Bosa, C. F., ChÁVez, D., Torres, L., ParÍS, A., Villamizar, L., and Cotes, A. (2004). Evaluation of natíve isolates of Nomuraea rileyi for the control of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Rev. Colomb. Entomol.* 30, 93–97. doi:10.25100/socolen.v30i1.9535

Cai, P., Gu, X., Yao, M., Zhang, H., Huang, J., Idress, A., et al. (2017). The optimal age and radiation dose for *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) eggs as hosts for mass-reared *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae). *Biol. Control* 108, 89–97. doi:10.1016/J.BIOCONTROL.2017.02.006

Cook, D. R., Leonard, B. R., and Gore, J. (2004). Field and laboratory performance of novel insecticides against armyworms (Lepidoptera: Noctuidae). *Fla. Entomol. Fla. Entomol. Soc.* 87, 433–439. doi:10.1653/0015-4040(2004)087[0433:FALPON]2.0.CO;2

Da, S. P. E., de, M. S. M. I., Ferreira-Keppler, R. L., Hamada, N., and Alencar, Y. B. (2009). Filamentous fungi associated with mosquito larvae (Diptera: Culicidae) in municipalities of the Brazilian amazon. *Neotrop. Entomol.* 38, 352–359. doi:10.1590/s1519-566x2009000300009

Dara, S. K. (2017). "Entomopathogenic microorganisms: Modes of action and role in IPM. E-Journal of Entomology and Biological," in *E-Journal of Entomology and Biological* (United States: University of California Agriculture and natural blogs).

Day, R., Abrahams, P., Bateman, M., Beale, T., Clottey, V., Cock, M., et al. (2017). Fall armyworm: Impacts and implications for Africa. *Outlooks Pest Manag.* 28, 196–201. doi:10.1564/v28_oct_02

Ebani, V. V., and Mancianti, F. (2021). Entomopathogenic fungi and bacteria in a veterinary perspective. *Biology* 10, 479. doi:10.3390/biology10060479

Ekesi, S., and Maniania, N. K. (2000). Susceptibility of *Megalurothrips sjostedti* developmental stages to *Metarhizium anisopliae* and the effects of infection on feeding, adult fecundity, egg fertility and longevity. *Entomol. Exp. Appl.* 94, 229–236. doi:10.1046/j.1570-7458.2000.00624.x

Fargues, J., Delmas, J. C., and Lebrun, R. A. (1994). Leaf consumption by larvae of the Colorado potato beetle (Coleoptera: Chrysomelidae) Infected with the Entomopathogen, *Beauveria bassiana*. *J. Econ. Entomol.* 87, 67–71. doi:10.1093/jee/87.1.67

Fargues, J., and Maniania, N. K. (1992). Variation in susceptibility of *Spodoptera littoralis* (Noctuidae) to *Nomuraea rileyi* (hyphomycetes). *Entomophaga* 37, 545–554. doi:10.1007/BF02372324

Foo, K., Sathiya Seelan, J. S., and Dawood, M. M. (2017). Microfungi associated with *Pteroptyx bearni* (Coleoptera: Lampyridae) eggs and larvae from kawang river, sabah (northern borneo). *Insects* 8, 66. doi:10.3390/insects8030066

Garcia, L. C., Raetano, C. G., and Leite, L. G. (2008). Application technology for the entomopathogenic nematodes *Heterorhabditis indica* and *Steinernema* sp. (Rhabditida: Heterorhabditidae and Steinernematidae) to control *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) in corn. *Neotrop. Entomol.* 37, 305–311. doi:10.1590/s1519-566x2008000300010

Gardner, W. A., Noblet, R., and Schwehr, R. D. (1984). The potential of microbial agents in managing populations of the fall armyworm (Lepidoptera: Noctuidae). *Fla. Entomol.* 67, 325–332.

Goergen, G., Kumar, P. L., Sankung, S. B., Togola, A., and Tamò, M. (2016). First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J E Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. *PLoS One* 11, e0165632. doi:10.1371/journal.pone.0165632

Gómez, J., Guevara, J., Cuartas, P., Espinel, C., and Villamizar, L. (2013). Microencapsulated *Spodoptera frugiperda* nucleopolyhedrovirus: Insecticidal activity and effect on arthropod populations in maize. *Biocontrol Sci. Technol.* 23, 829–846. doi:10.1080/09583157.2013.802288

Gu, X., Cai, P., Yang, Y., Yang, Q., Yao, M., Idrees, A., et al. (2018). The response of four braconid parasitoid species to methyl eugenol: Optimization of a biocontrol tactic to suppress *Bactrocera dorsalis*. *Biol. Control* 122, 101–108. doi:10.1016/J.BIOCONTROL.2018.04.002

Gui, G., Yue, L., Bin, C., and Ying, W. (2005). Virulence of entomophagous fungus *Cladosporium aphidis* (Thuemen) against 3 aphid species. *J. South. Agric. Uni.* 27, 184–187.

Guo, J., Zhao, J., He, K., Zhang, F., and Wang, Z. (2018). Potential invasion of the crop-devastating insect pest fall armyworm *Spodoptera frugiperda* to China. *Plant Prot.* 44, 1–10.

Hajek, A. E. (1989). Food consumption by *Lymantria dispar* (Lepidoptera: Lymantriidae) larvae infected with entomophaga maimaiga (zygomycetes: Entomophthorales). *Environ. Entomol.* 18, 723–727. doi:10.1093/ee/18.4.723

He, L., Zhao, S., and Wu, K. (2020). Study on the damage of fall armyworm, *Spodoptera frugiperda* to peanut. *Plant Prot.* 46, 28–33.

Herlinda, S., Efendi, R. A., Suharjo, R., Hasbi, H., Setiawan, A., Elfita, E., et al. (2020). New emerging entomopathogenic fungi Isolated from soil in South Sumatra (Indonesia) and their filtrate and conidial insecticidal activity againts Spodoptera litura. *J. Bio. Divers.* 21, 5102–5113. doi:10.13057/biodiv/d211115

Hussain, A., Tian, M., He, Y., and Ahmed, S. (2009). Entomopathogenic fungi disturbed the larval growth and feeding performance of Ocinara varians (Lepidoptera: Bombycidae) larvae. *Insect Sci.* 16, 511–517. doi:10.1111/j.1744-7917.2009.01272.x

Idrees, A., Afzal, A., Qadir, Z. A., and Li, J. (2022a). Bioassays of *Beauveria bassiana* isolates against the fall armyworm, *Spodoptera frugiperda*. *J. Fungi* 8, 717. doi:10.3390/jof8070717

Idrees, A., Qadir, Z. A., Afzal, A., Ranran, Q., and Li, J. (2022b). Laboratory efficacy of selected synthetic insecticides against second instar invasive fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae. *PLoS One* 17, e0265265. doi:10.1371/journal.pone.0265265

Idrees, A., Qadir, Z. A., Akutse, K. S., Afzal, A., Hussain, M., Islam, W., et al. (2021). Effectiveness of entomopathogenic fungi on immature stages and feeding performance of fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae. *Insects* 12, 1044. doi:10.3390/insects12111044

Idrees, A., Qasim, M., Ali, H., Qadir, Z. A., Idrees, A., Bashir, M. H., et al. (2016). Acaricidal potential of some botanicals against the stored grain mites. *Rhizoglyphus tritici. J. Entomology Zoology Stud.* 4, 611–617. Available at: https://www.mendeley.com/catalogue/d3ee9586-69e5-343d-90e2-4713ae1007c4/(Accessed November 11, 2021).

Idrees, A., Zhang, H., Luo, M., Thu, M., Cai, P., Islam, W., et al. (2017). Protein baits, volatile compounds and irradiation influence the expression profiles of odorant-binding protein genes in *Bactrocera dorsalis* (Diptera: Tephritidae). *Appl. Ecol. Environ. Res.* 15, 1883–1899. doi:10.15666/AEER/1504_18831899

Kellner, R. L. L. (2002). "The role of microorganisms for eggs and progeny," in *Chemoecology of insect eggs and egg deposition*. Editor T. M. Monika Hilker (Berlin): Blackwell), 149–164. doi:10.1002/9780470760253.ch6

Kumela, T., Simiyu, J., Sisay, B., Likhayo, P., Mendesil, E., Gohole, L., et al. (2019). Farmers' knowledge, perceptions, and management practices of the new invasive pest, fall armyworm (*Spodoptera frugiperda*) in Ethiopia and Kenya. *Int. J. Pest Manag.* 65, 1–9. doi:10.1080/09670874.2017.1423129

Liu, Z. K., Li, X. L., Tan, X. F., Yang, M. F., Idrees, A., Liu, J. F., et al. (2022). Sublethal effects of emamectin benzoate on fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Agriculture* 12, 959. doi:10.3390/agriculture12070959

Luo, M., Zhang, H., Du, Y., Idrees, A., He, L., Chen, J., et al. (2018). Molecular identification of cultivable bacteria in the gut of adult *Bactrocera tau* (Walker) and their trapping effect. *Pest Manag. Sci.* 74, 2842–2850. doi:10.1002/ps.5074

Maketon, M., Amnuaykanjanasin, A., and Kaysorngup, A. (2014). A rapid knockdown effect of *Penicillium citrinum* for control of the mosquito *Culex quinquefasciatus* in Thailand. *World J. Microbiol. Biotechnol.* 30, 727–736. doi:10.1007/s11274-013-1500-4

Meekes, E. T. M. (2001). *Entomopathogenic fungi against whiteflies: Tritrophic interactions between aschersonia species, trialeurodes vaporariorum and bemisia argentifolii, and glasshouse crops*. Ann Arbor, MI: Wageningen University and Research ProQuest Dissertations Publishing.

Migiro, L. N., Maniania, N. K., Chabi-Olaye, A., Wanjoya, A., and Vandenberg, J. (2011). Effect of infection by *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) on the feeding and oviposition of the pea leafminer *Liriomyza huidobrensis* (Diptera: Agromyzidae) on different host plants. *Biol. Control* 56, 179–183. doi:10.1016/j.biocontrol.2010.09.013

Monnerat, R., Martins, E., Queiroz, P., Ordúz, S., Jaramillo, G., Benintende, G., et al. (2006). Genetic variability of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) populations from Latin America is associated with variations in susceptibility to *Bacillus thuringiensis* cry toxins. *Appl. Environ. Microbiol.* 72, 7029–7035. doi:10.1128/aem.01454-06

Montezano, D. G., Sosa-Gómez, D. R., Specht, A., Roque-Specht, V. F., Sousa-Silva, J. C., Paula-Moraes, S. V. de, et al. (2018). Host plants of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the americas. *Afr. Entomol.* 26, 286–300. doi:10.4001/003.026.0286

Negrisoli, A. S., Garcia, M. S., Barbosa Negrisoli, C. R. C., Bernardi, D., and da Silva, A. (2010a). Efficacy of entomopathogenic nematodes (Nematoda: Rhabditida) and insecticide mixtures to control *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) in corn crops. *Crop Prot.* 29, 677–683. doi:10.1016/j.cropro.2010.02.002

Negrisoli, A. S., Garcia, M. S., and Negrisoli, C. R. C. B. (2010b). Compatibility of entomopathogenic nematodes (Nematoda: Rhabditida) with registered insecticides for *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) under laboratory conditions. *Crop Prot.* 29, 545–549. doi:10.1016/j.cropro.2009.12.012

Ondiaka, S., Maniania, N. K., Nyamasyo, G. H. N., and Nderitu, J. H. (2008). Virulence of the entomopathogenic fungi Beauveria bassiana and Metarhizium anisopliae to sweet potato weevil Cylas puncticollis and effects on fecundity and egg viability. *Ann. Appl. Biol.* 153, 41–48. doi:10.1111/j.1744-7348.2008.00236.x

Opisa, S., du Plessis, H., Akutse, K. S., Fiaboe, K. K. M., and Ekesi, S. (2018). Effects of entomopathogenic fungi and Bacillus thuringiensis-based biopesticides on Spoladea recurvalis (Lepidoptera: Crambidae). *J. Appl. Entomol.* 142, 617–626. doi:10.1111/jen.12512

Pires, L. M., Marques, E. J., Wanderley-Teixeira, V., Teixeira, Á. A. C., Alves, L. C., and Alves, E. S. B. (2009). Ultrastructure of Tuta absoluta parasitized eggs and the reproductive potential of females after parasitism by Metarhizium anisopliae. *Micron* 40, 255–261. doi:10.1016/j.micron.2008.07.008

Polanczyk, R. A., Silva, R. F. P. da, and Fiuza, L. M. (2000). Effectiveness of *Bacillus thuringiensis* strains against *Spodoptera frugiperda*(Lepidoptera: Noctuidae). *Braz. J. Microbiol.* 31, 164–166. doi:10.1590/S1517-83822000000300003

Qadir, Z. A., Idrees, A., Mahmood, R., Sarwar, G., Bakar, M. A., Ahmad, S., et al. (2021). Effectiveness of different soft Acaricides against honey bee ectoparasitic mite *varroa destructor* (Acari: Varroidae). *Insects* 12, 1032. doi:10.3390/insects12111032

Rwomushana, I., Bateman, M., Beale, T., Beseh, P., Cameron, K., Chiluba, M., et al. (2018). Fall armyworm: Impacts and implications for Africa. *Outlooks Pest Manag.* 28 (5), 196–201. doi:10.1564/v28_oct_02

Salvadori, J. D. M., Defferrari, M. S., Ligabue-Braun, R., Yamazaki Lau, E., Salvadori, J. R., and Carlini, C. R. (2012). Characterization of entomopathogenic nematodes and symbiotic bacteria active against *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and contribution of bacterial urease to the insecticidal effect. *Biol. Control* 63, 253–263. doi:10.1016/j.biocontrol.2012.08.002

Shahriari, M., Zibaee, A., Khodaparast, S. A., and Fazeli-Dinan, M. (2021). Screening and virulence of the entomopathogenic fungi associated with *Chilo suppressalis* walker. *J. Fungi.* 7, 34. doi:10.3390/jof7010034

Shapiro, S. S., and Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika* 52, 591–611. doi:10.2307/2333709

Sharanabasappa, A., Kalleshwaraswamy, C. M., Asokan, R., Swamy, H. M. M., Maruthi, M. S., Pavithra, H. B., et al. (2018). First report of the fall armyworm, *Spodoptera frugiperda* (JE Smith)(Lepidoptera: Noctuidae), an alien invasive pest on maize in India. *Pest Manag. Horti. Eco. GU* 24, 23–29. Available at: http://aapmhe.in/816.

Sparks, A. N. (1979). A review of the biology of the Fall Armyworm. *Fla. Entomol.* 62, 82–87. doi:10.2307/3494083

Stokstad, E. (2017). New crop pest takes Africa at lightning speed. *Science* 356, 473–474. doi:10.1126/science.356.6337.473

Sun, X., Hu, C., Jia, H., Wu, Q., Shen, X., Zhao, S., et al. (2021). Case study on the first immigration of fall armyworm, *Spodoptera frugiperda* invading into China. *J. Integr. Agric.* 20, 664–672. doi:10.1016/s2095-3119(19)62839-x

Tefera, T., and Pringle, K. L. (2003). Food consumption by *Chilo partellus* (Lepidoptera: Pyralidae) larvae infected with *Beauveria bassiana* and *Metarhizium anisopliae* and effects of feeding natural versus artificial diets on mortality and mycosis. *J. Invertebr. Pathol.* 84, 220–225. doi:10.1016/j.jip.2003.11.001

Thomas, M. B., Blanford, S., and Lomer, C. (1997). Reduction of feeding by the variegated grasshopper, *Zonocerus variegatus*, following infection by the fungal pathogen. *Metarhizium Flavoviride. Biocontrol Sci. Technol.* 7, 327–334. doi:10.1080/09583159730730

Tillman, P. G. (2010). Parasitism and predation of stink bug (Heteroptera: Pentatomidae) eggs in Georgia corn fields. *Environ. Entomol.* 39, 1184–1194. doi:10.1603/en09323

Tong, P. (2000). *History of maize science and technology in China*. Beijing: Chinese Science and Technology Press.

Trougakos, L. P., and Margaritis, L. H. (2002). "Novel morphological and physiological aspects of insect eggs," in *Chemoecology of insect eggs and egg deposition*. Editors M. Hilker and T. Meiners (Oxford, UK: Blackwell Publishing), 3–31. doi:10.1002/9780470760253.ch1

Xu, P., Zhang, D., Wang, J., Wu, K., Wang, X., Wang, X., et al. (2019). The host preference of *Spodoptera frugiperda* on maize and tobacco. *Plant Prot.* 45, 61–64.

Yang, X. L., Liu, Y. C., Luo, M. Z., Li, Y., Wang, W. H., Wan, F., et al. (2019). *Spodoptera frugiperda* (JE Smith) was first discovered in Jiangcheng county of Yunnan province in southwestern China. *Yunnan Agric.* 1, 72.

Yang, X., Sun, X., Zhao, S., Li, J., Chi, X., Jiang, Y., et al. (2020). Population occurrence, spatial distribution and sampling technique of fall armyworm *Spodoptera frugiperda* in wheat fields. *Plant Prot.* 46, 10–16. doi:10.16688/j.zwbh.2019586-en

Zhang, D., Zhao, S., Wu, Q., Li, Y., and Wu, K. (2021). Cold hardiness of the invasive fall armyworm, *Spodoptera frugiperda* in China. *J. Integr. Agric.* 20, 764–771. doi:10.1016/S2095-3119(20)63288-9

Zhang, L., Jin, M., Zhang, D., Jiang, Y., Liu, J., Wu, K., et al. (2019). Molecular identification of invasive fall armyworm *Spodoptera frugiperda* in Yunnan Province. *Plant Prot.* 45, 19–24. doi:10.16688/j.zwbh.2019121-en

Zhang, L., Shi, W., and Feng, M. (2014). Histopathological and molecular insights into the ovicidal activities of two entomopathogenic fungi against two-spotted spider mite. *J. Invertebr. Pathol.* 117, 73–78. doi:10.1016/j.jip.2014.02.005

frontiers | Frontiers in Physiology

TYPE Original Research
PUBLISHED 27 March 2023
DOI 10.3389/fphys.2023.1130389

OPEN ACCESS

EDITED BY
Ting Li,
Alabama State University, United States

REVIEWED BY
Jianhong Li,
Huazhong Agricultural University, China
Ran Wang,
Beijing Academy of Agriculture and Forestry Sciences, China

*CORRESPONDENCE
Jianhui Wu,
jhw@scau.edu.cn
Shaukat Ali,
aliscau@scau.edu.cn

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Invertebrate Physiology, a section of the journal Frontiers in Physiology

RECEIVED 23 December 2022
ACCEPTED 13 March 2023
PUBLISHED 27 March 2023

CITATION
Chen W, Li Z, Zhou C, Ali A, Ali S and Wu J (2023), RNA interference in cytochrome P450 monooxygenase (CYP) gene results in reduced insecticide resistance in *Megalurothrips usitatus* Bagnall. *Front. Physiol.* 14:1130389. doi: 10.3389/fphys.2023.1130389

RNA interference in cytochrome P450 monooxygenase (CYP) gene results in reduced insecticide resistance in *Megalurothrips usitatus* Bagnall

Weiyi Chen[1†], Zhaoyang Li[1†], Chenyan Zhou[1], Asad Ali[2], Shaukat Ali[1*] and Jianhui Wu[1*]

[1]Key Laboratory of Bio-Pesticide Innovation and Application, Engineering Research Center of Biological Control, College of Plant Protection, South China Agricultural University, Guangzhou, China, [2]Department of Agriculture, Abdul Wali Khan University, Mardan, Pakistan

Genes of the cytochrome P450 (CYP450) superfamily are known to be involved in the evolution of insecticide resistance. In this study, the transcriptomes of two *Megalurothrips usitatus* Bagnall (Thysanoptera: Thripidae) strains (resistant and susceptible) were screened for detoxification genes. *MusiDN2722* encodes a protein composed of 504 amino acid residues with a relative molecular mass of 57.3 kDa. Multiple sequence alignment and phylogenetic analysis showed that *MusiDN2722* is a member of the CYP450 family and has characteristics of the conserved CYP6 domain shared by typical CYP450 family members. RT-qPCR (real-time quantitative polymerase chain reaction) analysis showed that *MusiDN2722* was upregulated in the acetamiprid-resistant strain compared with the susceptible strain ($p < 0.05$), and the relative expression level was significantly higher at 48 h after exposure than at 24 h after exposure. The interference efficiency of the injection method was higher than that of the membrane-feeding method. Silencing of *MusiDN2722* through RNA interference significantly increased the sensitivity of *M. usitatus* to acetamiprid. Overall, this study revealed that *MusiDN2722* plays a crucial role in the resistance of *M. usitatus* to acetamiprid. The findings will not only advance our understanding of the role of P450s in insecticide resistance but also provide a potential target for the sustainable control of destructive pests such as thrips.

KEYWORDS

***Megalurothrips usitatus* (Bagnall), insecticide resistance, transcriptome, cytochrome P-450, RNA interference**

1 Introduction

The bean flower thrip *Megalurothrips usitatus* Bagnall (Thysanoptera: Thripidae) is a major pest of leguminous crops grown in southern China, especially in Hainan Province (Huang et al., 2018; Yang et al., 2021). A range of synthetic insecticides are extensively used for *M. usitatus* management, but its high reproduction rate and short generation time have induced the development of insecticide resistance. Acetamiprid, a second-generation nicotinoid insecticide, acts through antagonization of nAChR receptors, thus hindering nerve impulse transmission across the central nervous system of insects (Elbert et al., 2008; Phogat et al., 2022). In recent years, various thrip species (*Frankliniella occidentalis*, *Thrips*

tabaci, *Thrips hawaiiensis*, *Scolothrips takahashii*, and *M. usitatus*) have developed resistance to acetamiprid in different regions of the world (Mori and Gotoh, 2001; Chen and Yuan, 2011; Fu et al., 2016; Nazemi et al., 2016; Wang et al., 2016; Han et al., 2017; Zuo et al., 2017; Fu et al., 2019; Lin et al., 2021). The mechanism of resistance to neonicotinoids is mainly attributed to two factors: target insensitivity and increased metabolic detoxification (Puinean et al., 2010; Ihara et al., 2020). Enhanced detoxification mediated by insect cytochrome P450 monooxygenases (CYPs) is a major mechanism of resistance development.

CYP450s play an essential physiological role in the growth, development, and reproduction of insects (Scott, 1999; Cui et al., 2016). They are also involved in the biosynthesis and degradation pathways of endogenous compounds (such as pheromones, 20-hydroxyecdysone, and juvenile hormone (JH)) (Cifuentes et al., 2012; Roberto et al., 2017; Xu et al., 2020). Cytochrome P450 (CYP450) is the main detoxification enzyme in insects, and its action is considered to be one of the main mechanisms underlying resistance of insects to insecticides (Berge et al., 1998). CYP450 might be involved in resistance and cross-resistance mechanisms in the MEAM1 whitefly (*Bemisia tabaci* Gennadius) (Zhou et al., 2020). Three P450 genes (*CYP6CY14*, *CYP6DC1*, and *CYP6CZ1*) have been found to be involved in the development of resistance to acetamide in *Aphis gossypii* (Farman et al., 2020). By knocking out *CYP4PR1*, which is highly expressed in epidermal tissues, the susceptibility of pyrethroid-resistant *Triatoma infestans* can be increased (Dulbecco et al., 2021). Enhanced detoxification mediated by CYPs is the main mechanism of insecticide resistance development in *F. occidentalis* and *Thrips palmi* (Espinosa et al., 2005; Bao et al., 2014). However, the involvement of P450 genes in the development of insecticide resistance in an insect strain with an extremely high degree of resistance to neonicotinoids has not been elucidated in detail.

In this study, a highly acetamiprid-resistant *M. usitatus* strain (established in the laboratory through consecutive selection for 40 generations) was subjected to transcriptome analysis, followed by cloning of the P450 gene *MusiDN2722* to study the role of this gene in the development of acetamiprid resistance in *M. usitatus*. These results will provide basic information on the mechanism of neonicotinoid resistance in *M. usitatus* and thus can help with the formulation of management strategies for acetamiprid-resistant populations of *M. usitatus* in the field.

2 Materials and methods

2.1 Insect-rearing

Two *M. usitatus* Bagnall strains (one acetamiprid-resistant (AcR) and one susceptible (SS)) were used in this study. The SS strain was collected from Nanbin Farm, Sanya City, Hainan Province, in 2008 and was reared for 60 generations in the laboratory without exposure to any insecticide. The AcR strain was established from the SS population through continuous exposure to acetamiprid for 40 generations, using the leaf dip bioassay method (Rueda and Shelton, 2003). Both strains were reared on fresh cowpea pods in the laboratory at 26°C ± 1°C, under a photoperiod of 14:10 h (light:dark).

2.2 Bioassays

The median lethal concentration was determined using the leaf tube film method. Acetamiprid (25% water dispersible granules) was used in a commercially available formulation. Five graded concentrations of acetamiprid were used. Cowpea pods were dipped for 15 s in the designated concentration of insecticide or distilled water (the latter as a control) and placed in the shade until air dried. Emerging adult insects were transferred to the beans. Bioassays were performed in the laboratory at 26°C ± 1°C, under a photoperiod of 12:12 h (light:dark). Each concentration was performed in triplicate, and mortality was assessed after 2 days. LC_{50} (lethal concentration 50%) values were calculated via probit analysis using the SPSS software package (LeOra Software Inc., Berkeley, CA, United States). Resistance factor (RF) was estimated at LC_{50} as RR = LC_{50} of the AcR strain/LC_{50} of the SS strain; the 95% CI for RR was calculated following Preisler and Robertson (1989).

2.3 Transcriptome analysis and P450 gene selection

A total of 500 adult females of both the susceptible and resistant strains were collected for three biological replicates. Sample processing, extraction, and metabolite detection for transcriptome analysis were performed by Suzhou PANOMIX Biomedical Tech Co., Ltd. (Suzhou, China), following standard procedures, and the fragments per kilobase of transcript per million mapped reads (FPKM) of the assembled transcripts was calculated. Transcript expression abundance was calculated using the FPKM method (Mortazavi et al., 2008). Benjamini–Hochberg correction of the *p*-value for multiple tests was applied using the false discovery rate (FDR). FDR ≤0.001 and absolute value of the log2 ratio ≥2 were the thresholds for determining significance of differences in gene expression (Itai et al., 2001). Given the large number of P450s in the *M. usitatus* transcriptome, we first characterized gene expression using real-time quantitative polymerase chain reaction (RT-qPCR). Our goal was to identify genes with consistent differential expression.

2.4 Construction and identification of a recombinant plasmid with *MusiDN2722*

The SteadyPure Agarose Gel DNA Purification Kit (Accurate Biotechnology, China) was used to purify and recycle the cloned *MusiDN2722* PCR products. The primers used are listed in Supplementary Table S1. The following procedure was adopted to combine the vector components in a 5-μL reaction system. First, the reaction mixture was blended gently and placed in a PCR instrument at 25°C for 5 min. A measure of 5 ml of the transformation product was centrifuged for 1 min, and the entire bacterial solution was subsequently used to cover the Luria–Bertani solid culture. Next, 100 μl of the PCR product was mixed with Trans1-T1 phage-resistant chemically competent cells and aseptically coated on a Luria–Bertani (LB)/ampicillin plate. After growth, preparation, and plasmid DNA analysis, the plate was incubated at 37°C for 12 h; subsequently, a colony was harvested.

The colony was cultured in 5 mL of LB liquid medium with ampicillin; the cells were cultured overnight at 37°C. Thereafter, white monoclonals were collected in 10 μL of sterile water and vortexed. A measure of 1 μl of the mixture was mixed with 20 μL of PCR mixture, and positive clones were identified with M13 forward and M13 reverse primers. After these steps, the recombinant plasmid from the positive bacterial fluid was sequenced by Shanghai Sangon Biological Company to determine the clone. The GenBank nucleotide sequence was subtyped and homologously analyzed.

2.5 RNA extraction and RT-qPCR

Total RNA was extracted from 300 adults and nymphs of the AcR and SS strains of *M. usitatus* using the Total RNA TRIzol Extractor (Sangon Biotech, China, Shanghai). cDNA was constructed from the total RNA using the PrimeScript™ RT Reagent Kit with gDNA Eraser (TaKaRa, Tokyo, Japan). RT-qPCR was performed in CFX96 TOUCH (Bio-Rad) using TB Green® Premix Ex Taq™ II (Tli RNaseH Plus; TaKaRa, Japan). Gene-specific primers were designed using Premier 5.0 and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). Experiments were performed thrice with different RNA preparations for each strain. The following cycling conditions were used: 95°C for 30 s; 40 cycles of 95°C for 5 s, 60°C for 30 s, 60°C for 30 s; and 95°C for 1 s for plate reading. Reaction fluorescence was continuously monitored after the cycling protocol using the dissociation temperature of the PCR products at a temperature transition rate of 0.1°C/s to generate a melting curve. Relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method (Pfaffl, 2001). The RT-qPCR product was resolved via 1.0% agarose gel electrophoresis, and a DNA fragment of approximately 500 bp was obtained. These results indicated that P450 was expressed in both SS and AcR strains of *M. usitatus*.

2.6 Bioinformatic analysis

The sequencing results were submitted to the NCBI (National Center for Biotechnology Information), and the target gene sequences were predicted using the open reading frame (ORF) and conserved domain. The amino acid sequence of the protein encoded by *MusiDN2722* was predicted and analyzed using bioinformatics software applications. The physicochemical properties of the target protein were predicted using ProtParam software (SIB, Swiss Institute of Bioinformatics). The transmembrane region of the target protein was predicted using the TMHMM-2.0 online tool (Department of Health Technology). The signal peptide of the target protein was predicted using the Signal P5.0 server. Finally, the phylogenetic relationships of the target proteins were predicted using MEGA 7.

2.7 RNA interference

Specific primers for dsRNA synthesis were designed based on the cDNA sequence of *MusiDN2722* and the fragment sequence of a green fluorescent protein (GFP) containing T7 polymerase promoter sequences at both ends. Approximately 50 newly emerged 3-day-old adult females were collected and placed in a specially designed device. Ten biological replicates were set up. Two approaches were employed for RNA interference: membrane feeding and microinjection of dsRNA. In the membrane-feeding approach, the mouth of a tube was covered with a thin film of BuddyTape (Aglis, Japan). Thereafter, 30 μL of dsRNA was added to the membrane and sealed with Parafilm (Sangon Biotech). After 2 consecutive days of feeding, live adults were collected and half of them were subjected to fluorescence qPCR to verify the silencing efficiency. The remaining half of the live adults were treated with acetamiprid at LC_{50} for bioassays. In the microinjection approach, dsRNA was injected using a microinjector between the mesothoracic shield plate and abdominal segment shield plate into female adult worms.

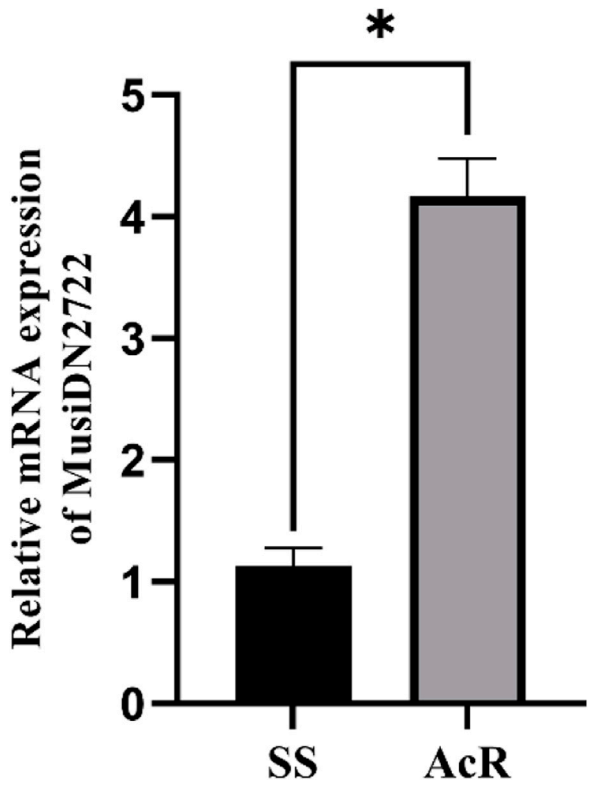


FIGURE 1
Expression of *MusiDN2722* in two strains. *significant difference ($p < 0.05$).

2.8 Data analysis

All results are expressed in the form mean ± standard error; IBM SPSS Statistics 20 software (SPSS, Chicago, IL, United States) was used for statistical analyses. A Student's *t*-test or one-way analysis of variance (ANOVA) was used to compare the differences between samples or among multiple samples in RT-qPCR and bioassays, respectively. Differences were considered significant at $p > 0.05$.

10.3389/fphys.2023.1130389

```
1       CGGCGGGTGGCGGCGGGCGGAGGCGAGGGCAGCGGGGCGTCAACATGTGAGTCGTATATAGCGCCGCGGGCTGGAGCGGCAGCAGTACCC
91      ACAGCGTGCTGCAGACACCAACATGGCGCTGACCATCCTCTTGACGGCGGCCGTGGCCGTCCTCGCCCTCGTCTACCTGTTCTTCTCGCA
31                              M  A  L  T  I  L  L  T  A  A  V  A  V  L  A  L  V  Y  L  F  F  S  H
181     CCGCTTCCAGCACTGGAGCAAGCGCGGCGTGCCCTTCCCCAAGCCGACGCCCTTCTTCGGCAACTTCGGGCCCACGATCCGGGGCAAGAG
61        R  F  Q  H  W  S  K  R  G  V  P  F  P  K  P  T  P  F  F  G  N  F  G  P  T  I  R  G  K  R
271     GCCGTTCACGAAGCTGGCGCTGGACCTGGCGGTGCCGTTCCGCGAGCAGGGCCTGGGCTACTGCGGCATCTACCAATGGTGCAAGCCCAT
91        P  F  T  K  L  A  L  D  L  A  V  P  F  R  E  Q  G  L  G  Y  C  G  I  Y  Q  W  C  K  P  I
361     CCTCATGGTGTGGGACCCGGAGATGGTGAAGCAGATCGTGTGCAAGGACTTCAGCAGCTTCCACGACCGCGGCATGCCCACCAACGACAG
121       L  M  V  W  D  P  E  M  V  K  Q  I  V  C  K  D  F  S  S  F  H  D  R  G  M  P  T  N  D  S
451     CGACCCCCTCAGCCTGCACCTGTTCAACCTGAGCGGCCCGAAGTGGCGGAACCTGCGCAACAGGCTCAGCCCCACCTTCACGTCCGGCAA
151       D  P  L  S  L  H  L  F  N  L  S  G  P  K  W  R  N  L  R  N  R  L  S  P  T  F  T  S  G  K
541     GCTCAAGATGATGCTGCCGCTGATGAGTGACATCGGTGACAACCTCAGCACCACCCTGTCCGAGGAGGCGAAGCGCAGCGAGTCGCACGA
181       L  K  M  M  L  P  L  M  S  D  I  G  D  N  L  S  T  T  L  S  E  E  A  K  R  S  E  S  H  E
631     GGTGGACGTCAGCAGCCTGCTGTCCCGGTTCGCGACGGACATCATCGGCTCGGTGGCGTTCGGCATCCAGTGCAACTGCCTCCGCGACGA
211       V  D  V  S  S  L  L  S  R  F  A  T  D  I  I  G  S  V  A  F  G  I  Q  C  N  C  L  R  D  E
721     GAACAACGAGTTCCTGGCCATGTCCCGCCAGATCTTCCAGCAGACGCCCGCCCAGTTCGTGCGCTTCCTGCTGGACTCGCTGCACCCCAA
241       N  N  E  F  L  A  M  S  R  Q  I  F  Q  Q  T  P  A  Q  F  V  R  F  L  L  D  S  L  H  P  K
811     GCTGGGCCGGCTGCTGCCGTTCAAGATCATGTTCAACAGCGTGCACAACTTCTTCATCACGCTGATGCGGGACACGGTCAAGTACCGCGA
271       L  G  R  L  L  P  F  K  I  M  F  N  S  V  H  N  F  F  I  T  L  M  R  D  T  V  K  Y  R  E
901     GGAGAACAAGGTGGAGCGCAACGACTTCGTGCAGCTCATGATGCAGCTGCGCGACCAGGACCTGGCCAACGCCGACCCGCTCAACCACAT
301       E  N  K  V  E  R  N  D  F  V  Q  L  M  M  Q  L  R  D  Q  D  L  A  N  A  D  P  L  N  H  M
991     GGAGCTGACGCCCGGCGTGATGGCGGCGCAGGGCTTCATCTTCTTCATCGCCGGCCTCGAGTCGACCTCCAACACCATCGCCTACTGCCT
331       E  L  T  P  G  V  M  A  A  Q  G  F  I  F  F  I  A  G  L  E  S  T  S  N  T  I  A  Y  C  L
1081    CAGCAAGCTGGCCCAGGACCCCGCCCTCCAGGACCGCGTGGCCGGCGAGGTGCGCGACGTGCTCCGGCAGCACGACGGCCAGATGACGTA
361       S  K  L  A  Q  D  P  A  L  Q  D  R  V  A  G  E  V  R  D  V  L  R  Q  H  D  G  Q  M  T  Y
1171    CGAGGCCCTCAAGAAGATGGAGCTCGTCAACTGCACCGCGCTCGAGGGCCTGCGCCTCTGGCCCCCCGTCGGCGTGCTCATCCGCAAGTG
391       E  A  L  K  K  M  E  L  V  N  C  T  A  L  E  G  L  R  L  W  P  P  V  G  V  L  I  R  K  C
1261    CAACGAGACGACCAAGGTCGGCGACGTGCTCATCGAGAAGGACATGCCCATCTTCATCGTCTCGGACGCCATGCACACCGACGAGAACAT
421       N  E  T  T  K  V  G  D  V  L  I  E  K  D  M  P  I  F  I  V  S  D  A  M  H  T  D  E  N  I
1351    CTTCCCCGAGCCGCAGCGCTTCGACCCGGACCGGTTCTCGGCCGAGAACCGGTCCCAGCGGCACCCCTACTCGTTCCTGCCGTTCGGCGA
451       F  P  E  P  Q  R  F  D  P  D  R  F  S  A  E  N  R  S  Q  R  H  P  Y  S  F  L  P  F  G  E
1441    GGGGCCCAGGATCTGCATCGCCGAGCGCTTCGCGCTGCTCGAGATGAAGCTCGCCCTCGCCGTGCTGCTGAGGGACTTCACCTTCTCGAC
481       G  P  R  I  C  I  A  E  R  F  A  L  L  E  M  K  L  A  L  A  V  L  L  R  D  F  T  F  S  T
1531    CGGCCCCAGCTTCGAGGCCGACGCCGAGCTCGACCCCTCCGCCTTCTTCCCCAGGCCCAAGAACGGCTTCAAGCTGCGCGTCGAGGCCAG
511       G  P  S  F  E  A  D  A  E  L  D  P  S  A  F  F  P  R  P  K  N  G  F  K  L  R  V  E  A  R
1621    GACGTAGGCCGCAGCTTGCTGCAGGTGTGATTCTTAGACTCGCATCAGTCGTGTGTGTGTGTGTGTGTGTAAGGGAGAGAGATACTGT
541       T  *
1711    ATTTGTGTGTGTGTGTGTGTGAAAGGAGGTAGTGCTTGTCCGTGTCTTGATAAGGATTATCGAGATCATGACTATGACATATTGAGAATG
1801    TACGAGCATTGGGTGTGACATATGGATTAGGCTTGGGCGCTGATTTTTATTTTGTTTTGTTTGTTGTCTGTTCGGATGACCTTTGATATA
1891    ACATCATTGTATCAGTGTGAATTTCGTCATCCCTTACGGGCAAATTGTGAACTTGTGACCCTGTGATACTACAAAATGTTTATGTGTTAC
1981    CGTACGACAGAGCGTGTCCATTAATGAAGTCTATGATCCTCACTTTGTTTGTGTTGTATTTACGTTGCCCCTTTACATGTGTATATTTTC
2071    ATGTCATATGACTTCTTTGATATTTTCAAATCTCTTATAAATAGTATTATACAGTATATCATTTTCAGCAAT
```

FIGURE 2
Nucleotide and deduced amino acid sequence of *MusiDN2722*.

3 Results

3.1 Analysis of expression profile

After 40 generations of selection, the screening acetamiprid concentration was determined to be 4,700 mg/L, and the LC_{50} values of acetamiprid for the SS and AcR strains were 85.676 and 1439.425 mg/L, respectively. The AcR strain developed a moderate level of resistance to acetamiprid, with a 16.78-fold resistance ratio.

The RT-qPCR analysis showed that the expression of *MusiDN2722* was 4.17 times higher in the AcR strain than in the SS strain (Figures 1, 2). Transcriptomic sequencing analysis of the susceptible and resistant strains (SS and AcR) showed that there were more than 40 million original sequences. The Trinity software tool was used to concatenate the filtered sequences, and 21,740 unigenes were obtained (Supplementary Figure S1); the total length was 38343948 bp. The results of differential expression analysis based on the FPKM values showed that there were 167 upregulated and 139 downregulated genes in AcR strains compared with those in the SS strain (Supplementary Figure S2). The results of GO (Gene Ontology) term enrichment analysis indicated enrichment of genes under the following terms: binding, catalytic activity, cellular process, metabolic process, single-organism process, cell, cell junction, and organelle (Supplementary Figure S3). The results of KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis indicated enrichment of genes under the following terms: carbohydrate metabolism, translation, folding, sorting and degradation, signal transduction, transport and catabolism, and the endocrine system (Supplementary Figure S4). The P450 superfamily genes were identified in the transcriptome analysis of the two strains of *M. usitatus*. We list the top 10 CYP450 genes with a fold difference greater than 2 in Tables 1 and 2. We selected a gene of interest (TRINITY_DN2722_c0_g1) for full characterization in this study; this gene was named *MusiDN2722*.

3.2 Bioinformatics analysis

3.2.1 Prediction of physicochemical properties

Analysis using the ProtParam tool showed that the molecular formula of *MusiDN2722* was $C_{2583}H_{4033}N_{699}O_{723}S_{28}$. The full-length cDNA sequence of *MusiDN2722* was 2075 bp, with the CYP6 conserved domain of the P450 superfamily. Sequence analysis showed that the ORF of *MusiDN2722* was 1515 bp and this encodes a protein of 504 amino acids with a relative molecular weight of 57.3 KDa and a PI (isoelectric point) of 11.06. The total number of negatively charged residues (Asp + Glu) carried by the protein was 61. The total number of positively charged residues (Arg + Lys) was 109. The total mean value of hydrophilicity was −0.128, and the instability coefficient was 39.59, indicating that *MusiDN2722* is hydrophilic and stable (Figure 3A). Analysis using the SignalP 4.1 and TMHMN 2.0 tools revealed that *MusiDN2722* had no signal peptide (Figure 3B) but had one transmembrane structure, which was in the range of 2–21 bp (Figure 3C).

TABLE 1 Genes annotated as cytochrome P450.

Gene ID	Log_2Fold	*p*-value	NR-annotation
TRINITY_DN4541_c3_g1	17.42010275	1.98E-01	Cytochrome p450
TRINITY_DN899_c0_g1	5.393972239	5.31E-01	Cytochrome P450 6k1
TRINITY_DN2722_c0_g1	3.54462	1.46E-01	Cytochrome p450
TRINITY_DN12852_c0_g1	3.452228214	6.82E-01	Cytochrome P450 6a2-like isoform X1
TRINITY_DN9313_c0_g1	2.904800048	9.00E-01	Cytochrome P450
TRINITY_DN1932_c1_g2	2.827139838	2.88E-01	Cytochrome P450 4C1
TRINITY_DN222_c4_g1	2.409297348	5.25E-01	Cytochrome P450
TRINITY_DN27581_c0_g2	2.230435956	7.39E-01	Cytochrome P450 9e2-like
TRINITY_DN22321_c0_g1	2.200350449	3.25E-01	Cytochrome P450 301a1

TABLE 2 Median lethal concentration (LC_{50}) of acetamiprid against *M. usitatus*.

Strain	LC_{50}(mg/L)	95%FL	Chi-square	Slope (±SE)	Resistance ratio
SS	85.676	60.57–111.61	0.602	1.045 ± 0.142	1.0
AcR	1439.425	1064.52–3316.60	1.637	0.663 ± 0.135	16.78

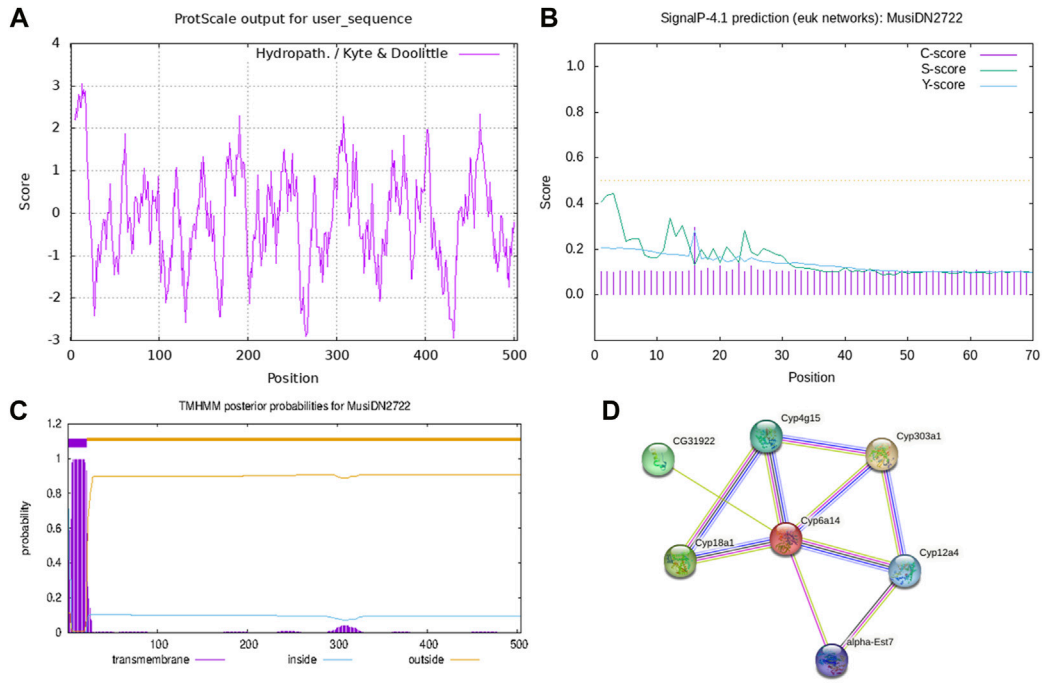


FIGURE 3
Domain analysis of *MusiDN2722*. **(A)** Hydrophobicity analysis. **(B)** Signal peptide analysis. **(C)** Prediction of transmembrane domain structures. **(D)** Prediction of protein.

3.2.2 *MusiDN2722* target protein prediction

We analyzed *MusiDN2722* using the STRING protein interaction database; the results showed that *MusiDN2722* may be related to the cytochrome C oxide subunit proteins Cyp303a (NP_0012859777.1), CYP18a1 (CAL69954.1), CYP4g15 (NP_727531.2), CYP12a4 (NP_650783.2), CG31922 (NP_722687.1), and alpha-EST7 (NP_524261.1) (Figure 3D).

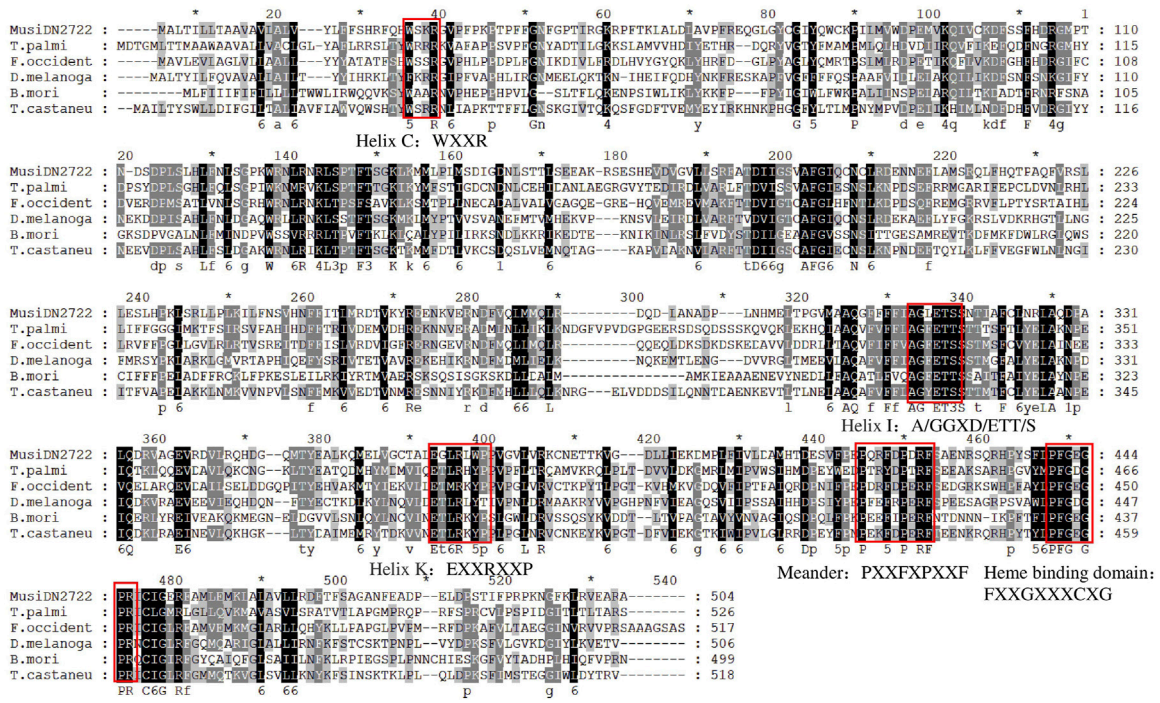


FIGURE 4
Multiple sequence alignment of *M. usitatus* cytochrome P450s. Included species and corresponding Genbank accession numbers: *T. palmi, Thrips palmi* (XP_034255739.1 CYP6a2); *F. occident, Frankliniella occidentalis* (KAE8752265.1 CYP6); *D. melanoga, Drosophila melanogaster* (AAF58185.2 CYP6a8); *B. mori, Bombyx mori* (XP_037874445.1 CYP6k1).

3.2.3 Multiple sequence alignment and phylogenetic tree analysis of *MusiDN2722*

Multi-sequence comparison results showed that six CYP450s had a common CYP450 feature sequence located in the spiral C region ("WXXR" sequence), the spiral K region ("EXXRXXP" sequence), and the Meander region ("PPXXF" sequence). Above the CYP450s, the characteristic sequence "FXXGXXXCXG" appeared in the heme-binding region and the sequence "A/GGXD/ETT/S" in the spiral I region. Phylogenetic analysis revealed that *MusiDN2722* was most closely related to CYP450 from *F. occidentalis* (Figure 4). The amino acid sequences of 37 CYP450 proteins from 26 other insects were analyzed phylogenetically (Figure 4). Based on the results of the evolutionary tree, the *MusiDN2722* gene cloned in this study belongs to the CYP6 family. The evolutionary distance between *MusiDN2722* and *FoccCYP6A14* was small; they shared 84.8% amino acid similarity and were clustered in the same branch as *Thrips palmi TpCYP6A13* and *TpCYP6A2*. This finding suggests that *MusiDN2722* is evolutionarily conserved.

3.3 *MusiDN2722* expression at different developmental stages and in different tissues of *M. usitatus*

RT-qPCR analysis of *M. usitatus* at different developmental stages indicated the expression of *MusiDN2722* in different instars. Expression was significantly higher during instars from the third larval stage to the adult stages than during the 1st and 2nd instar stages, and peaked during the 4th instar and adult stages. Expression was 1.67–3.23-fold higher at the 4th instar and female adult stages than at the other stages (Figures 5, 6A, 7). Expression in the head was used as a baseline (relative gene expression = 1) for comparison of gene expression in different tissues. The results of a relative gene expression test using RT-qPCR revealed that *MusiDN2722* was predominantly expressed in the thorax and abdomen, with levels 16.73- and 12.57-fold higher than the level in the head (Figure 6B).

3.4 Effects of membrane feeding and microinjection of ds*MusiDN2722* on ds*MusiDN2722* gene expression in *M. usitatus*

After 24 h, insects administered ds*MusiDN2722*, dsGFP, and 10% hydromel exhibited survival rates of 87.70%, 89.49%, and 88.54%, respectively. After 48 h, the survival rates were 78.53%, 81.19%, and 79.73%, respectively (Figure 7A1). At 24 and 48 h after microinjection for RNA interference, the survival rate of insects administered air (no injection solution), PBS, dsGFP, and ds*MusiDN2722* was assessed. As shown in Figure 6B, after 24 h, the survival rates of these insects were 80.00%, 81.48%, 68.00%, and 62.07%, respectively; after 48 h, the survival rates were 52.00%, 51.85%, 40.00%, and 41.38%, respectively. Analysis using SPSS revealed no significant difference between insects

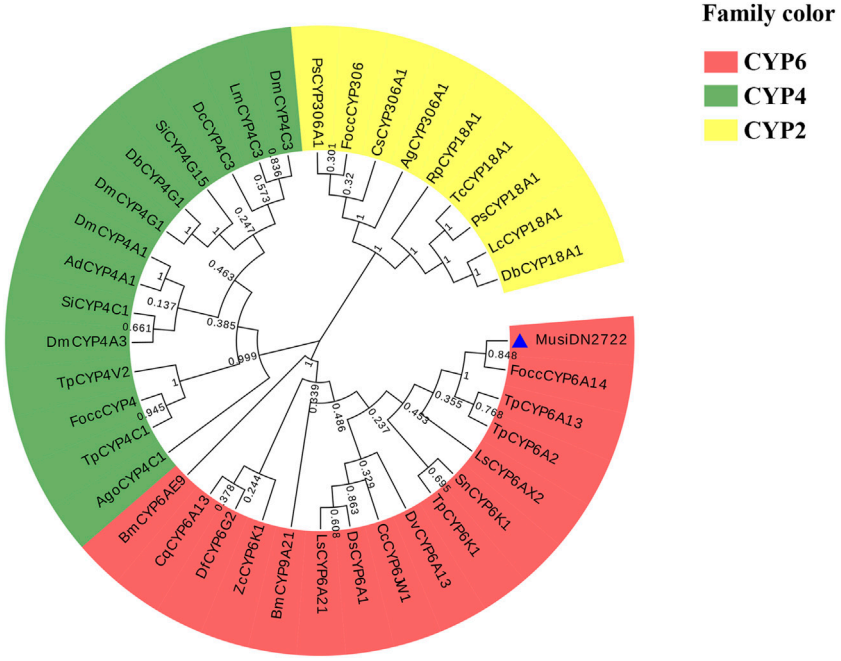


FIGURE 5
Phylogenetic analysis of *MusiDN2722*. Focc, *Frankliniella occidentalis*; Tp, *Thrips palmi*; Sn, *Schistocerca nitens*; Cc, *Ceratitis capitata*; Ls, *Laodelphax striatellus*; Dv, *Diabrotica virgifera*; Bm, *Bombyx mori*; Ds, *Drosophila simulans*; Df, *Drosophila ficusphila*; Zc, *Zerene cesonia*; Cq, *Culex quinquefasciatus*; Ls, *Lucilia sericata*; Bm, *Bombyx mori*; Tc, *Tribolium castaneum*; Lc, *Lucilia cuprina*; Rp, *Rhopalosiphum padi*; Cs, *Chilo suppressalis*; Ps, *Phyllotreta striolata*; Ag, *Anopheles gambiae*; Db, *Drosophila busckii*; Ad, *Anopheles darlingi*; Dm, *Drosophila melanogaster*; Si, *Solenopsis invicta*; Lm, *Locusta migratoria*; Dc, *Diaphorina citri*; Ago, *Aphis gossypii*.

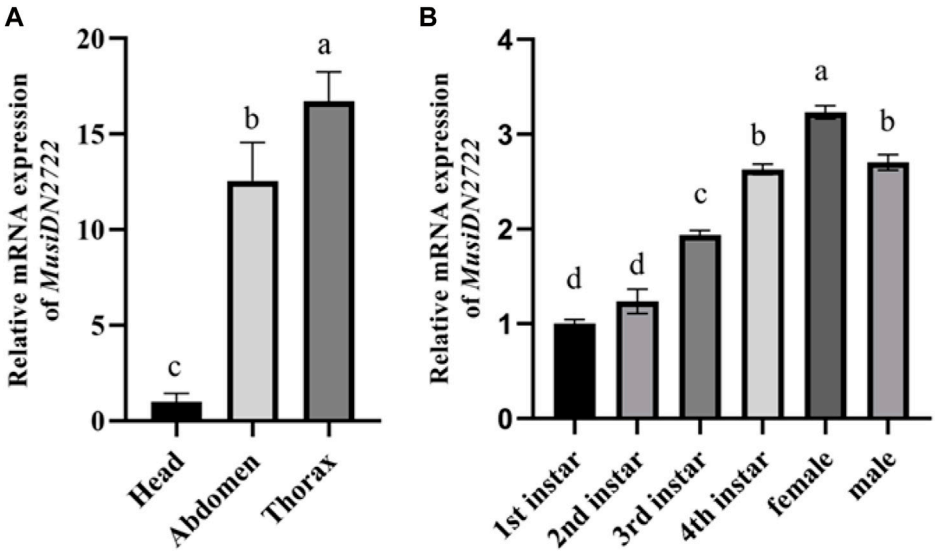


FIGURE 6
Relative levels of expression of *MusiDN2722* **(A)** in different adult tissues and **(B)** at different developmental stages of *M. usitatus*. Data are represented in the form mean ± SEM. Different letters indicate a significant difference at $p < 0.05$.

administered air and PBS or between those administered dsGFP and ds*MusiDN2722*, but the survival rate of the latter was considerably lower than that of the former (Figure 7B1).

The control group was fed and injected with dsGFP. RT-qPCR analysis showed that *MusiDN2722* expression in the membrane-fed insects, compared with that occurring in the

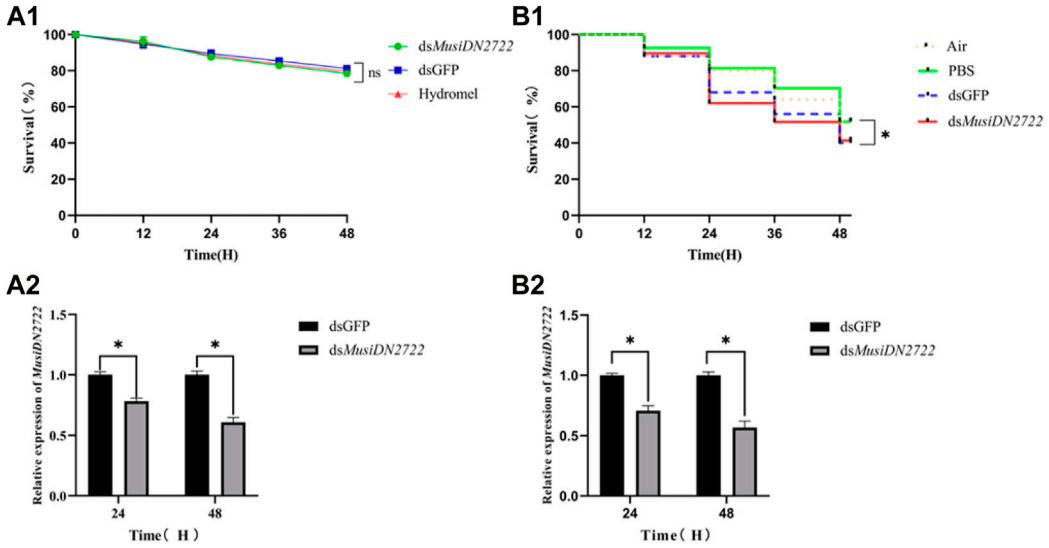


FIGURE 7
Survival rates after RNAi by different methods and gene expression of *MusiDN2722* at different times. **(A)** Feeding method; **(B)** injection method. Data are represented in the form mean ± SEM. *significant difference ($p < 0.05$).

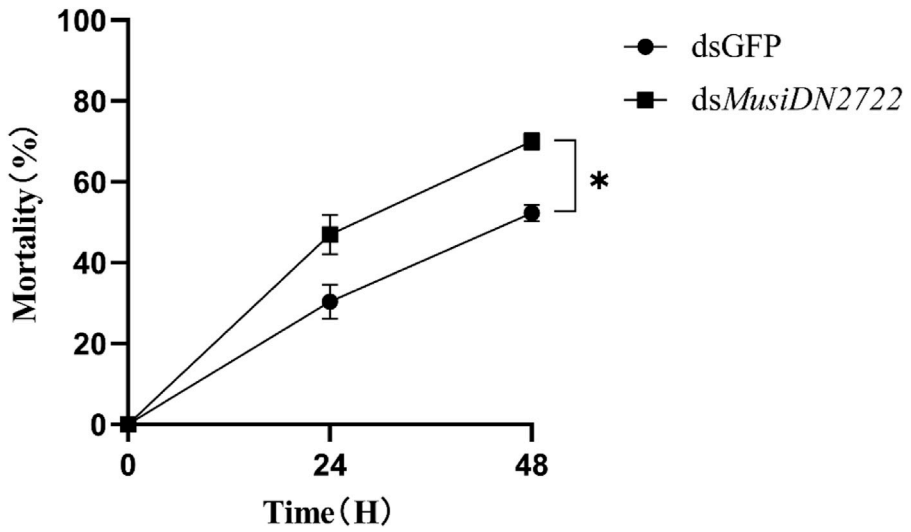


FIGURE 8
Mortality following dsRNA interference, 24 h and 48 h after treatment with acetamiprid LC_{50}. Data are represented in the form mean ± SEM. *significant difference ($p < 0.05$).

control group fed dsGFP, was 21.76% and 39.21% after 24 and 48 h, respectively (Figure 7A2). This finding suggests that interference via the membrane-feeding method was effective to some degree. ds*MusiDN2722* was microinjected into the cavity of *M. usitatus*. At 24 and 48 h after the injection, gene expression was 29.35% and 43.20%, respectively, of that occurring in the control group, and the difference between the groups was significant ($p < 0.05$) (Figure 7B2).

3.5 *MusiDN2722* modulates acetamiprid resistance in *M. usitatus*

The knockdown of *MusiDN2722* substantially increased the mortality of adults of the ACR strain relative to that of control adults upon exposure to 1878.99 mg/L acetamiprid. Toxicity bioassay showed that membrane-fed *M. usitatus* individuals were more susceptible to acetamiprid than the controls at 24 and 48 h

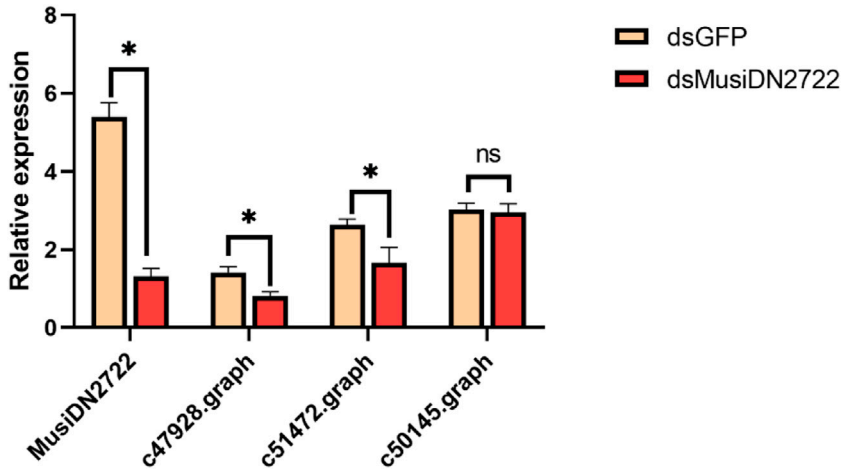


FIGURE 9
Expression of related genes after silencing of target genes.

(Figure 8). As shown in the figure, the mortality rate of the dsGFP group at 24 and 48 h was 30.38% and 52.31%, respectively, and that of the ds*MusiDN2722* group at 24 and 48 h was 46.98% and 68.29%, respectively. Compared with the dsGFP group, mortality increased by 23.4%.

3.6 Interacting protein prediction

Based on the results of prediction analysis using the STRING database, the corresponding genes were selected from the transcriptome for expression analysis. After silencing of *MusiDN2722*, there was a significant decrease in gene expression. CYP18A1, CYP12A4, and CYP4G15 correspond to transcripts C47928.graph, C51472.graph, and C50145.graph, respectively. According to the qPCR results, expression of C47928.graph and C51472.graph significantly decreased after silencing of *MusiDN2722*. C50145.graph exhibited no difference in expression before and after treatment (Figure 9).

4 Discussion

Owing to the widespread use of neonicotinoid insecticides for the control of *M. usitatus* and growing concerns regarding decreasing sensitivity, it is necessary to monitor resistance to these chemicals and elucidate the underlying mechanisms of resistance. *Megalurothrips usitatus* is a major threat to cowpea crops cultivated in Hainan and Guangdong provinces, China, and basic information on the mechanisms and pathways involved in insecticide resistance in *M. usitatus* is lacking. In this study, we observed increased susceptibility of *M. usitatus* to acetamiprid when expression of ds*MusiDN2722* was inhibited through RNA interference, indicating the possible involvement of *MusiDN2722* in the development of resistance and suggesting a possible target gene for genetic control of *M. usitatus*.

Monooxygenase-mediated detoxification is a common mechanism by which insects become resistant to insecticides (Scott, 1999). Studies have shown that the P450 genes related to insecticide resistance are mainly concentrated in the CYP3 (including CYP6 and CYP9) and CYP4 families (Feyereisen, 2012). In this study, the *MusiDN2722* sequence cloned from *M. usitatus* was used to construct a phylogenetic tree and was found to belong to the CYP6 clade, the family members of which have also been shown to play important roles in the detoxification and metabolism of toxic substances (Pan et al., 2018; Han et al., 2022). Through comparison with CYP genes of the same family in other insects, it was found that *MusiDN2722* shared the characteristic CYP450 sequence with other model insects, containing highly conserved hydrogen-bonding regions, including Helix-C (WxxxR), Helix-I (AGxxT), Helix-K (ExxR), Meander (FxxGxRxxxG), and the heme-binding domain (PxxFxPxxF) (Feyereisen, 2005).

The patterns of expression of detoxifying enzyme-coding genes at various stages of growth and development and in various tissues can, to a certain extent, reveal the functions of genes (Chung et al., 2009). These distinct expression patterns indicate that the proteins are involved in pesticide resistance and breakdown of secondary plant compounds (Ohkawa et al., 1999). Similarly to the findings of previous research (Xu et al., 2018; Hou et al., 2021), our findings revealed that *MusiDN2722* is expressed in both larvae and adults. For instance, it has been shown that mature worms (*Nilaparvata lugens*) express the P450 gene *CYP6ER1* (Mao et al., 2022). P450 activity differs between adult males and females. Adult females of *Culex pipiens quinquefasciatus* express the P450 gene at a higher level than adult males (Wang et al., 2014). This outcome was also validated in our research. Similarly, in *Chilo suppressalis*, the greatest level of expression of the P450 gene was observed in female adults (Bai

et al., 2018). With age, thrips are increasingly exposed to damaging compounds in the external environment. To adapt to the environment, adults, particularly females, should be expected to express *MusiDN2722* at higher levels to detoxify and metabolize exogenous chemicals for survival (FENG, 2020). This is a form of adaptive evolution in insecticide resistance that occurs in mosquitoes. Additionally, the quantity and quality of P450 gene expression vary across insect tissues. If the P450 gene is overexpressed in the thorax and abdomen of resistant adults, it may be implicated in insect midgut detoxification (Zhao et al., 2021). The insect thorax may feature P450-related functional sites and binding sites (Yang et al., 2016). For instance, the P450 gene is essential for ecdysteroid production in the prothoracic gland of the silkworm *Bombyx mori* and the fruit fly *Drosophila melanogaster* (Ryusuke et al., 2004). These previous findings may explain why expression of *MusiDN2722* is substantially higher in the chest and abdomen than in the head. In contrast, other studies have shown that in insects such as *B. tabaci* (Liu et al., 2020) and *Lygus pratensis* (Ma et al., 2022), P450 expression is substantially higher in the head than in the chest and abdomen. These findings imply that the expression profiles of these genes are insect-specific.

Genes of the insect CYP6 subfamily play crucial roles in plant–insect interactions, particularly in the case of polyphagous insect pests (Nelson, 1998). Recently, putative functions of *CYP6AB14* and *CYP6AB60* in detoxifying harmful plant compounds in *Spodoptera litura* have been revealed (Pottier et al., 2012; Wang et al., 2015). The resistance of *B. tabaci* to neonicotinoid insecticides involves upregulation of *CYP6CM1* (Jones et al., 2011). In our study, silencing of *MusiDN2722* led to increased sensitivity of *M. usitatus* to acetamiprid, suggesting that *MusiDN2722* plays an important role in metabolizing neonicotinoid pesticides, thus affecting the toxicity tolerance of *M. usitatus*. Seventy-four CYP genes have been found in the potato beetle *Leptinotarsa decemlineata*, and six CYP6 family genes (*CYP6BH2*, *CYP6BJ1*, *CYP6BQ17*, *CYP6EG1*, *CYP6EH1*, and *CYP6EJ1*) are involved in the detoxification process of cyhalothrin (Wan et al., 2013). In our study, we found that *MusiDN2722* knockdown resulted in downregulation of *CYP18A1* and *CYP12A4*. This finding indicates that insect resistance may involve more than one detoxification enzyme. The involvement of more than one gene in insecticide resistance has also been reported in insects such as *Musca domestica* L. (Liu and Scott, 1996), *D. melanogaster* (Meigen) (Pedra et al., 2004), *Helicoverpa armigera* (Yang et al., 2006), and *Plutella xylostella* (Bautista et al., 2007).

Heritable RNAi through dsRNA expression is not possible in most insect species; therefore, loss-of-function experiments are mainly performed by introducing dsRNA from outside the insect body (Yu et al., 2013). In this study, *MusiDN2722* of *M. usitatus* was silenced using two methods: membrane feeding and microinjection. The results showed that interference in the gene of interest was successfully achieved *via* both methods. However, the survival rate of insects was significantly higher under the membrane-feeding method than under the microneedle injection method. This result is consistent with a previous finding (Prentice et al., 2017) and provides a technical means for the subsequent study of gene function in large thrips and small insects. These results also suggest that we need to consider various factors in future application processes to improve the effect of RNA interference. In fact, there are many other ways to deliver dsRNA, such the delivery of nucleic acid drugs (DNA or RNA) to insects via the targeted delivery and controlled release functions of nanocarriers (Nadeau, 2017; Zhang et al., 2021), but these methods may be challenging in the case of small insects such as thrips.

In the wild, insects usually detoxify toxins from plants by overexpressing detoxification enzymes. Although we have demonstrated using bioassays that P450-mediated detoxification certainly plays a major role in neonicotinoid resistance in *M. usitatus*, this does not rule out the possibility that other P450 monooxygenases and target-site resistance to imidacloprid might also play a role. However, several questions remain unanswered. It is still unknown how many different P450s contribute to resistance in a certain strain and how many significant amino acid changes occur in P450s, warranting further research.

5 Conclusion

In summary, *MusiDN2722* was identified as a P450-encoding gene in *M. usitatus*. RT-qPCR analysis revealed high *MusiDN2722* expression in females and in the thorax of *M. usitatus*. Both membrane-feeding and microinjection strategies successfully knocked down *MusiDN2722* and enhanced the sensitivity of common thrips to acetamiprid. Our findings lay a foundation for the determination of the long-term susceptibility of *M. usitatus* to neonicotinoid pesticides and for preservation of the field efficacy of this class of insecticides.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI, BankIt2661356 Seq1 OQ200384.

Author contributions

JW, AA and AS designed and revised the manuscript. WC, ZL, and CZ performed the experiments. WC and ZL analyzed the data. WC and CZ prepared the materials used in the study. WC drafted the manuscript. JW provided financial support for the project leading to this publication. All authors have read and approved the manuscript.

Funding

This work was supported by grants from the Guangdong Province Natural Science Foundation General Program (No. 2021A1515011058).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors, and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2023.1130389/full#supplementary-material

References

Bai, Q., Lu, Y. H., Zheng, X. S., and Lv, Z. X. (2018). Spatiotemporal expression profiling of two P450 genes, 438 and 439, in rice stem borer, (Lepidoptera: Crambidae). *Plant Prot. Sci.* 30, 521–527. doi:10.3969/j.issn.1004-1524.2018.04.01

Bao, W. X., Narai, Y., Nakano, A., Kaneda, T., Murai, T., and Sonoda, S. (2014). Spinosad resistance of melon thrips, *Thrips palmi*, is conferred by G275E mutation in α6 subunit of nicotinic acetylcholine receptor and cytochrome P450 detoxification. *Pestic. Biochem. Phys.* 112, 51–55. doi:10.1016/j.pestbp.2014.04.013

Bautista, M. A. M., Tanaka, T., and Miyata, T. (2007). Identification of permethrin-inducible cytochrome P450s from the diamondback moth, *Plutella xylostella* (L.) and the possibility of involvement in permethrin resistance. *Pestic. Biochem. Physiol.* 87, 85–93. doi:10.1016/j.pestbp.2006.06.004

Berge, J., Feyereisen, R., and Amichot, M. (1998). Cytochrome p450 monooxygenases and insecticide resistance in insects. *PhilosT R. Soc. B* 353, 1701–1705. doi:10.1098/rstb.1998.0321

Chen, X. L., Yuan, L. Z., Du, Y. Z., Zhang, Y. J., and Wang, J. J. (2011). Cross-resistance and biochemical mechanisms of abamectin resistance in the Western flower thrips, *Frankliniella occidentalis*. *Pestic. Biochem. Phys.* 101, 34–38. doi:10.1016/j.pestbp.2011.07.001

Chung, H., Sztal, T., Pasricha, S., Sridhar, M., Batterham, P., and Daborn, P. J. (2009). Characterization of *Drosophila Melanogaster* cytochrome P450 genes. *Natl. A Sci.* 14, 5731–5736. doi:10.1073/PNAS.0812141106

Cifuentes, D., Chynoweth, R., Guillé, J. N., and Bielza, P. (2012). Novel cytochrome P450 genes, CYP6EB1 and CYP6EC1, are over-expressed in acrinathrin-resistant *Frankliniella occidentalis* (thysanoptera: Thripidae). *J.* Econ. Entomol. 105, 1006–1018. doi:10.1603/EC11335

Cui, S. F., Wang, L., Ma, L., and Geng, X. Q. (2016). P450-mediated detoxification of botanicals in insects. *Phytoparasitica* 44, 585–599. doi:10.1007/s12600-016-0550-1

Dulbecco, A. B., Moriconi, D. E., and Pedrini, N. (2021). Knockdown of CYP4PR1, a cytochrome P450 gene highly expressed in the integument tissue of *Triatoma infestans*, increases susceptibility to deltamethrin in pyrethroid-resistant insects. *Pestic. Biochem. Phys.* 2021, 104781. doi:10.1016/j.pestbp.2021.104781

Elbert, A., Haas, M., Springer, B., Thielert, W., and Nauen, R. (2008). Applied aspects of neonicotinoid uses in crop protection. *Pest Manag. Sci.* 64, 1099–1105. doi:10.1002/ps.1616

Espinosa, P. J., Contreras, J., Quinto, V., Grávalos, C., Fernández, E., and Bielza, P. (2005). Metabolic mechanisms of insecticide resistance in the Western flower thrips, *Frankliniella occidentalis* (Pergande). *Pest Manag. Sci.* 61, 1009–1015. doi:10.1002/ps.1069

Farman, U., Hina, G., Kaleem, T., Nicolas, D., Gao, X. W., and Song, D. L. (2020). Thiamethoxam induces transgenerational hormesis effects and alteration of genes expression in *Aphis gossypii*. *Pestic. Biochem. Phys.* 2020, 104557. doi:10.1016/j.pestbp.2020.104557

Feng, Q. L. (2020). Physiology and interaction of insects with environmental factors. *J. Integr. Agr.* 19, 1411–1416. doi:10.1016/S2095-3119(20)63200-2

Feyereisen, R. (2012). Insect CYP genes and P450 enzymes. *Insect Mol. Biol.*, 236–316. doi:10.1016/B978-0-12-384747-8.10008-X

Feyereisen, R. (2005). *Insect cytochrome P450*. Oxford: Elsevier, 1–77. doi:10.1016/B0-44-451924-6/00049-1

Fu, B. L., Liu, J. F., Qiu, H. Y., Tang, L. D., Lin, J., and Zeng, D. Q. (2016). Monitoring insecticide resistance in field populations of thrips hawaiiensis(morgan) in hainan. *Chin. Appl. Entomol.* 53 (2), 403–410. doi:10.7679/j.issn.2095-1353.2016.053

Fu, B., Qiu, H., Li, Q., Tang, L., Zeng, D., Liu, K., et al. (2019). Analysis of seasonal and annual field-evolved insecticide resistance in populations of *Thrips hawaiiensis* in banana orchards. *J. Pest Sci.* 92, 1293–1307. doi:10.1007/s10340-019-01112-w

Han, D., Lei, L., and Chen, J. (2017). Toxicity and control effect of nine insecticides on thrip compound of mango. *Chin. Agric. Sci. Bull.* 33 (16), 141–145. doi:10.11924/j.issn.1000-6850.casb16060104

Han, H., Yang, Y. Y., Hu, J., Wang, Y. X., Zhao, Z. G., Ma, R. Y., et al. (2022). Identification and characterization of CYP6 family genes from the oriental fruit moth (*Grapholita molesta*) and their responses to insecticides. *Insects* 17, 13–300. doi:10.3390/insects13030300

Hou, W. T., Staehelin, C., Elzaki, M. E, A., Hafeez, M., Luo, Y. S., and Wang, R. L. (2021). Functional analysis of CYP6AE68, a cytochrome P450 gene associated with indoxacarb resistance in *Spodoptera litura* (Lepidoptera: Noctuidae). *Pestic. Biochem. Phys.* 2021, 104946. doi:10.1016/j.pestbp.2021.104946

Huang, W. K., Kong, X. Y., Ke, Y. C., Wang, S., Li, Q. J., Fu, Q. W., et al. (2018). Research progress on thrips *Megalurothrips usitatus* (bagrall). *China Veget* 2020, 30–31. doi:10.1186/s41938-020-00225-0

Ihara, M., Furutani, S., Shigetou, S., Shimada, S., Niki, K., Komori, Y., et al. (2020). Cofactor-enabled functional expression of fruit fly, honeybee, and bumblebee nicotinic receptors reveals picomolar neonicotinoid actions. *P Natl. Acad. Sci. U. S. A.* 117, 16283–16291. doi:10.1073/pnas.2003667117

Itai, B., Russell, L., Yuval, P., and Oded, S. (2011). Special invited paper: Uniform spanning forests. *Ann. Probab.* 29, 1–65. doi:10.1017/9781316672815.011

Jones, C. M., Daniels, M., Andrews, M., Slater, R., Lind, R. J., Gorman, K., et al. (2011). Age-specific expression of a P450 monooxygenase (CYP6CM1) correlates with neonicotinoid resistance in *Bemisia tabaci*. *Pestic. Biochem. Phys.* 101, 53–58. doi:10.1016/j.pestbp.2011.07.004

Lin, T., Zeng, Z., Chen, Y., You, Y., Hu, J., Yang, F., et al. (2021). Compatibility of six reduced-risk insecticides with *Orius strigicollis* (Heteroptera: Anthocoridae) predators for controlling *Thrips hawaiiensis* (Thysanoptera: Thripidae) pests. *Ecotox Environ. Safe* 226, 112812. doi:10.1016/j.ecoenv.2021.112812

Liu, N., and Scott, J. G. (1996). Genetic analysis of factors controlling elevated cytochrome P450, CYP6D1, cytochrome b5, P450 reductase and monooxygenase activities in LPR house flies, *Musca domestica*. *Biochem. Genet.* 34, 133–148. doi:10.1007/bf02396246

Liu, S., He, C., Liang, J., Su, Q., Zhang, Y., Wang, S., et al. (2020). Molecular characterization and functional analysis of the halloween genes and cyp18a1 in bemisia tabaci med. *Pestic. Biochem. Phys.* 167, 104602. doi:10.1016/j.pestbp.2020.104602

Ma, Y., Zhang, W. B., Zhang, H. L., Ma, Y. H., Han, H. B., Pang, B. P., et al. (2022). Cloning of CYP6A13 and sodium channel gene LPVSSC and their involvement in analysis on the resistance of lambda-cyhalothrin in *Lygus pratensis* (Hemiptera: Miridae). *J. Environ. Entomol.* 44, 1252–1263. doi:10.3969/j.issn.1674-0858.2020.05.19

Mao, K., Jin, R., Ren, Z., Zhang, J., Li, Z., He, S., et al. (2022). miRNAs targeting CYP6ER1 and CarE1 are involved in nitenpyram resistance in *Nilaparvata lugens*. *Insect Sci.* 2022, 177–187. doi:10.1111/1744-7917.12910

Mori, K., and Gotoh, T. (2001). Effects of pesticides on the spider mite predators, *Scolothrips takahashii* (Thysanoptera: Thripidae) and *Stethorus japonicus* (Coleoptera: Coccinellidae). *Int. J. Acarol.* 27, 299–302. doi:10.1080/01647950108684270

Mortazavi, A., Williams, B. A., McCue, K., Schaeffer, L., and Wold, B. (2008). Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nat. Methods* 5, 621–628. doi:10.1038/nmeth.1226

Nadeau, E. (2017). *RNA delivery properties of porous silica nanoparticles in* spodoptera frugiperda *cells*. UKnowledge: University of Kentucky. Binding, Protection.

Nazemi, A., Khajehali, J., and Leeuwen, T. V. (2016). Incidence and characterization of resistance to pyrethroid and organophosphorus insecticides in *Thrips tabaci* (Thysanoptera: Thripidae) in onion fields in Isfahan, Iran. *Pestic. Biochem. Phys.* 129, 28–35. doi:10.1016/j.pestbp.2015.10.013

Nelson, D. R. (1998). Metazoan cytochrome P450 evolution. Comp. Biochem. Physiol. *Part C. Pharmacol. Toxicol. Endocrinol.* 121, 15–22. doi:10.1016/s0742-8413(98)10027-0

Ohkawa, H., Tsujii, H., Shimoji, M., Imajuku, Y., and Imaishi, H. (1999). Cytochrome P450 biodiversity and plant protection. *J. Pestic. Sci.* 24, 197–203. doi:10.1584/jpestics.24.197

Pan, Y., Chai, P., Zheng, C., Xu, H., Wu, Y., Gao, X., et al. (2018). Contribution of cytochrome P450 monooxygenase CYP380C6 to spirotetramat resistance in *Aphis gossypii* Glover. *Pestic. Biochem. Phys.* 148, 182–189. doi:10.1016/j.pestbp.2018.04.015

Pedra, J. H., McIntyre, L. M., Scharf, M. E., and Pittendrigh, B. R. (2004). Genome-wide transcription profile of field and laboratory selected dichlorodiphenyltrichloroethane (DDT)-resistant *Drosophila*. *Proc.* Natl. Acad. Sci. U.S.A. 101, 7034–7039. doi:10.1073/pnas.0400580101

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.* 29, e45. doi:10.1093/nar/29.9.e45

Phogat, A., Singh, J., Kumar, V., and Malik, V. (2022). Toxicity of the acetamiprid insecticide for mammals: A review. *Environ. Chem. Lett.* 20, 1453–1478. doi:10.1007/s10311-021-01353-1

Pottier, M. A., Bozzolan, F., Chertemps, T., Jacquin, Joly. E., Lalouette, L., Siaussat, D., et al. (2012). Cytochrome P450s and cytochrome P450 reductase in the olfactory organ of the cotton leafworm *Spodoptera littoralis*. *Insect Mol. Biol.* 21, 568–580. doi:10.1111/j.1365-2583.2012.01160.x

Preisler, H. K., and Robertson, J. L. (1989). Analysis of time-dose-mortality data[J]. *J. Econ. Entomol.* 82, 1534–1542. doi:10.1093/jee/82.6.1534

Prentice, K., Christiaens, O., Pertry, I., Bailey, A., Niblett, C., Ghislain, M., et al. (2017). RNAi-based gene silencing through dsRNA injection or ingestion against the African sweet potato weevil *Cylas puncticollis* (Coleoptera: Brentidae). *Pest Manag. Sci.* 73, 44–52. doi:10.1002/ps.4337

Puinean, A. M., Foster, S. P., Oliphant, L., Denholm, I., Field, L. M., Millar, N. S., et al. (2010). Amplification of a cytochrome P450 gene is associated with resistance to neonicotinoid insecticides in the aphid *Myzus persicae*. *Plos Genet.* 6, e1000999. doi:10.1371/journal.pgen.1000999

Roberto, F., Shane, D., Trent, P., Richard, A. J., and Philip, B. (2017). Partitioning the roles of CYP6G1 and gut microbes in the metabolism of the insecticide imidacloprid in *Drosophila melanogaster*. *Sci. Rep-UK* 7, 11339. doi:10.1038/s41598-017-09800-2

Rueda, A., and Shelton, A. M. (2003). Development of a bioassay system for monitoring susceptibility in *Thrips tabaci*. *Pest Manag. Sci.* 59, 553–558. doi:10.1002/ps.680

Ryusuke, N., Takahiro, M., Takuji, Y., Toshiki, N., Kazuei, M., Yoshinori, F., et al. (2004). CYP306A1, a cytochrome P450 enzyme, is essential for ecdysteroid biosynthesis in the prothoracic glands of *Bombyx* and *Drosophila*. *J. Biol. Chem.* 279, 35942–35949. doi:10.1074/jbc.M404514200

Scott, J. G. (1999). Cytochromes P450 and insecticide resistance. *Insect Biochem. Molec* 29, 757–777. doi:10.1016/S0965-1748(99)00038-7

Wan, P. J., Shi, X. Q., ShiKong, Y., Zhou, L. T., Guo, W. C., Tursun, A., et al. (2013). Identification of cytochrome P450 monooxygenase genes and their expression profiles in cyhalothrin-treated Colorado potato beetle, *Leptinotarsa decemlineata*. *Pestic. Biochem. Phys.* 107, 360–368. doi:10.1016/j.pestbp.2013.10.004

Wang, R. L., Xia, Q. Q., Baerson, S. R., Ren, Y., Wang, J., Su, Y. J., et al. (2015). A novel cytochrome P450 CYP6AB14 gene in *spodoptera litura* (lepidoptera: Noctuidae) and its potential role in plant allelochemical detoxification. *J. Iinsect Physiol.* 75, 54–62. doi:10.1016/j.jinsphys.2015.02.013

Wang, W., Chen, S. L., and Cui, F. (2014). Temporal and spatial expression of the GSTe1 and CYP9J40 genes in *Culex pipiens quinquefasciatus*. *China Appl. Entomol.* doi:10.7679/j.issn.2095-1353.2014.179

Wang, Z. H., Gong, Y. J., Jin, G. H., Li, B. Y., Chen, J. C., Kang, Z. J., et al. (2016). Field-evolved resistance to insecticides in the invasive Western flower thrips Frankliniella occidentalis (Pergande) (Thysanoptera: Thripidae) in China. *Pest Manag. Sci.* 72 (7), 1440–1444. doi:10.1002/ps.4200

Xu, L., Mei, Y., Liu, R. Q., Chen, X. L., Li, D. Z., and Wang, C. Q. (2020). Transcriptome analysis of *Spodoptera litura* reveals the molecular mechanism to pyrethroids resistance. *Pestic. Biochem. Phys.* 2020, 104649. doi:10.1016/j.pestbp.2020.104649

Xu, L., Zhao, J., Sun, Y., Xu, D., Xu, G., Xu, X., et al. (2018). Constitutive overexpression of cytochrome P450 monooxygenase genes contributes to chlorantraniliprole resistance in *Chilo suppressalis* (Walker). *Pest Manag. Sci.* 75, 718–725. doi:10.1002/ps.5171

Yang, L., Shao, Y., Li, F., Chen, D. X., Li, F. Y., and Wu, S. Y. (2021). Advances on biological control of thrips pests. *Chin. J. Bio Control* 37, 393–405. doi:10.16409/j.cnki.2095-039x.2021.03.033

Yang, N, N., Zhang, Y. J., Yang, X., Huang, D. Y., Long, T., and Wan, P. Differential expression of the detoxification enzyme genes in different developmental stages of the whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Acta Entomol. Sin.* 59, 1166–1173. doi:10.16380/j.kcxb.2016.11.003

Yang, Y. H., Chen, S., Wu, S. W., Yue, L. N., and Wu, Y. D. (2006). Constitutive overexpression of multiple cytochrome P450 genes associated with pyrethroid resistance in *Helicoverpa armigera*. *J. Econ. Entomol.* 99, 1784–1789. doi:10.1093/jee/99.5.1784

Yu, N., Christiaens, O., Liu, J. S., Niu, J. Z., Cappelle, K., Caccia, S., et al. (2013). Delivery of dsRNA for RNAi in insects: An overview and future directions. *Insect Sci.* 20, 4–14. doi:10.1111/j.1744-7917.2012.01534.x

Zhang, Y. H., Ma, Z. Z., Zhou, H., Chao, Z. J., Yan, S., and Shen, J. (2021). Nanocarrier-delivered dsrna suppresses wing development of green peach aphids. *Insect Sci.* 29, 669–682. doi:10.1111/1744-7917.12953

Zhao, Q. N., Huang, M. J., Wei, X. G., Yang, J., Du, T. H., Yin, C., et al. (2021). Cloning of cytochrome P450 gene CYP6EM1 and its function in imidacloprid resistance in white fly. *Bemisia Abaci*. *Acta Phytophy Sin.* 171–177. doi:10.16688/j.zwbh.2020279

Zhou, C., Cao, Q., Li, G., and Ma, D. Y. (2020). Role of several cytochrome P450s in the resistance and cross-resistance against imidacloprid and acetamiprid of *Bemisia tabaci* (Hemiptera: Aleyrodidae) MEAM1 cryptic species in Xinjiang, China. *Pestic. Biochem. Phys.* 163, 209–215. doi:10.1016/j.pestbp.2019.11.017

Zuo, T., Shuai, L., and Zhang, B. (2017). Prevention and control of Western flower thrips *Frankliniella occidentalis* by treatment with low doses of pesticides and high temperatures. *J. Plant Prot.* 44 (04), 687–692. doi:10.13802/j.cnki.zwbhxb.2017.2016037

frontiers | Frontiers in Physiology

TYPE Original Research
PUBLISHED 30 March 2023
DOI 10.3389/fphys.2023.1155455

OPEN ACCESS

EDITED BY
Xiaoming Xia,
Shandong Agricultural University, China

REVIEWED BY
Pei Liang,
China Agricultural University, China
Jianhong Li,
Huazhong Agricultural University, China
Shaoying Wu,
Hainan University, China

*CORRESPONDENCE
Xuegui Wang,
wangxuegui@sicau.edu.cn

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Invertebrate Physiology, a section of the journal Frontiers in Physiology

RECEIVED 31 January 2023
ACCEPTED 14 March 2023
PUBLISHED 30 March 2023

CITATION
Hasnain A, Zhang S, Chen Q, Xia L, Wu Y, Gong C, Liu X, Jian P, Zhang L and Wang X (2023), Effects of chlorantraniliprole on the life history traits of fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae).
Front. Physiol. 14:1155455.
doi: 10.3389/fphys.2023.1155455

Effects of chlorantraniliprole on the life history traits of fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

Ali Hasnain[1,2,3†], Shuirong Zhang[1,2†], Qinghua Chen[4], Lijuan Xia[5], Yutong Wu[2], Changwei Gong[1,2], Xuemei Liu[1,2], Pu Jian[1,2], Lei Zhang[6] and Xuegui Wang[1,2*]

[1]State Key Laboratory of Crop Gene Exploration and Utilization in Southwest China, Sichuan Agricultural University, Chengdu, China, [2]College of Agriculture, Sichuan Agricultural University, Chengdu, China, [3]College of Plant Protection, Nanjing Agricultural University, Nanjing, China, [4]Key Laboratory of Integrated Pest Management on Crops in Southwest, Institute of Plant Protection, Sichuan Academy of Agricultural Sciences, Ministry of Agriculture, Chengdu, China, [5]Talent Development Service Center, Sichuan Provincial Department of Agriculture and Rural Affairs, Chengdu, China, [6]Department of Entomology, China Agricultural University, Beijing, China

Introduction: *Spodoptera frugiperda* is an important nomadic agricultural pest with a diverse host range and resistance against several insecticides. The current study investigated the life history traits of two strains of the field-collected population against chlorantraniliprole using an age-stage two-sex life table.

Method: For this, we established the chlorantraniliprole-susceptible (Crp-SUS G_{12}), and chlorantraniliprole-reduced susceptible (Crp-RES G_{12}) strains derived from the sixth generation of the QJ-20 population having a resistance ratio (RR) of 10.39-fold, compared with the reported susceptible population.

Results: The results showed that the chlorantraniliprole-reduced susceptible strain attained a 4.0-fold RR, while the chlorantraniliprole-susceptible strain attained an RR of 0.85-fold, having overlapped fiducial limits (FLs) with the referred susceptible baseline. Meanwhile, the present study revealed that the development time of the susceptible strain was significantly longer than that of the reduced susceptible strain. Similarly, the mean longevity, adult pre-oviposition period (APOP), and total pre-oviposition period (TPOP) of the female chlorantraniliprole-susceptible strain were considerably longer than those of the female chlorantraniliprole-reduced susceptible strain. Contrarily, the population parameters, including the intrinsic rate of increase (r), finite rate of increase (λ), and net reproductive rate (R), of the chlorantraniliprole-susceptible strain were considerably lower than those of the chlorantraniliprole-reduced susceptible strain, while the mean generation time (T) of the chlorantraniliprole-susceptible strain was substantially longer than the chlorantraniliprole-reduced susceptible strain. The age-stage characteristic survival rate (s_{xj}) and age-stage characteristic life expectancy (e_{xj}) of the chlorantraniliprole-susceptible strain were longer than those of the chlorantraniliprole-reduced susceptible strain, but the age-stage-specific reproductive value (v_{xj}) of the chlorantraniliprole-susceptible strain was shorter than that of the chlorantraniliprole-reduced susceptible strain. Moreover, the contents of vitellogenin (Vg) and VgR in the chlorantraniliprole-reduced

susceptible strain were higher than those in the chlorantraniliprole-susceptible strain.

Discussion: These findings showed that reducing susceptibility to chlorantraniliprole promoted population growth in *S. frugiperda*. Therefore, this study could provide conceptual support for the integrated pest management (IPM) approach to control *S. frugiperda* in the field.

KEYWORDS

***Spodoptera frugiperda*, resistance, life history traits, chlorantraniliprole, vitellogenin**

1 Introduction

The fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera, Noctuidae), a devastative agricultural pest in its innate range, North and South America, and during the last 10 years, has become a significant invasive pest on a global scale. Numerous variables, including a high reproductive capacity, long-distance migration, and multiple host plants, have been implicated in the widespread FAW (Barros et al., 2010; Westbrook et al., 2016). Additionally, it may move from overwintering areas to other suitable climate zones without entering diapause (Sparks, 1979; Hardke et al., 2015). FAW was first reported in China in January 2019, and there are several reports on severe damage caused by FAW to the fields of maize, rice, and wheat, threatening the food supply and causing yield loss in China (Xiao, 2021; Zhang et al., 2021). The continuous application of insecticides causes resistance in the pest population over time (Denholm and Devine, 2013). For FAW, the resistance development has been reported for about 29 different insecticides from America by 2017 (Gutierrez-Moreno et al., 2019) and is attributed to attaining exceptional deviations in physiology, behavior, reproduction, longevity, and biology (Haynes, 1988; Teke and Mutlu, 2021; Liu et al., 2022). The life history parameters, including insect fertility, mortality, and lifespan, can be better studied by using the age-stage two-sex life table because it computes the data by considering both sexes in the calculations, as compared to the conventional life table, which only focuses on the female population (Abdel-Khalek and Momen, 2022; Younas et al., 2022). Many studies have reported the usage of an age-stage two-sex life table in elaborating the life history traits of *S. frugiperda* that were reared on different host plants (Guo et al., 2021; Xie et al., 2021) and the effects of spinetoram on its growth and fecundity (Gao et al., 2021). Similarly, several other studies were conducted to elaborate the impact of different insecticides on the life history traits of insects, such as *Spodoptera litura* (Rehan et al., 2015), *Oxycarenus hyalinipennis* (Lygaeidae; Hemiptera) (Ullah et al., 2016), and *Musca domestica* (Shah et al., 2015). Vitellogenesis is vital for the reproduction of oviparous insects since it includes the synthesis and absorption of vitellogenin (Vg), a source of nutrients for embryonic development as it stimulates oocyte maturation (Roy et al., 2018; Wu et al., 2020), which is required for reproduction (Schneider, 1996; Ge et al., 2019). Therefore, studying the difference between the two strains was crucial because insecticidal stress also affects fecundity.

Chlorantraniliprole, an anthranilic diamide, works by modulating ryanodine receptors (RyRs) in the sarcoplasmic reticulum membrane, causing permanent paralysis in insect bodies owing to acute muscular contraction caused by excessive Ca^{2+} release in the cytosol that causes insect feeding cessation and death (Nauen, 2006). It has been reported to eradicate various insect orders, including Coleoptera, Diptera, and Lepidoptera (Sattelle et al., 2008). As a new pesticide for managing lepidopterous pests, i.e., *Spodoptera exigua* and *Tryporyza incertulas*, it is considered a great alternative in integrated pest management (IPM) (Lai et al., 2011). However, prolonged use of chlorantraniliprole may impair the ecosystem; thus, its adverse effects on FAW must be evaluated to optimize the use and reduce environmental damage.

This study aimed to investigate the effects of chlorantraniliprole on the life history traits of susceptible and reduced susceptible strains of the field-collected population of *S. frugiperda* from Southwest China and the difference in vitellogenin content of the female strains. Moreover, the degree of chlorantraniliprole ferocity and its influence on all larval instars of *S. frugiperda* could appropriately highlight its efficacy. Therefore, the effects of chlorantraniliprole on the life stages (larva, pupa, and adult) of both male and female *S. frugiperda* could be used as a reference for its effective management under field conditions.

2 Materials and methods

2.1 Insects and insecticides

The field population of *S. frugiperda* was collected from Qianjiang (QJ-20), located in the southwestern part of China (N 29°32′01, E 108°46′15), during the summer of 2020. The bioassay result showed a moderate resistance with an RR of 10.39-fold against chlorantraniliprole. About 150–200 larvae were procured at the sample location. The larvae and adults were raised on synthetic food and 10% sugar syrup, respectively. Subsequently, we used sodium hypochlorite solution (0.2%–0.3%) to disinfect the fresh eggs and pupae. After the hatching of eggs, larvae at all growth stages were grown under a controlled environment at temperature, relative humidity (RH), and photoperiod of 26°C ± 1°C, 70%–80%, and 16:8 (light: dark), respectively. The target insecticide used for screening was 95% chlorantraniliprole (Corteva Agriscience, Indianapolis, United States).

2.2 Bioassays

The toxicity of chlorantraniliprole was determined in the third instar of *S. frugiperda* by topical application. At first, we determined the dose range for chlorantraniliprole in a preliminary experiment by using a series of its concentration (i.e., 200, 100, 50, and 10 mg a.i. L^{-1}). Later,

technical chlorantraniliprole was dissolved and diluted to a series of concentrations using acetone (i.e., ranged from 1.05 to 21.06 mg a.i. L^{-1}) to determine the LD_{50} values of the subsequent generations of the two strains (established from the field population). A 50-µL micro-syringe, coupled with a micro-applicator (PB600-1 Repeating Dispenser, Hamilton Company), was used to apply 1 µL droplet of each prepared concentration on the dorsal side of the frontal thorax of the third-instar larva (average weight of 0.006 g) (Gutierrez-Moreno et al., 2019). Five different insecticidal concentrations and control (each in triplicate) were prepared, with 12 larvae in each replication. The LD_{50} values were expressed in µg g^{-1} (active ingredient/larval weight). A measure of 1 µL of acetone per larva was applied for the control treatment. The treated larvae were placed on a 12-compartment plate with enough food, with experiments performed in triplicate for each concentration. Mortality was observed at 48 h after treatment. The larvae that expressed severe inebriation signs (slow movement, twitching, feeding interruption, and severe growth inhibition) were considered dead.

2.3 Establishment of susceptible and reduced susceptible strains against chlorantraniliprole

Based on bioassay observations, the two strains derived from QJ-20 (RR = 10.39-fold) against chlorantraniliprole were prepared using the approach reported by Wang et al. (2006). About 200 F_6-generation larvae of QJ-20 were divided equally into two groups: the susceptible strain (Crp-SUS G_{12}) and the reduced susceptible strain (Crp-RES G_{12}), using a single-pair mating method. The former generations were kept in the laboratory, without insecticidal contact, to free the field-collected population from biotic or abiotic stress.

For establishing the Crp-RES G_{12} strain, the screening doses, i.e., LD_{70} of each generation (6.31, 8.42, 9.47, 11.57, 12.62, and 13.68 mg a.i. L^{-1} from G_7 to G_{12} generation, respectively) were prepared. About 250–300 third-instar larvae were treated topically with 1 µL droplet of each designed concentration on the dorsal side of the frontal thorax of the third-instar larva for each generation using a 50-µL micro-syringe (Hamilton Company, Reno, NV), coupled with a micro-applicator (PB600-1 Repeating Dispenser, Hamilton Company). After 48 h, the survived larvae were shifted into the glass tubes with a fresh artificial diet and raised for the next generation.

However, for the Crp-SUS G_{12} strain, male and female adults were coupled independently, and about 50 pairs were prepared during each generation. The progeny of about 20 pairs was considered for selection in every generation. A total of 20 third-instar larvae were given the same dosage of chlorantraniliprole (i.e., 5.26 mg a.i. L^{-1}) in pair. Subsequently, after 48 h treatment, the mortality was checked, and the larvae with >80% mortality were raised for obtaining the next generation.

2.4 Life history traits

Based on the generational screening result of the two strains, a prominent resistance difference (i.e., 4.0-fold) was progressively developed. Then, about 100 adults of each strain were kept in a clean cage covered with a clean muslin cloth. At the time of high fecundity, we randomly selected at least five egg masses and allowed them to air-dry at room temperature. Later, 100 larvae from the hatched eggs were raised separately in an artificial diet in a pre-labeled glass tube with 1.8 cm diameter and 10 cm height. Larvae at all growth stages were monitored daily and transferred to a six-compartment dish/plate. All the eggs and pupae were disinfected using sodium hypochlorite solution.

The fresh adults (developed from artificially raised larvae) were paired, and each pair was grown separately in plastic cups (500 mL; 9.5 cm diameter and 13.7 cm height) to establish a family. Each cup contained a wet cotton ball soaked daily with 10% sugar solution. Eventually, 30 families of each strain were established, and the population characteristics, including longevity, fecundity, and developmental time, were recorded every day until the couple's death. The eggs were precisely counted and recorded. The life parameters, including time and duration of each growth stage, the emergence, life span, mating, and fecundity intensity of the adults, were recorded. The dataset of the values was analyzed to establish the life table.

2.5 Protein contents of Vg and VgR

The ovaries from five adult female adults of each strain were collected 2 days after their emergence (Zhen et al., 2018), weighed. A measure of 1 mL of PBS (pH 7.3) was added and then manually homogenized. The supernatant was then collected by centrifuging the mixture at 2,500 g for 20 min. The protein contents of Vg and VgR were determined according to the instructions of the ELISA kit (Shanghai Enzyme Biotechnology Co., Ltd., Product Code: mlbio104703 for insect vitellogenin and mlbio104704 for insect vitellogenin receptor): Insect Vg (or VgR) was added to a microtiter plate well that had been coated with a particular insect Vg (or VgR) antibody and then combined with an antibody that had been labeled with horseradish peroxidase (HRP) to form an antibody–antigen–enzyme–antibody complex. After thoroughly cleaning the plate, 3, 3′5, 5′-tetramethyl benzidine (TMB) substrate solution was added until the substrate turned blue, signifying HRP enzyme catalysis. The reaction was then stopped by adding sulfuric acid solution. The concentrations of Vg (or VgR) were calculated by comparing the absorbance (OD) of the samples to the standard curve using a microplate reader (Model 680 Microplate Reader, Bio-Rad) that measured the absorbance (OD) at 450 nm.

2.6 Statistical analysis

The POLO 2.0 program (LeOra Software, www.leorasoftware.com) was used to calculate the slope, LD_{50}, 95% fiducial limits (FLs), and chi-square (X^2) value of the insecticide after 48 h of treatment (Wang et al., 2018). The susceptible baseline value was referred from Wang et al. (2021). The raw data of the life table were examined using age-stage two-sex life table computer software. The basic parameters, including age-specific survival rate (l_x), age-stage survival rate (s_{xj}), finite rate of increase (λ), reproductive value (v_{xj}), intrinsic rate of increase (r), net reproductive rate (R_0), and mean generation time (T), were analyzed. Based on the confidence interval of the differences, the adult longevity, fecundity, adult pre-oviposition period (APOP), total pre-oviposition period (TPOP),

TABLE 1 Dose–mortality response (μg/larva) for the chlorantraniliprole-susceptible strain.

Generation	Survival %	n[a]	Slope ± SE	LD_{50} (95% FL) (μg/g)	X^2 (df)	*p*	RR[b]
SUS*	—	—	1.139 ± 0.234	0.410 (0.229–0.602)	3.425 (18)	0.9990	1.00
Crp-SUS G_7	67.85	216	3.088 ± 0.418	1.050 (0.868–1.302)	21.77 (16)	0.1507	2.56
Crp-SUS G_8	59.12	216	3.074 ± 0.376	0.790 (0.675–0.918)	10.45 (16)	0.8420	1.93
Crp-SUS G_9	50.00	216	2.761 ± 0.329	0.567 (0.470–0.669)	7.69 (16)	0.9575	1.38
Crp-SUS G_{10}	39.35	216	2.460 ± 0.304	0.505 (0.409–0.606)	8.88 (16)	0.9182	1.23
Crp-SUS G_{11}	35.19	216	2.701 ± 0.323	0.449 (0.365–0.534)	13.79 (16)	0.6143	1.09
Crp-SUS G_{12}	28.24	216	2.705 ± 0.335	0.349 (0.276–0.421)	10.99 (16)	0.8101	0.85

Note: The parental generation selected for screening was G_6, and the median lethal dose (LD_{50}) was expressed as micrograms of active ingredient per larva. n[a], number of larvae used in bioassay; SUS*, baseline referred to Wang et al. (2021); RR[b], LD_{50} of the generation/LD_{50} of SUS.

TABLE 2 Dose–mortality response (μg/larva) for the chlorantraniliprole-reduced susceptible strain.

Generation	Survival %	n[a]	Slope ± SE	LD_{50} (95% FL) (μg/g)	X^2 (df)	*p*	RR[b]
SUS*	—	—	1.139 ± 0.234	0.410 (0.229–0.602)	3.425 (18)	0.9990	1.00
Crp-RES G_7	57.94	216	3.864 ± 0.459	0.778 (0.676–0.882)	11.73 (16)	0.7623	1.89
Crp-RES G_8	55.56	216	5.091 ± 0.638	1.112 (0.998–1.225)	4.08 (16)	0.9987	2.71
Crp-RES G_9	60.32	216	4.812 ± 0.593	1.231 (1.068–1.413)	23.56 (16)	0.0995	3.0
Crp-RES G_{10}	59.26	216	3.943 ± 0.525	1.409 (1.252–1.595)	5.09 (16)	0.9952	3.44
Crp-RES G_{11}	63.89	216	4.409 ± 0.596	1.540 (1.382–1.737)	5.22 (16)	0.9945	3.76
Crp-RES G_{12}	68.05	216	5.576 ± 0.752	1.643 (1.499–1.825)	10.85 (16)	0.8186	4.0

Note: The parental generation selected for screening was G_6, and the median lethal dose (LD_{50}) was expressed as micrograms of active ingredient per larva. n[a], number of larvae used in bioassay; SUS*, baseline referred to Wang et al. (2021); RR[b], LD_{50} of the generation/LD_{50} of SUS.

developmental growth, and other population parameters (r, λ, R_0, and T) were compared using the paired bootstrap test with 10,000 random resamplings. The intrinsic rate of increase (r) and the finite rate of increase (λ) are the two critical metrics for estimating the capacity for population expansion, used to indicate the population's fitness. A sigma plot (SigmaPlot 12.0) was used for the graphical representation of survival rate and reproductive value curves.

3 Results

3.1 Screening of the Crp-RES G_{12} and Crp-SUS G_{12} strains from the field population

The third-instar larvae were screened for toxicity of the two strains: Crp-SUS G_{12} and Crp-RES G_{12}. The increased toxicity to chlorantraniliprole was observed for Crp-SUS G_{12} (LD_{50}: 0.349 μg·g^{-1}), having almost similar susceptibility to the referred susceptible strain as observed by the overlapping FL (fiducial limit) of its LD_{50} values (Table 1). In contrast, the Crp-RES G_{12} strain showed reduced susceptibility to chlorantraniliprole (LD_{50}: 1.63 μg·g^{-1}), and the resistance ratio increased by four-fold compared to the susceptible strain. Moreover, no overlapping of CI for LD_{50} was observed between Crp-RES G_{12} and susceptible strains (Table 2).

3.2 Developmental times and population parameters of various life stages of Crp-RES G_{12} and Crp-SUS G_{12} strains

The life history traits of both strains were significantly different from each other. The developmental times of varying life stages of Crp-SUS G_{12} were considerably longer than those of Crp-RES G_{12} ($p < 0.05$) (Table 3). The mean longevity of male and female adults, APOP (adult pre-oviposition period), and TPOP (total pre-oviposition period) for Crp-SUS G_{12} were significantly longer than those for the Crp-RES G_{12} ($p < 0.05$), while the fecundity of Crp-SUS G_{12} was considerably lower than that of Crp-RES G_{12} ($p < 0.05$) (Table 4). However, the mean generation time (T) of the Crp-SUS G_{12} strain was much longer than that of the Crp-RES G_{12} strain, but the intrinsic rate of increase (r), finite rate of increase (λ), and net reproduction rate (R_0) of the Crp-SUS G_{12} strain were all significantly lower than those of the Crp-RES G_{12} strain (Table 5).

TABLE 3 Developmental time durations of various life stages of the Crp-RES G_{12} and Crp-SUS G_{12} strains.

Stages	Crp-RES G_{12}		Crp-SUS G_{12}	
	n	Mean ± SE (days)	*n*	Mean ± SE (days)
Egg	100	5.95 ± 0.03 b	100	6.0 ± 0.02 a
First instar	100	3.81 ± 0.05 b	100	4.58 ± 0.07 a
Second instar	99	3.94 ± 0.08 b	100	4.67 ± 0.09 a
Third instar	92	5.12 ± 0.08 b	100	6.18 ± 0.06 a
Fourth instar	85	5.6 ± 0.09 b	98	6.44 ± 0.06 a
Fifth instar	84	5.98 ± 0.09 b	96	5.24 ± 0.08 a
Sixth instar	82	6.01 ± 0.90 a	96	5.92 ± 0.12 b
Pupa	68	18.49 ± 0.27 b	66	22.94 ± 0.46 a
Adult	68	10.6 ± 0.28 b	66	14.82 ± 0.65 a

Means in the same row followed by different letters are significantly different ($p < 0.05$) using Tukey's test in SPSS software.

TABLE 4 Adult longevity and female fecundity for the Crp-RES G_{12} and Crp-SUS G_{12} strains.

Parameter	Crp-RES G_{12}		Crp-SUS G_{12}	
	n	Mean ± SE (days)	*n*	Mean ± SE (days)
Total longevity (M)	34	65.65 ± 0.79 b	38	79.24 ± 1.24 a
Total longevity (F)	34	65.09 ± 0.62 b	28	73.43 ± 1.05 a
APOP	33	3.73 ± 0.20 b	27	4.04 ± 0.26 a
TPOP	33	58.21 ± 0.45 b	27	62.89 ± 0.71 a
Fecundity	34	414.26 ± 31.03 a	28	304.43 ± 21.92 b

The standard errors (SE) of the mean values were estimated using 10,000 bootstrap replications. Means in the same row followed by different letters are significantly different ($p < 0.05$) using Tukey's test in SPSS software. APOP, adult pre-oviposition period; TPOP, total pre-oviposition period.

TABLE 5 Population parameters for the Crp-RES G_{12} and Crp-SUS G_{12} strains.

Parameter	Original		Bootstrap (mean ± SE)	
	Crp-RES G_{12}	Crp-SUS G_{12}	Crp-RES G_{12}	Crp-SUS G_{12}
r	0.08	0.07	0.082 ± 0.003 a	0.067 ± 0.003 b
λ	1.09	1.07	1.085 ± 0.003 a	1.069 ± 0.003 b
R_0	140.85	85.24	140.86 ± 22.16 a	85.18 ± 14.92 b
T	60.47	65.69	60.47 ± 0.56 b	65.71 ± 0.79 a

The standard errors (SE) of the mean values were estimated using 10,000 bootstrap replications. Means in the same row followed by different letters are significantly different ($p < 0.05$) using Tukey's test in SPSS software. *r*, intrinsic rate of increase (d^{-1}); λ, finite rate of increase (d^{-1}); R_0, net reproductive rate (d^{-1}); *T*, mean generation time.

3.3 Age-stage-specific survival rate (s_{xj}) and reproductive value (v_{xj}) of the Crp-RES G_{12} and Crp-SUS G_{12} strains

The Crp-SUS G_{12} and Crp-RES G_{12} strains overlapped at various developmental phases and showed earlier terminating curves for female larvae than male larvae in the displayed peaks for each developmental stage. These peaks were more significant in the Crp-SUS G_{12} strain than in the Crp-RES G_{12} strain, indicating that male adults of the former had better survival rates. However, Crp-RES G_{12} female adults showed identical survival rates to male adults, but Crp-SUS G_{12} female larvae showed a lower peak and consequently poorer survival rates (Figure 1). During the pupal stage, the v_{xj} value of the Crp-SUS G_{12} individuals was lower than that of the Crp-RES G_{12} individuals. When the female adults emerged, the Crp-RES G_{12} strain's plotted curve increased somewhat higher than the Crp-SUS G_{12} strain before significantly declining. In the Crp-SUS G_{12} strain, the maximum v_{xj} value was 241.1 d^{-1} on the 57th day, but in the Crp-RES G_{12} strain, it was 301.74 d^{-1} on the 56th day (Figure 2).

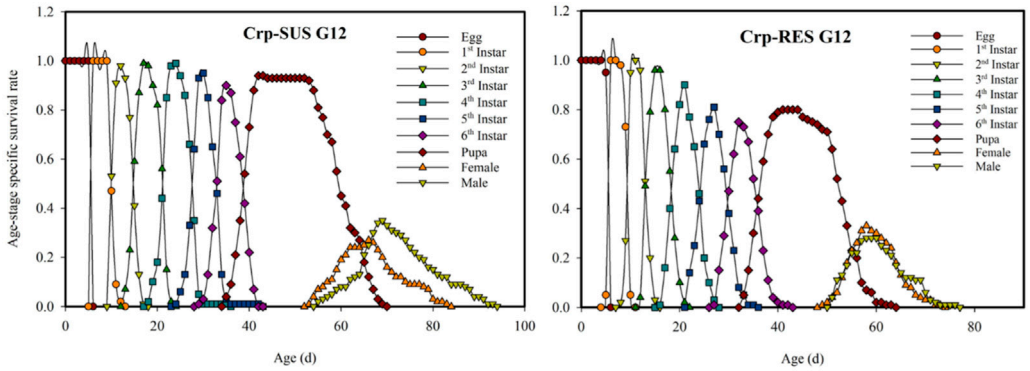


FIGURE 1
Age-stage-specific survival rate (s_{xj}) for the Crp-RES G_{12} and Crp-SUS G_{12} strains of *Spodoptera frugiperda*.

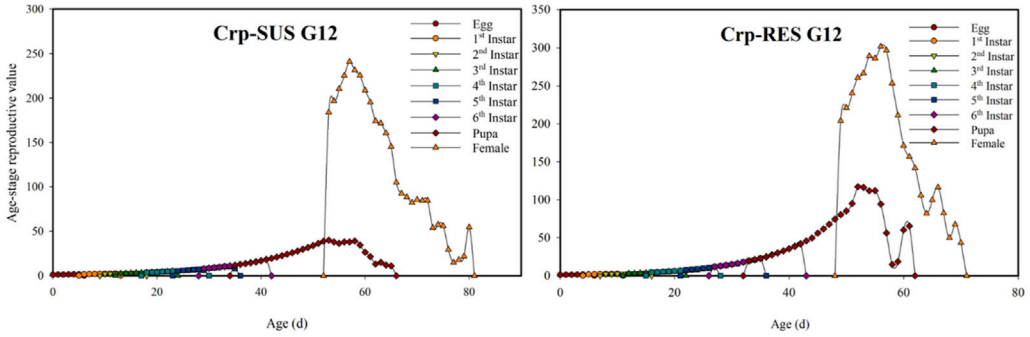


FIGURE 2
Age-stage reproductive value (v_{xj}) for the Crp-RES G_{12} and Crp-SUS G_{12} strains of *S. frugiperda*.

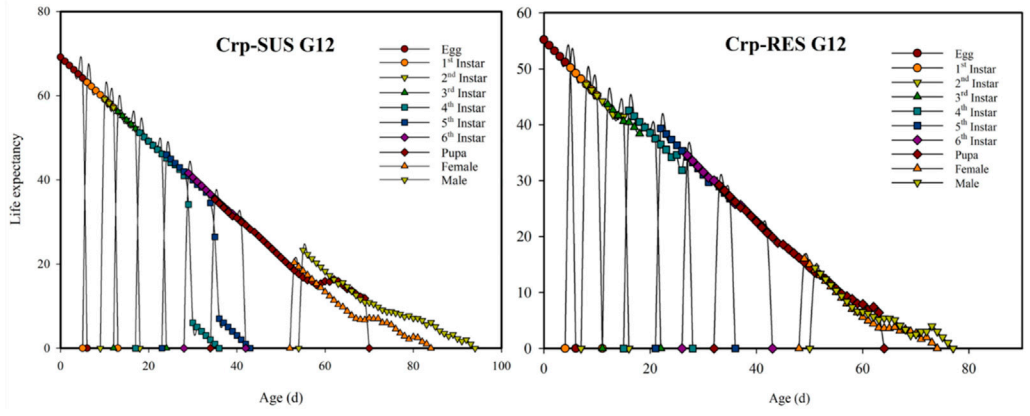


FIGURE 3
Age-stage-specific life expectancies (e_{xj}) for the Crp-RES G_{12} and Crp-SUS G_{12} strains of *S. frugiperda*.

3.4 Age-stage-specific life expectancies (e_{xj}) and age-specific survival rate (l_x) of the Crp-RES G_{12} and Crp-SUS G_{12} strains

In this research, the Crp-SUS G_{12} strain had a higher age-stage-specific life expectancy (e_{xj}) than the Crp-RES G_{12} strain (Figure 3). The age-specific survival rates (l_x) for these strains indicated that the l_x value decreased more quickly for the Crp-RES G_{12} strain at 15 d than the Crp-SUS G_{12} strain. In addition, the highest mean fecundity (m_x) for Crp-RES G_{12} was 140.85 eggs compared to the Crp-SUS G_{12} that showed a mean fecundity of 85 eggs (Figure 4).

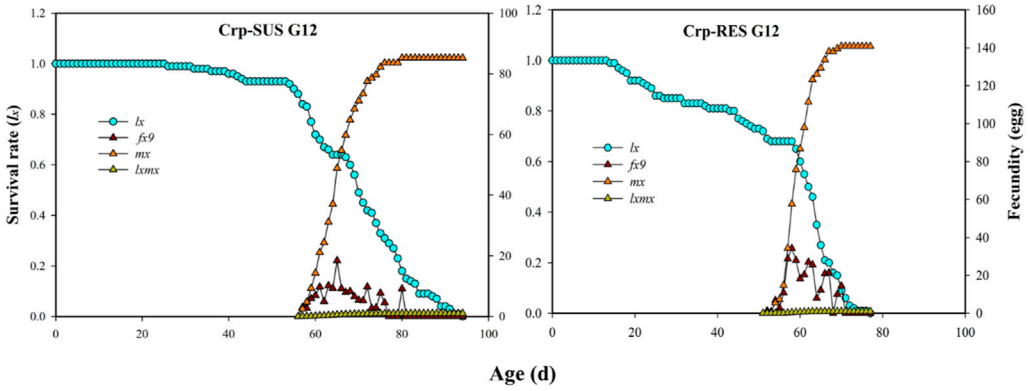


FIGURE 4
Age-specific survival rate (l_x), female age-specific fecundity (f_{x9}), age-specific fecundity of the total population (m_x), and age-specific net maternity ($l_x m_x$) of the Crp-RES G_{12} and Crp-SUS G_{12} strains of *S. frugiperda*.

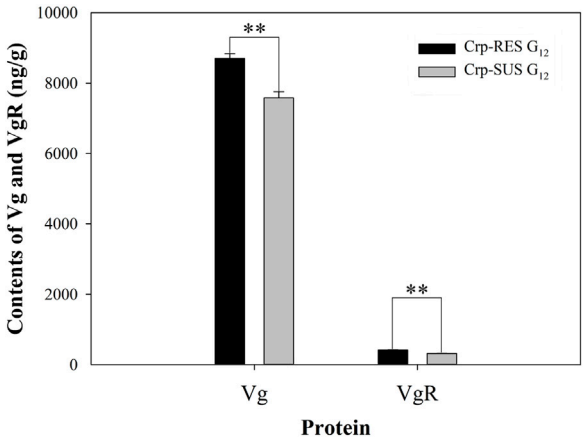


FIGURE 5
Contents of Vg and VgR for Crp-RES G_{12} and Crp-SUS G_{12} strains were presented as the mean of three replications ± SE. The asterisks above the bars indicate statistical differences between Vg and VgR contents of both strains (Student's *t*-test, $^{**}p < 0.01$).

3.5 Contents of Vg and VgR in the Crp-RES G_{12} and Crp-SUS G_{12} strains

The results indicated that the Vg content in the Crp-RES G_{12} strain (8,702.9 ng/g) was significantly more than that in the Crp-SUS G_{12} strain (7,581.7 ng/g) ($p < 0.01$). Similarly, the VgR content in Crp-RES G_{12} (414.6 ng/g) was significantly more than that in Crp-SUS G_{12} (318.8 ng/g) ($p < 0.01$) (Figure 5).

4 Discussion

The development of insect life tables is crucial for pest control methods when evaluating insect population dynamics (Gabre et al., 2005). Numerous biological and non-biological elements, including temperature, light, and the host plants, cause changes in insect populations (Tuan et al., 2014). Chemicals are currently considered one of the most effective integrated pest control strategies. However, the pervasive use of pesticides has led to substantial resistance, which is now a significant factor altering the parameters of insects' life tables. Adequate knowledge about the life cycle, survival rate, and reproduction can be helpful in developing efficient management strategies against insect pests (Harcourt, 1969). In this study, we established the susceptible and reduced susceptible strains of *S. frugiperda* against chlorantraniliprole, and resistance ratios were gradually decreased (RR = 0.85 -fold) and increased (RR = 4.0 -fold) in both strains, respectively. Based on the results, all life stages of the Crp-SUS G_{12} strain had significantly longer time duration than those of the Crp-RES G_{12} strain, which is consistent with the findings of Abbas et al. (2012) in which imidacloprid application enhanced the emergence rate of healthy adults, developmental time, and fecundity of *S. litura*. In contrast, it was also reported that the fecundity of *S. litura* was reduced under the influence of emamectin benzoate, along with an increase in the larval duration and developmental time (Zaka et al., 2014), which was similar in the case of the Crp-SUS G_{12} strain in this study. Similarly, the thiamethoxam-selected individuals of *Mythimna separata* had prolonged larval and developmental times (Yasoob et al., 2018). This tendency suggests that different pesticides can alter the insect life history characteristics, which are also affected by many other variables, including the temperature (Chi et al., 2016), host plants (Nematikalkhoran et al., 2018), and insecticides (Steinbach et al., 2017).

Furthermore, chlorantraniliprole significantly affected the total pre-oviposition period (TPOP), mean longevities of male (M) and female (F) adults, and developmental times (T) of Crp-RES G_{12}. These were positively related to the variations in intrinsic rate of increase (r), net reproductive rate (R), and finite rate of increase (λ). The fecundity and reproduction of both strains were greatly affected by chlorantraniliprole in this study. The plotted curves of the age-specific survival rate (l_x) showed that the resistant strain showed the fitness cost of its normal survival rate and adapted to increase its eggs for better survival while shortening its life span. However, the Crp-SUS G_{12} strain modified itself toward only better survival and tried to maintain an average life duration. The age-stage reproductive value (v_{xj}) also revealed the same trend with increased life span and

decreased fecundity in the Crp-SUS G_{12} strain and *vice versa*. Similar results were also reported by Huang et al. (2019) that the bistrifluron-resistant strain of *S. litura* had higher values of population metrics along with an increase in fecundity than the bistrifluron-susceptible strain. However, in contrast, Han et al. (2012) showed that the fecundity decreased dramatically, along with the decrease in the values of *R*, *r*, and λ, when the sublethal doses of chlorantraniliprole were applied to the larvae of *Plutella xylostella*. Still, as in our study, we established Crp-RES G_{12} by screening LD_{70} of each generation (which is more than the median lethal dosage), and thus its effects are different from the sublethal doses. Moreover, the varied amounts of pesticides may contribute to differences between past and present results, based on the diverse species of insects evaluated and the pesticide application method.

Moreover, the fecundity in female adults is primarily governed by vitellogenin (Vg), and its receptor (VgR), as a precursor to Vg, is involved in the sequestration of Vg into the oocytes through endocytosis during the development and subsequently crucial for Vg uptake and oocyte maturation (Lu et al., 2015). The results of our study showed that the protein contents of Vg and VgR for Crp-RES G_{12} were higher than those for Crp-SUS G_{12}, which supported the findings of Chen et al. (2020) that under the influence of triflumezopyrim, Vg and VgR contents increased in the subsequent F_4 generation of *Sogatella furcifera*. In addition, Ge et al. (2011) reported that Vg content in the female larvae of *Nilaparvata lugens* significantly increased under the influence of triazophos. Similarly, Liu et al. (2016) also reported that the resistant strain of *Tetranychus cinnabarinus* treated with fenpropathrin had more protein content than the sensitive strain.

These results from life table data proved that the reduction in susceptibility against chlorantraniliprole positively affected the life history traits of *S. frugiperda*. However, to comprehend the impact of chlorantraniliprole on the life parameters of the individuals of Crp-SUS G_{12} and Crp-RES G_{12}, further screening and follow-up research can be carried out to get more resistant individuals. In addition, as an alternate plan to integrated pest management, to prevent chlorantraniliprole from promoting the population dynamics of *S. frugiperda*, alternative no cross-resistance chemical insecticides or natural control agents should be used.

5 Conclusion

This study about the life history traits revealed that female adults of the reduced susceptible strain increased their fertility while lowering their life span to withstand chlorantraniliprole. In contrast, female adults of the susceptible strain decreased their fecundity while increasing their life span. These findings may serve as a foundation for subsequent research on chlorantraniliprole resistance in *S. frugiperda* and can contribute to developing the integrated pest management (IPM) program for fall armyworms in the future.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Author contributions

AH and XW: Conceptualization and methodology. AH, SZ, and XW: Writing the original draft. AH, QC, LX, YW, CG, LZ, and XL: Investigation and formal analysis. LZ, PJ, and XW: Supervision, review, and editing. XW: Project administration and funding acquisition.

Funding

The authors gratefully acknowledge the financial support of the Science and Technology Program of Sichuan, China (2022YFSY0034).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer PL declared a shared affiliation with the author LZ to the handling editor at the time of review.

Publisher's note

References

Abbas, N., Shad, S. A., and Razaq, M. (2012). Fitness cost, cross resistance and realized heritability of resistance to imidacloprid in *Spodoptera litura*, (Lepidoptera: Noctuidae). *Pestic. Biochem. Physiol.* 103, 181–188. doi:10.1016/j.pestbp.2012.05.001

Abdel-Khalek, A. A., and Momen, F. M. (2022). Biology and life table parameters of *Proprioseiopsis lindquisti* on three eriophyid mites (Acari: Phytoseiidae: Eriophyidae). *Persian J. Acarol.* 11, 59–69. doi:10.22073/pja.v11i1.68574

Barros, E. M., Torres, J. B., Ruberson, J. R., and Oliveira, M. D. (2010). Development of *Spodoptera frugiperda* on different hosts and damage to reproductive structures in cotton. *Entomol. Exp. Appl.* 137, 237–245. doi:10.1111/j.1570-7458.2010.01058.x

Chen, L., Wang, X., Zhang, Y., Yang, R., Zhang, S., Xu, X., et al. (2020). The population growth, development and metabolic enzymes of the white-backed planthopper, *Sogatella furcifera* (Hemiptera: Delphacidae) under the sublethal dose of triflumezopyrim. *Chemosphere* 247, 125865. doi:10.1016/j.chemosphere.2020.125865

Chi, B., Zheng, X., Liang, X., and Liu, Y. (2016). Temperature-dependent demography of *Agriphila aeneociliella* (Lepidoptera: Crambidae), a new insect pest of wheat in China. *Agric. For. Entomol.* 18, 189–197. doi:10.1111/afe.12151

Denholm, I., and Devine, G. (2013). Insecticide resistance. *Encycl. Biodivers.* 3, 298–307. doi:10.1016/B978-0-12-384719-5.00104-0

Gabre, R. M., Adham, F. K., and Chi, H. (2005). Life table of *Chrysomya megacephala* (fabricius) (Diptera: Calliphoridae). *Acta Oecol* 27, 179–183. doi:10.1016/j.actao.2004.12.002

Gao, Z., Chen, Y., He, K., Guo, J., and Wang, Z. (2021). Sublethal effects of the microbial-derived insecticide spinetoram on the growth and fecundity of the fall armyworm (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 114, 1582–1587. doi:10.1093/jee/toab123

Ge, L. Q., Cheng, Y., Wu, J. C., and Jahn, G. C. (2011). Proteomic analysis of insecticide triazophos-induced mating-responsive proteins of *Nilaparvata lugens* stål (Hemiptera: Delphacidae). *J. Proteome Res.* 10, 4597–4612. doi:10.1021/pr200414g

Ge, L. Q., Zheng, S., Gu, H. T., Zhou, Y. K., Zhou, Z., Song, Q. S., et al. (2019). Jinggangmycin-induced UDP-glycosyltransferase 1-2-like is a positive modulator of fecundity and population growth in *Nilaparvata lugens* (stål) (Hemiptera: Delphacidae). *Front. Physiol.* 10, 747. doi:10.3389/fphys.2019.00747

Guo, J., Zhang, M., Gao, Z., Wang, D., He, K., and Wang, Z. (2021). Comparison of larval performance and oviposition preference of *Spodoptera frugiperda* among three host plants: Potential risks to potato and tobacco crops. *Insect Sci.* 28, 602–610. doi:10.1111/1744-7917.12830

Gutierrez-Moreno, R., Mota-Sanchez, D., Blanco, C. A., Whalon, M. E., Teran-Santofimio, H., Rodriguez-Maciel, J. C., et al. (2019). Field-evolved resistance of the fall armyworm (Lepidoptera: Noctuidae) to synthetic insecticides in Puerto Rico and Mexico. *J. Econ. Entomol.* 112, 792–802. doi:10.1093/jee/toy372

Han, W., Zhang, S., Shen, F., Liu, M., Ren, C., and Gao, X. (2012). Residual toxicity and sublethal effects of chlorantraniliprole on *Plutella xylostella* (Lepidoptera: Plutellidae). *Pest Manag. Sci.* 68, 1184–1190. doi:10.1002/ps.3282

Harcourt, D. G. (1969). The development and use of life tables in the study of natural insect populations. *Annu. Rev. Entomol.* 14, 175–196. doi:10.1146/annurev.en.14.010169.001135

Hardke, J. T., Lorenz, G. M., and Leonard, B. R. (2015). Fall armyworm (Lepidoptera: Noctuidae) ecology in southeastern cotton. *J. Integr. Pest Manag.* 6, 10. doi:10.1093/jipm/pmv009

Haynes, K. F. (1988). Sublethal effects of neurotoxic insecticides on insect behavior. *Annu. Rev. Entomol.* 33, 149–168. doi:10.1146/annurev.en.33.010188.001053

Huang, Q., Wang, X., G., Yao, X., Gong, C., and Shen, L. (2019). Effects of bistrifluron resistance on the biological traits of *Spodoptera litura* (fab.) (Noctuidae: Lepidoptera). *Ecotoxicology* 28, 323–332. doi:10.1007/s10646-019-02024-2

Lai, T. C., Li, J., and Su, J. Y. (2011). Monitoring of beet armyworm *Spodoptera exigua* (Lepidoptera: Noctuidae) resistance to chlorantraniliprole in China. *Pestic. Biochem. Physiol.* 101, 198–205. doi:10.1016/j.pestbp.2011.09.006

Liu, X., Shen, G. M., Xu, H. R., and He, L. (2016). The fenpropathrin resistant *Tetranychus cinnabarinus* showed increased fecundity with high content of vitellogenin and vitellogenin receptor. *Pestic. Biochem. Physiol.* 134, 31–38. doi:10.1016/j.pestbp.2016.04.010

Liu, Z. K., Li, X. L., Tan, X. F., Yang, M. F., Idrees, A., Liu, J. F., et al. (2022). Sublethal effects of emamectin benzoate on fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Agriculture* 12, 959. doi:10.3390/agriculture12070959

Lu, K., Shu, Y. H., Zhou, J. L., Zhang, X. Y., Zhang, X. Y., Chen, M. G., et al. (2015). Molecular characterization and RNA interference analysis of vitellogenin receptor from *Nilaparvata lugens* (Stål). *J. Insect Physiol.* 73, 20–29. doi:10.1016/j.jinsphys.2015.01.007

Nauen, R. (2006). Insecticide mode of action: Return of the ryanodine receptor. *Pest Manag. Sci.* 62, 690–692. doi:10.1002/ps.1254

Nematikalkhoran, M., Razmjou, J., Borzoui, E., and Naseri, B. (2018). Comparison of life table parameters and digestive physiology of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) fed on various barley cultivars. *J. Insect Sci.* 18 (2), 31. doi:10.1093/jisesa/iey022

Rehan, A., Freed, S., and Abbas, N. (2015). Fitness cost of methoxyfenozide and the effects of its sublethal doses on development, reproduction, and survival of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). *Neotrop. Entomol.* 44, 513–520. doi:10.1007/s13744-015-0306-5

Roy, S., Saha, T. T., Zou, Z., and Raikhel, A. S. (2018). Regulatory pathways controlling female insect reproduction. *Annu. Rev. Entomol.* 63, 489–511. doi:10.1146/annurev-ento-020117-043258

Sattelle, D. B., Cordova, D., and Cheek, T. R. (2008). Insect ryanodine receptors: Molecular targets for novel pest control chemicals. *Invert. Neurosci.* 8 (3), 107–119. doi:10.1007/s10158-008-0076-4

Schneider, W. J. (1996). Vitellogenin receptors: Oocyte-specific members of the low-density lipoprotein receptor supergene family. *Int. Rev. Cytol.* 166, 103–137. doi:10.1016/s0074-7696(08)62507-3

Shah, R. M., Shad, S. A., and Abbas, N. (2015). Mechanism, stability and fitness cost of resistance to pyriproxyfen in the house fly, *Musca domestica* L. (Diptera: Muscidae). *Pestic. Biochem. Physiol.* 119, 67–73. doi:10.1016/j.pestbp.2015.02.003

Sparks, A. N. (1979). A review of the biology of the fall armyworm. *Fla. Entomol.* 62, 82–87. doi:10.2307/3494083

Steinbach, D., Moritz, G., and Nauen, R. (2017). Fitness costs and life table parameters of highly insecticide-resistant strains of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) at different temperatures. *Pest Manag. Sci.* 73 (9), 1789–1797. doi:10.1002/ps.4597

Teke, M. A., and Mutlu, Ç. (2021). Insecticidal and behavioral effects of some plant essential oils against *Sitophilus granarius* L. and *Tribolium castaneum* (Herbst). *J. Plant Dis. Prot.* 128, 109–119. doi:10.1007/s41348-020-00377-z

Tuan, S. J., Lee, C. C., and Chi, H. (2014). Population and damage projection of *Spodoptera litura* (F.) on peanuts (*Arachis hypogaea* L.) under different conditions using the age-stage, two-sex life table. *Pest Manag. Sci.* 70, 805–813. doi:10.1002/ps.3618

Ullah, S., Shad, S. A., and Abbas, N. (2016). Resistance of dusky cotton bug, *Oxycarenus hyalinipennis* costa (lygaidae: Hemiptera), to conventional and novel chemistry insecticides. *J. Econ. Entomol.* 109 (1), 345–351. doi:10.1093/jee/tov324

Wang, H. H., Lv, S. L., Zhao, R., Liang, P., Zhang, S., Gao, X. W., et al. (2021). Establishment of the relative susceptible baselines of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae to commonly used insecticides. *Acta. Entomol. sini.* 64, 1427–1432. doi:10.16380/j.kcxb.2021.12.008

Wang, W., Mo, J., Cheng, J., Zhuang, P., and Tang, Z. (2006). Selection and characterization of spinosad resistance in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). *Pestic. Biochem. Physiol.* 84, 180–187. doi:10.1016/j.pestbp.2005.07.002

Wang, X. G., Xiang, X., Yu, H. L., Liu, S., Yin, Y., Cui, P., et al. (2018). Monitoring and biochemical characterization of beta-cypermethrin resistance in *Spodoptera exigua* (Lepidoptera: Noctuidae) in Sichuan province, China. *Pestic. Biochem. Physiol.* 146, 71–79. doi:10.1016/j.pestbp.2018.02.008

Westbrook, J. K., Nagoshi, R. N., Meagher, R. L., Fleischer, S. J., and Jairam, S. (2016). Modeling seasonal migration of fall armyworm moths. *Int. J. Biometeorol.* 60, 255–267. doi:10.1007/s00484-015-1022-x

Wu, Z., Yang, L., He, Q., and Zhou, S. (2020). Regulatory mechanisms of vitellogenesis in insects. *Front. Cell Dev. Biol.* 8, 593613. doi:10.3389/fcell.2020.593613

Xiao, Y. T. (2021). Research on the invasive pest of fall armyworm (*Spodoptera frugiperda*) in China. *J. Integr. Agric.* 20, 633–636. doi:10.1016/s2095-3119(21)63623-7

Xie, W., Zhi, J., Ye, J., Zhou, Y., Li, C., Liang, Y., et al. (2021). Age-stage, two-sex life table analysis of *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) reared on maize and kidney bean. *Chem. Biol. Technol. Agric.* 8, 44. doi:10.1186/s40538-021-00241-8

Yasoob, H., Abbas, N., Li, Y., and Zhang, Y. (2018). Selection for resistance, life history traits and the biochemical mechanism of resistance to thiamethoxam in the maize armyworm, *Mythimna separata* (Lepidoptera: Noctuidae). *Phytoparasitica* 46, 627–634. doi:10.1007/s12600-018-0692-4

Younas, H., Razaq, M., Farooq, M. O., and Saeed, R. (2022). Host plants of *Phenacoccus solenopsis* (Tinsley) affect parasitism of *Aenasius bambawalei* (Hayat). *Phytoparasitica* 50, 669–681. doi:10.1007/s12600-022-00980-w

Zaka, S. M., Abbas, N., Shad, S. A., and Shah, R. M. (2014). Effect of emamectin benzoate on life history traits and relative fitness of *Spodoptera litura*, (Lepidoptera: Noctuidae). *Phytoparasitica* 42, 493–501. doi:10.1007/s12600-014-0386-5

Zhang, D. D., Xiao, Y. T., Xu, P. J., Yang, X. M., Wu, Q. L., and Wu, K. M. (2021). Insecticide resistance monitoring for the invasive populations of fall armyworm, *Spodoptera frugiperda* in China. *J. Integr. Agric.* 19 (0), 783–791. doi:10.1016/s2095-3119(20)63392-5

Zhen, C. A., Miao, L., and Gao, X. W. (2018). Sublethal effects of sulfoxaflor on biological characteristics and vitellogenin gene (*AlVg*) expression in the mirid bug, *Apolygus lucorum* (Meyer-Dür). *Pestic. Biochem. Physiol.* 144, 57–63. doi:10.1016/j.pestbp.2017.11.008

frontiers | Frontiers in Physiology

TYPE Correction
PUBLISHED 12 May 2023
DOI 10.3389/fphys.2023.1211689

OPEN ACCESS

APPROVED BY
Frontiers Editorial Office,
Frontiers Media SA, Switzerland

*CORRESPONDENCE
Xuegui Wang,
wangxuegui@sicau.edu.cn

†These authors have contributed equally to this work and share first authorship

RECEIVED 25 April 2023
ACCEPTED 04 May 2023
PUBLISHED 12 May 2023

CITATION
Hasnain A, Zhang S, Chen Q, Xia L, Wu Y, Gong C, Liu X, Jian P, Zhang L and Wang X (2023), Corrigendum: Effects of chlorantraniliprole on the life history traits of fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae).
Front. Physiol. 14:1211689.
doi: 10.3389/fphys.2023.1211689

Corrigendum: Effects of chlorantraniliprole on the life history traits of fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

Ali Hasnain[1,2,3†], Shuirong Zhang[1,2†], Qinghua Chen[4], Lijuan Xia[5], Yutong Wu[2], Changwei Gong[1,2], Xuemei Liu[1,2], Pu Jian[1,2], Lei Zhang[6] and Xuegui Wang[1,2*]

[1]State Key Laboratory of Crop Gene Exploration and Utilization in Southwest China, Sichuan Agricultural University, Chengdu, China, [2]College of Agriculture, Sichuan Agricultural University, Chengdu, China, [3]College of Plant Protection, Nanjing Agricultural University, Nanjing, China, [4]Key Laboratory of Integrated Pest Management on Crops in Southwest, Institute of Plant Protection, Sichuan Academy of Agricultural Sciences, Ministry of Agriculture, Chengdu, China, [5]Talent Development Service Center, Sichuan Provincial Department of Agriculture and Rural Affairs, Chengdu, China, [6]Department of Entomology, China Agricultural University, Beijing, China

KEYWORDS

***Spodoptera frugiperda*, resistance, life history traits, chlorantraniliprole, vitellogenin**

A Corrigendum on
Effects of chlorantraniliprole on the life history traits of fall armyworm Spodoptera frugiperda (Lepidoptera: Noctuidae)

by Hasnain A, Zhang S, Chen Q, Xia L, Wu Y, Gong C, Liu X, Jian P, Zhang L and Wang X (2023). Front. Physiol. 14:1155455. doi: 10.3389/fphys.2023.1155455

In the published article, there was an error in the **Acknowledgments** statement. The Acknowledgments statement should have been a **Funding** statement and the grant number "(22SYSX0138)" has further been corrected to "(2022YFSY0034)." The correct **Funding** statement appears below.

"The authors gratefully acknowledge the financial support of the Science and Technology Program of Sichuan, China (2022YFSY0034)."

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

Publisher's note

frontiers | Frontiers in Physiology

TYPE Original Research
PUBLISHED 13 April 2023
DOI 10.3389/fphys.2023.1160873

OPEN ACCESS

EDITED BY
Qingli Shang,
Jilin University, China

REVIEWED BY
Zhaojiang Guo,
Institute of Vegetables and Flowers (CAAS), China
Youssef Dewer,
Agricultural Research Center, Egypt

*CORRESPONDENCE
Xiaoping Yu,
yxp@cjlu.edu.cn
Xuping Shentu,
stxp@cjlu.edu.cn

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Invertebrate Physiology, a section of the journal Frontiers in Physiology

RECEIVED 07 February 2023
ACCEPTED 29 March 2023
PUBLISHED 13 April 2023

CITATION
Feng X, Li D, Wang H, Yu X and Shentu X (2023), Fitness costs of resistance to insecticide pymetrozine combined with antimicrobial zhongshengmycin in *Nilaparvata lugens* (Stål). *Front. Physiol.* 14:1160873. doi: 10.3389/fphys.2023.1160873

Fitness costs of resistance to insecticide pymetrozine combined with antimicrobial zhongshengmycin in *Nilaparvata lugens* (Stål)

Xupiaoyang Feng†, Danting Li†, Hongfeng Wang, Xiaoping Yu* and Xuping Shentu*

Zhejiang Provincial Key Laboratory of Biometrology and Inspection and Quarantine, College of Life Science, China Jiliang University, Hangzhou, China

The brown planthopper, *Nilaparvata lugens* (Stål), is a major pest of rice crops, and its control is critical for food security. Pymetrozine has been recommended as an alternative to imidacloprid for controlling *N. lugens*, but the pest has developed high resistance to it, making its prohibition and restriction urgent. To address this issue, we conducted a study using a mixture of pymetrozine and zhongshengmycin with the effective ratio of 1:40, to evaluate the fitness costs in *N. lugens*. Our results showed that *N. lugens* had a relative fitness of 0.03 under this ratio, with significantly reduced longevity, female and male adult periods, total pre-oviposition days, and fecundity. Moreover, the expression levels of the uricase gene (EC1.7.3.3) and farnesyl diphosphate farnesyl transferase gene (EC2.5.1.21) were reduced in *N. lugens*. These genes are involved in urea metabolism and steroid biosynthesis pathway, respectively, and their suppression can interfere with the normal nutritional function of *N. lugens*. Our study demonstrates that the combination of chemical insecticides and antimicrobials can delay the development of resistance and improve the efficiency of pest control. This information is valuable for researchers developing management strategies to delay the development of pymetrozine resistance in *N. lugens*.

KEYWORDS

***Nilaparvata lugens* (Stål), pymetrozine, zhongshengmycin, fitness costs, two-sex life table**

1 Introduction

The brown planthopper, *Nilaparvata lugens* (Stål), is a monophagous rice pest belonging to the planthopper family and is considered one of the most critical pests in Asia (Bottrell and Schoenly, 2012). *N. lugens* feeds on rice phloem sap (Zhang, 2007), and as a sap-sucking insect, it harbors endosymbionts that are thought to play a vital role in host development (Fan et al., 2015). Due to its monophagy, *N. lugens* harbors obligatory yeast-like symbionts (YLS) that cannot be cultured *in vitro* (Pang et al., 2012). YLS is known to support sterol biosynthesis and nitrogen recycling in the host and are closely related to the life activities of *N. lugens* (Xue et al., 2014).

The impact of *N. lugens* infestation on rice crops in Asia is immeasurable. Chemical pesticides are currently the most commonly used method for *N. lugens* management, but this has led to increasing resistance and serious environmental pollution (Liu et al., 2018). Several

studies have reported the development of resistance to a range of insecticides by *N. lugens*, including imidacloprid, buprofezin, thiamethoxam, dinotefuran, clothianidin, isoprocarb, chlorpyrifos, and nitenpyram (Zhang et al., 2014; Bass et al., 2015).

Pymetrozine was first developed in 1988 and released on the market in 1994, it has contact and systemic activities and acts as an agonist of nicotinic acetylcholine receptors (Li et al., 2021). It is highly effective against a wide range of sap-feeding pests by irreversibly blocking the stylets of *N. lugens*, causing them to stop feeding and eventually starve to death. Pymetrozine has a long-lasting effect, but its ability to knock down pests is not strong (He et al., 2011a). While it is one of the recommended alternatives to imidacloprid for controlling *N. lugens* (He, et al., 2011b), its frequent use leads to high levels of resistance in insects, which are likely to continue to increase. Therefore, the use of pymetrozine is now restricted and prohibited. To delay the development of resistance in insects, the use of mixed insecticides is a common method to prolong the service life of insecticides. Pymetrozine mixture products are generally compatible with neonicotinoid insecticides. Research has shown that the maximum co-toxicity coefficient to the third instar nymphs of *N. lugens* can reach 2,813.04 when the compound ratio of pymetrozine and dinotefuran is 2:1, and the synergistic effect of the two mixtures is remarkable (Wang et al., 2013). Additionally, zhongshengmycin, a fungicide, can inhibit symbiotic microorganisms in the fat body of *N. lugens*, thereby increasing the mortality of *N. lugens* (Shi et al., 2021; Song et al., 2021). Thus, we propose a novel method of using a mixture of pymetrozine and a fungicide such as zhongshengmycin to control pests by inhibiting their symbionts.

Insecticide-resistant insects often exhibit increased energy consumption or disturbances in their metabolic balance, resulting in a certain fitness cost (Ullah et al., 2020a). Resistant populations often have slower developmental rates, reduced survival rates and fecundity (Zhang et al., 2018; Liao et al., 2019; Li et al., 2020), these adverse effects of fitness costs on the growth, development, and reproduction of resistant insects are important factors that limit the development of resistance. Therefore, studying the cost of resistance fitness provides theoretical support for formulating resistance control measures (Liao et al., 2019). Studies have also shown that *N. lugens* is at risk of developing resistance to several insecticides, including imidacloprid, fipronil, nitenpyram, chlorpyrifos, and sulfenalazine (Ullah et al., 2020b). However, due to the obvious fitness cost of resistant populations, once selection pressure is lost, the sensitivity of *N. lugens* to an agent can recover quickly after stress. Therefore, when controlling *N. lugens* in the field, the frequency of application of the abovementioned pesticides should be strictly controlled, long-term single use should be avoided, and other types of insecticides without cross-resistance should be used in rotation to effectively delay the development of *N. lugens* resistance (Shi et al., 2011). Fitness costs vary among different species and depend on the types of pesticide treatments and living conditions. Understanding the fitness costs of insecticide resistance can help conduct a more comprehensive evaluation of new pesticide mixtures and design an integrated pest management program.

Previous studies have revealed that the genetic capacities for nitrogen recycling of both *N. lugens* and YLS are complementary (Brentassi et al., 2017), the pathways of the two organisms are highly complementary, with the uricase gene (EC1.7.3.3) being the only shared gene (Xue et al., 2014). The cooperation between the enzymes encoded by the two genomes contributes to the successful conversion of urate into precursors for the biosynthesis of essential amino acids, this nitrogen-recycling pathway serves as an additional source of amino acids for *N. lugens*, indicating that this integrative system is an adaptation *via* host-symbiont co-evolution that enables *N. lugens* to exist solely on a diet of nutritionally limited and unbalanced rice phloem sap. Researchers have also demonstrated that *N. lugens* and YLS have developed an interdependent system for steroid biosynthesis. The ability of YLS to supply sterol may explain how *N. lugens* can survive on a sterol-free diet. In general, the first enzymes of many metabolic pathways are rate-limiting. Therefore, in this experiment, the first gene of the steroid biosynthetic pathway in the YLS genome for the farnesyl diphosphate farnesyl transferase gene (EC2.5.1.21) was selected (Xue et al., 2014; Fan et al., 2015).

In this study, susceptible populations of *N. lugens* without laboratory exposure to chemical pesticides were used to evaluate the fitness costs of pymetrozine, zhongshengmycin, and the pymetrozine–zhongshengmycin mixture. The findings of this study are of great significance for delaying the development of resistance in *N. lugens* and formulating more efficient pest management programs, the results can serve as a reference for establishing an effective control system for *N. lugens*, thereby providing valuable information to researchers in the field.

2 Materials and methods

2.1 Experimental materials

2.1.1 Insect rearing

The susceptible rice variety TN1 was used in the trials. After soaking for 24 h, seed germination was accelerated for 48 h, and the seeds were evenly spread in an acrylic tray containing perlite. The rice seedlings were grown to three tillers in an artificial climate room and were used to feed *N. lugens*. The test insects were sourced from a rice field in Hangzhou, Zhejiang, China. They were maintained on TN1 in an artificial climate room for more than 10 generations under standard conditions of 26°C ± 1°C, 70%–80% relative humidity (RH), and a 16 h:8 h light/dark photoperiod.

2.1.2 Chemicals

Pymetrozine [(E)-4,5-dihydro-6-methyl-4-(3-pyridylmethyleneamino)-1,2,4-triazin-3(2H)-one] (96.6% active ingredient w/w) was purchased from Jiangsu Weunite Fine Co., Ltd. Zhongshengmycin [1-N-glucosidine-2-amino-L-lysine-2-deoxygulosamine] (12% active ingredient w/w) was provided by Fujian Kaili Biotechnology Co., Ltd.

2.2 Fitness comparison

Four treatment groups were investigated in this experiment: a control treatment group (*N. lugens* population feeding on rice plants treated with 0.1% Triton X-100 water solution), a pymetrozine treatment group (*N. lugens* population feeding on rice plants treated with pymetrozine at LC_{50}), a zhongshengmycin treatment group (*N. lugens* population feeding on rice plants treated with

zhongshengmycin at LC_{50}), and a mixture treatment group (*N. lugens* population feeding on rice plants treated with a 1: 40 pymetrozine-zhongshengmycin mixture). Separate life tables were constructed for the *N. lugens* populations in the four treatment groups using the age-stage, two-sex life-table approach (Chi et al., 2020; Liu et al., 2020). Approximately 1000 *N. lugens* adults from each group were transferred into a clean acrylic frame (40 cm length, 30 cm width, 50 cm height) with fresh and healthy rice seedlings for oviposition. *N. lugens* were isolated from other insect sources. After 24 h, all adults were removed, and 100 neonate nymphs were randomly housed. Each test insect was numbered and observed individually in transparent plastic test tubes (2.5 cm diameter, 15 cm height) containing fresh rice seedlings until adulthood. Unmated male and female adults were simultaneously paired in tubes containing two fresh rice seedlings and covered with a fine gauze top. During this process, when paired males died, healthy males from the corresponding treatment group were supplemented in a timely manner until the female died. All test tubes were maintained at 26°C ± 1°C and 70%–80% RH under a 16 h: 8 h light/dark photoperiod. The nymphs were considered dead if they were unable to move after a slight push with a fine brush. The number of unhatched eggs on the rice seedlings was counted using a microscope after the newly hatched nymphs were counted. Population characteristics, including the development times of the different stages, longevity, and fecundity (female eggs), were recorded daily to establish the life tables.

2.3 Statistical analysis

The life-table data for the *N. lugens* individuals were analyzed using an age-stage, two-sex life-table theory (Qin et al., 2021). Through calculations, the adult pre-oviposition period (APOP), total pre-oviposition period (TPOP), population parameters, and other life-table parameters were obtained. Population parameters include the average generation period (T), intrinsic growth rate (rm), net reproduction rate (R_0), and weekly growth rate (λ). The relative fitness was calculated based on the relative fitness (R_f) = R_0/R_0 of the control group (Fujii et al., 2020; Zhang et al., 2018). The age-stage-specific survival rate (S_{xj}), age-specific survival rate (l_x), female age-specific fecundity (f_x), age-specific fecundity (m_x), and age-specific maternity (l_xm_x) were plotted using GraphPad Prism 9.

2.4 Real-time quantitative PCR analysis

After 72 h, 15 surviving individuals from each treatment group were isolated for RNA extraction and RT-qPCR analysis (Chen et al., 2017). Total RNA from males was extracted using a TaKaRa MiniBEST universal RNA Extraction Kit (TaKaRa, Dalian, China), and the cDNA template for qPCR was synthesized using a Perfect Real-Time PrimeScript™ RT Reagent Kit with a gDNA Eraser (TaKaRa). The quality of the extracted RNA was determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, United States) and confirmed *via* agarose gel electrophoresis. The qPCR reagent was SYBR® Premix ExTaq™ II (Tli RNaseHPlus) (TaKaRa). The specific primers for EC1733 qPCR were EC1733-F and EC1733-R, and the primers for EC25121 qPCR were EC25121-F and EC25121-R. β-actin was used as the qPCR internal reference, with the primers actin-F and actin-R (Supplementary Table S1) (Xue et al., 2014). A 20 μL reaction system was used for qPCR using a StepOnePlus™ real-time PCR System (Applied Biosystems, Carlsbad, CA, United States). Three independent biological replicates and three technical replicates were set up. The relative transcript levels of EC1.7.3.3 and EC2.5.1.21 in different samples were determined using the $2^{-\Delta\Delta CT}$ method (Maroniche et al., 2011; Zheng et al., 2021).

3 Results

3.1 Development period comparison

The life history parameters of the *N. lugens* populations in the control group and three treatment groups were summarized (Table 1). Although inconsistent differences were observed between the treatment groups for *N. lugens* in the 1–5 instar nymph stages, the nymph life span in the three treatment groups was longer than that in the control group, with the preadult life span being longest in the mixture treatment group. Among the four groups, the adult period and longevity of *N. lugens* were shortest in the mixture treatment group, and the APOP was significantly longer in the mixture treatment group than in the control and other treatment groups. The number of eggs laid in the mixture treatment group was the lowest, which was significantly lower than that in the control group and the other two treatment groups. These findings suggest that the combination of pymetrozine and zhongshengmycin (1:40) had more detrimental effects on the growth parameters, development status, survival, and reproductive ability of *N. lugens* than either insecticide alone.

3.2 Population parameter comparison

Population parameters, including T, rm, R_0, and λ, were analyzed for each treatment group using TWOSEX MSChart software (Table 2). The T (day) of *N. lugens* in the mixture treatment group was 20.05 days, indicating that the population treated with the mixture required the shortest time to complete a generation cycle. Meanwhile, the rm (d^{-1}) of *N. lugens* in the mixture treatment group was 0.06, indicating that the population growth trend in this group was the slowest among all the groups. The λ (d^{-1}) of *N. lugens* in the mixture treatment group was 1.062/d, with this population producing the fewest offspring per individual under conditions of unlimited resources. The R_o (d^{-1}) of *N. lugens* in the mixture treatment group was 3.34, indicating that this population had the smallest multiplication after one generation cycle. The R_f value for the mixture treatment group was 0.0349, calculated from the R_0 value, which was the lowest among the four groups. These results demonstrate that fitness costs were associated with insecticide resistance in *N. lugens*, and that the combination of pymetrozine and zhongshengmycin can effectively inhibit the development, survival, and progeny reproduction of *N. lugens* populations.

TABLE 1 Developmental period of *N. lugens* after treatment with different pesticides and mixture.

Stage	Treatment			
	Water mean ± SE[b]	Pymetrozine mean ± SE	Zhongshengmycin mean ± SE	Mixture[a] mean ± SE
First instar (day)	2.46 ± 0.05a[c]	2.77 ± 0.08ab	2.75 ± 0.10ab	2.90 ± 0.10b
Second instar (day)	2.30 ± 0.05a	2.85 ± 0.07bc	2.60 ± 0.11ab	3.10 ± 0.10c
Third instar (day)	2.73 ± 0.05a	3.81 ± 0.08b	3.45 ± 0.11c	4.10 ± 0.10d
Fourth instar (day)	3.17 ± 0.07a	3.62 ± 0.11a	3.60 ± 0.11a	3.60 ± 0.16a
Fifth instar (day)	3.46 ± 0.07a	2.54 ± 0.11b	2.80 ± 0.11b	3.00 ± 0.15b
Pre-adult (day)	14.12 ± 0.10a	15.58 ± 0.17b	15.20 ± 0.16b	16.70 ± 0.26c
Female adult (day)	15.52 ± 0.58a	8.62 ± 0.45b	6.30 ± 0.68b	6.83 ± 0.79b
Male adult (day)	17.33 ± 0.70a	7.62 ± 0.87b	5.70 ± 0.99b	7.50 ± 0.87b
Longevity (day)	30.55 ± 0.46a	23.69 ± 0.50b	21.20 ± 0.54b	23.80 ± 0.66b
APOP (day)	2.98 ± 0.14a	4.54 ± 0.144b	4.00 ± 0.19ab	4.50 ± 0.29b
TPOP (day)	19.19 ± 0.29a	15.77 ± 0.20b	19.25 ± 0.62a	18.50 ± 0.29a
Fecundity (eggs female)	603.93 ± 14.18a	204.38 ± 8.83b	134.13 ± 13.37bc	74.00 ± 7.04c

[c]Note: [a]Mixture tratment: the effective component mass ratio of pymetrozine to zhongshengmycin is 1:40.
[cb]Data in th table are expressed as the means ± SE, and the numerals in brackets represent the number of repetitions.
[c]Means followed by different letters within a column are significantly different according to paired bootstrap test ($p < 0.05$).

TABLE 2 Population parameters of *N. lugens* after treatment with different pesticides and mixture.

Parameter	Water	Pymetrozine	Zhongshengmycin	Mixture[a]
T (day)	22.74	21.04	21.36	20.05
rm (d^{-1})	0.201	0.156	0.125	0.060
λ (d^{-1})	1.223/d	1.169/d	1.133/d	1.062/d
R_0 (offspring individual^{-1})	95.71 ± 3.59	26.39 ± 1.22	14.51 ± 0.98	3.34 ± 0.89
R_f[b]		0.276	0.152	0.035

[a]Note: Mixture treatment: the effective component mass ratio of pymetrozine to zhongshengmycin is 1:40.
[b]$R_f = R_0$ of the other treatment group/R_0 of control treatment group.

3.3 Life table curve comparison

The age-stage survival rate (S_{xj}) represents the probability that a newly laid egg will survive to age x and stage j (Figure 1). In this experiment, *N. lugens* with the same incubation period were selected for subsequent records to minimize the differences caused by different incubation periods. The curves for the mixture and the zhongshengmycin treatment groups show similar patterns. However, the survival of *N. lugens* in different incubation periods were higher in the zhongshnegmycin treatment group than the mixture treatment group. The age-specific survival rate (l_x) without considering the different stages, which is a simplification of S_{xj}, also showed a similar pattern for the mixture and zhongshengmycin treatments (Figure 2A). The l_x value was found to decrease in the late growth stage, which is the period of high mortality for *N. lugens* populations. The l_x of the pymetrozine treatment decreased first, followed by the mixture and zhongshengmycin treatments, and last, by the control, which had a shorter plateau period that delayed the downward trend. These results verify that pymetrozine does not have a strong ability to knock down pests. The overall trends of the m_x (Figure 2C), l_xm_x (Figure 2B), and f_x (Figure 2D) curves initially increase and then decrease. However, in terms of the m_x values, the following trend was observed: water treatment > pymetrozine treatment > zhongshengmycin treatment > mixture treatment (Figure 2C). For the entire observation period, the age-specific fecundity, age-specific maternity, and female age-specific fecundity of *N. lugens* in the mixture treatment group were lower than those of the other three treatment groups. (Figures 2B, D). These results indicate that the mixture treatment had the best control efficiency against *N. lugens*.

3.4 Expression of EC1.7.3.3 and EC2.5.1.21 in *N. lugens*

The results indicate that the expression level of the uricase gene (EC1.7.3.3) (gene ID: NLU006642.1) was significantly

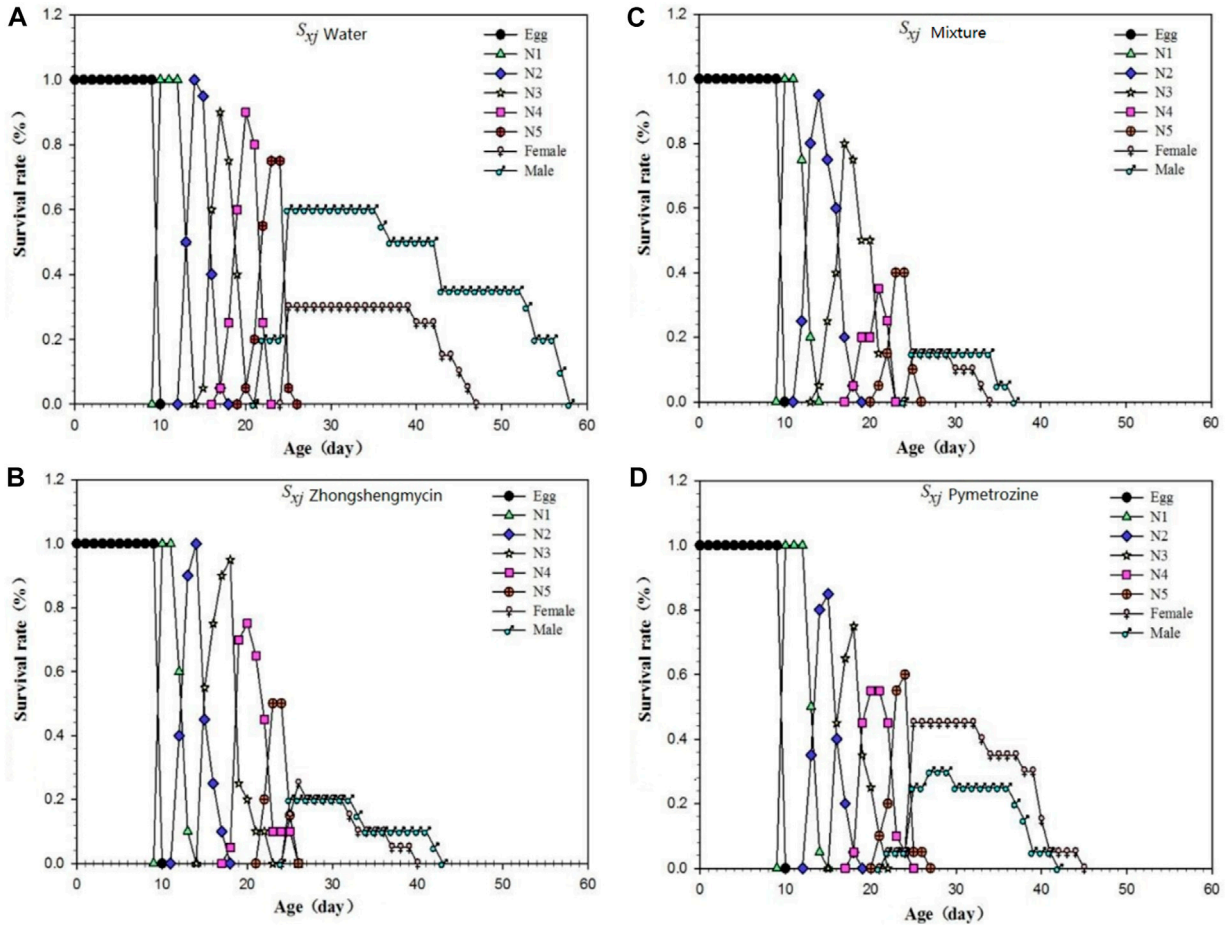


FIGURE 1
Age-stage-specific survival rate of *N. lugens* **(A)** Age-stage-specific survival rate (S_{xj}) of *N. lugens* in the water treatment; **(B)** age-stage-specific survival rate (S_{xj}) of *N. lugens* in the zhongshengmycin treatment; **(C)** age-stage-specific survival rate (S_{xj}) of *N. lugens* in the mixture treatment; **(D)** age-stage-specific survival rate (S_{xj}) of *N. lugens* in the pymetrozine treatments.

higher in the control group than in the zhongshengmycin and mixture treatment groups. Notably, the expression level in the mixture treatment group was lower than that for the zhongshengmycin treatment group (Figure 3A). EC1.7.3.3 is involved in both the nitrogen recycling and ammonia assimilation pathways in *N. lugens*, and the pathways would be compromised if either *N. lugens* or its YLS lost just one of the current genes that encode the enzymes involved in these pathways. The addition of zhongshengmycin affects YLS, which indirectly affects the expression of this gene. The mixture treatment not only inhibits YLS but also affects *N. lugens*, leading to a more pronounced effect on the expression of the uricase gene.

The results also show that the expression level of the farnesyl diphosphate farnesyl transferase gene (EC 2.5.1.21) (gene ID: A1835) was lower in the control treatment group than in the zhongshengmycin and mixture treatment groups (Figure 3B). The ability of YLS to supply sterol may explain how *N. lugens* can survive on a sterol-free diet. Thus, the inhibition of YLS by zhongshengmycin as an antimicrobial agent indirectly affected the expression of EC2.5.1.21 in *N. lugens*.

4 Discussion

Although pymetrozine has no direct insecticidal activity, it has a unique toxicological mechanism (He et al., 2011b; Yang et al., 2016). Electrical penetration graph evidence showed that pymetrozine toxicity to *N. lugens* occurs through the inhibition of phloem feeding. In addition, pymetrozine interferes with the reproductive behavior of *N. lugens* and hearing in insects (Liu et al., 2018; Wang et al., 2020). Subsequent studies on fruit flies identified the Nan-Iav TRPV channels as the target of pymetrozine and its analog pyrifluquinazon (Wang et al., 2019). The metabolic resistance mechanism of *N. lugens* to pymetrozine involves overexpression of cytochrome P450 *CYP6CS1* (Wang L. et al., 2021). Zhongshengmycin, on the other hand, is a low-toxicity aminoglycoside antibiotic produced by *Streptomyces lavendulae* var and is widely used as a chemical bactericide in the control of phytopathogens. Previous studies have shown that zhongshengmycin can effectively control vegetable bacterial diseases, rice bacterial leaf blight, and fruit tree diseases. Moreover, it has good herbicidal and fungicidal properties, making it a control agent in numerous new mixture studies. In

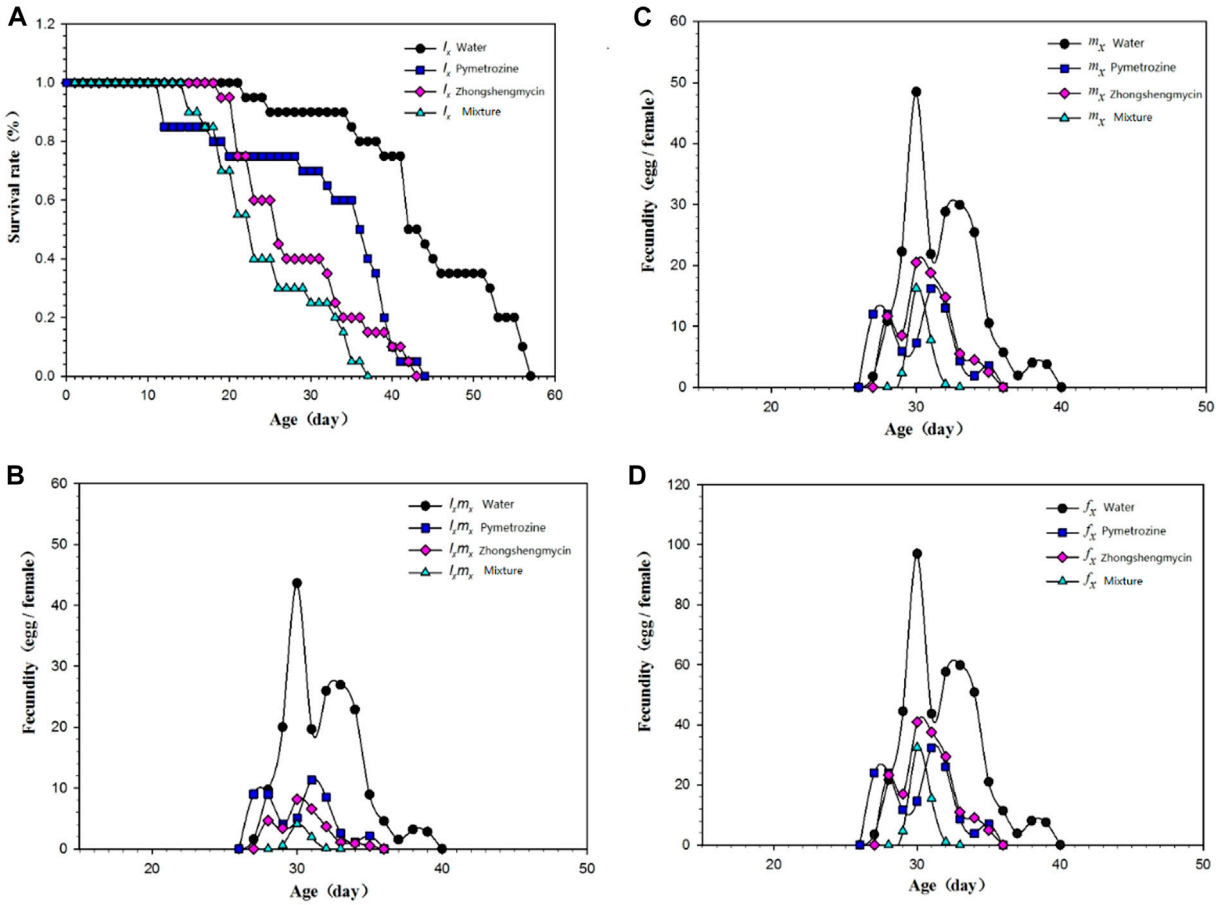


FIGURE 2
Age-specific survival rate and fecundity of *N. lugens* **(A)** Age-specific survival rate (l_x) of *N. lugens* in different treatments; **(B)** age-specific maternity (l_xm_x) of *N. lugens* in different treatments; **(C)** age-specific fecundity of *N. lugens* in different treatments (m_x); **(D)** female age-specific fecundity (f_x) of *N. lugens* in different treatments.

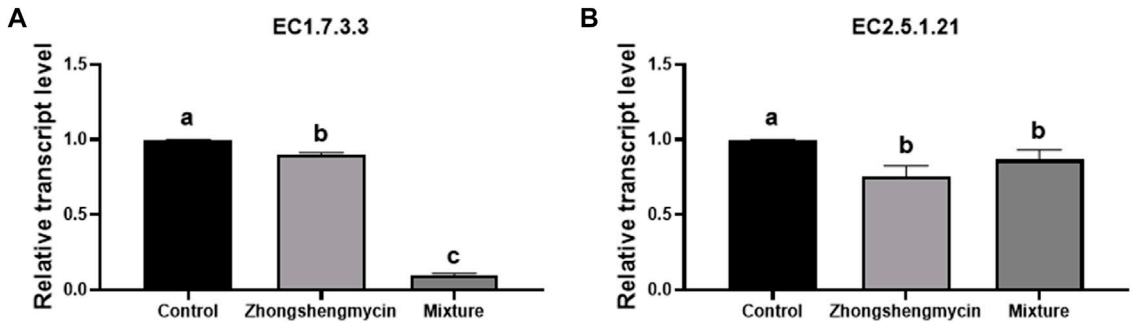


FIGURE 3
Expression level of EC1.7.3.3 and EC2.5.1.2.1 in *N. lugens* **(A)** Expression of EC1.7.3.3 in *N. lugens* with different treatments, **(B)** expression of EC2.5.1.2.1 in *N. lugens* with different treatments. After 72 h, 15 surviving individuals from each treatment group were isolated for RNA extraction and RT-qPCR analysis. Data are presented as the means ± standard error. Statistical analyses were conducted using one-way ANOVA and unpaired two-tailed Student's t-test using GraphPad Prism Software 8.0.2 (GraphPad Software, San Diego, CA, United States). Lowercase letters indicate significant differences ($p < 0.05$).

addition, zhongshengmycin has been found to inhibit *Xanthomonas oryzaepv. oryzae*, causing changes in bacterial communities (Wang Q. et al., 2021). While zhongshengmycin is effective in controlling plant pathogens, it can also affect the microbiome in the fat body through feeding behavior, which can influence the mortality of *N. lugens* (Shi et al., 2021).

The present study is the first to investigate the combined effects of pymetrozine and the antimicrobial agent zhongshengmycin on controlling *N. lugens*. The effective ingredient mass ratio of 1: 40 pymetrozine and zhongshengmycin showed a significant synergistic effect on controlling *N. lugens*. This effect is likely due to the antimicrobial properties of zhongshengmycin. Furthermore, the relationship between pymetrozine and zhongshengmycin is not just additive or amplifying but may involve complex interactions. The mixture not only improved the control effect of pymetrozine but also reduced the amount of pymetrozine used. The mechanisms of action of pymetrozine and zhongshengmycin are distinct, and the two agents do not exhibit cross-resistance. Typically, pesticide combinations involve different pesticides, different fungicides, or biological and chemical pesticides (He et al., 2013). The combination of an insecticide and an antimicrobial agent used in this experiment is relatively novel. The synergistic effect produced by the combination of agents can lead to a better control effect than using a single agent. This approach expands the insecticidal spectrum and reduces the dosage of the primary agent, thereby allowing the pesticide to exert its maximum effect at the lowest dosage.

Insecticide resistance often comes with fitness costs, which have been observed in many insect pests, including *Bradysia odoriphaga*, *Thrips hawaiiensis*, *Plutella xylostella*, *N. lugens*, and *Musca domestica*, and are considered a crucial in the evolution of resistance (Ullah et al., 2020a). Fitness costs accompanied by high energy costs or other significant disadvantages are generally observed during the development of pesticide resistance (Kliot and Ghanim, 2012). Our study on fitness costs shows that after treatment with insecticides, the pre-adult period of *N. lugens* was prolonged, the adult period and lifespan were shortened, and the survival rate and fecundity levels decreased. Understanding the age differentiation in the population and the differences in survival rates and fecundity at different stages can help improve of biological control of pests. Population parameters, including T, rm, R_0, and λ, significantly decreased after treatment with the pymetrozine-zhongshengmycin mixture, indicating that it effectively inhibits the growth rate of *N. lugens*. The mixture showed greater control of *N. lugens* populations than pymetrozine and zhongshengmycin alone, which is consistent with the shorter lifespan and lower fecundity was observed in insecticide-resistant insects screened for sulfoxaflor or nitenpyram resistance (Liao et al., 2019). To cope with the toxicity of insecticides, organisms require sufficient energy and resources for adaptation and survival. The fitness costs of insecticide resistance are considered an important factor limiting the evolution of resistance. These findings can be useful in developing effective resistance management strategies.

N. lugens has been shown to have a close symbiotic relationship with endosymbionts. Recent studies have shown that the ability to migrate and resistance to external environmental conditions are related to the abundance and variability of endosymbionts. In particular, *N. lugens* provides a habitat for endosymbionts, which in turn, contribute to its nutrition, growth, development, and ability to adapt to the environment. YLS is one of these endosymbiotic bacteria; it can synthesize essential amino acids, steroids, and vitamins to ensure the nutritional supply of *N. lugens* and the normal functioning of a number of important physiological functions (Vigneron et al., 2012; Laughton et al., 2016; Horgan et al., 2021). Thus, controlling *N. lugens* infection on rice by inhibiting symbionts using antimicrobials is feasible. There are complementary metabolic pathways between *N. lugens* and its symbionts, among which the uricase gene is a typical deletion gene involved in the nitrogen cycle and ammonia assimilation metabolic pathway. The uricase gene (EC 1.7.3.3) is present in the YLS and *N. lugens* genomes, and contributes to the conversion of uric acid to form the precursor of essential amino acid biosynthesis. It provides essential nutrients for the survival of *N. lugens* which are monophagous. Among the genes involved in the steroid biosynthesis pathway, the farnesyl diphosphate farnesyl transferase gene (EC 2.5.1.21) exists in the YLS genome, which converts farnesyl pyrophosphate into squalene in the branch chain of terpenoid biosynthesis, and is a key gene in sterol biosynthesis. To provide sterol nutrients necessary for the survival of *a* single diet of *N. lugens*. The metabolic genome verification of *N. lugens* and its symbionts showed that they were complementary. The complementarity of nutritional pathways provided a theoretical basis for understanding the interactions among *N. lugens* and its symbionts, and emphasized the potential direction for effective control of *N. lugens*. After being treated with zhongshengmycin, the relative expression level of EC 1.7.3.3 (geneID:NLU006642.1), which is involved in nitrogen cycling and the ammonia assimilation pathway was significantly decreased compared with the control group, while the relative expression level of EC 2.5.1.21 (geneID: A1835), which is involved in the steroid biosynthesis pathway, was extremely significantly decreased. The metabolic genome verified that *N. lugens* and YLS are highly complementary, and EC 1.7.3.3 is the only shared gene between *N. lugens* and YLS, which plays an important role in the urea metabolism pathway. At the same time, there is an interdependent steroid biosynthesis system between *N. lugens* and YLS, and EC 2.5.1.21 is only in YLS, which is closely related to sterol and sterol synthesis. Therefore, we can speculate that the decreased gene expression may be due to the inhibition of zhongshengmycin on the *N. lugens* and YLS genes, affecting the genes encoding related enzymes in this biological pathway and destroying the coevolutionary reciprocal relationship between *N. lugens* and YLS.

It is important to note that laboratory experiments may not fully reflect field conditions. Variations in the target population, differences in the degree of anti-drug resistance, and experimental errors can all result in different outcomes. Nonetheless, these results can serve as a useful early warning for pest managers and have practical implications for assessing the efficacy of new pesticides. However, it is necessary to verify the actual control effect of the mixture in the field through field efficacy tests. The relationship between the development of resistance and the cost of fitness can be a foundation for the development of new pesticides. Our study provides valuable insights for researchers to develop effective management strategies for delaying the development of pymetrozine resistance and establishing a sustainable control system for *N. lugens*.

5 Conclusion

This study demonstrates that combining pymetrozine with zhongshengmycin enhances the insecticidal effect of pymetrozine and its quick-acting properties. Additionally, the results indicate that antibiotics can impact YLS by inhibiting the expression of genes involved in critical pathways. These findings deepen our

understanding of the impact of insect symbionts on insect life activities. Given that field populations of *N. lugens* have shown increasing resistance to pymetrozine, it is crucial to use pymetrozine prudently. In light of this resistance, a cautious approach should be taken when using pymetrozine. Furthermore, considering the fitness cost of pests, developing more mixtures could reduce the use of pymetrozine while improving control effectiveness.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Author contributions

All authors listed have made substantial, direct, and intellectual contributions to the work and approved it for publication.

Funding

This study was funded by the National Natural Science Foundation of China (U21A20223) and Zhejiang Provincial Programs for Science and Technology Development (2022C02047 and 2023SNJF034).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2023.1160873/full#supplementary-material

References

Bass, C., Denholm, I., Williamson, M. S., and Nauen, R. (2015). The global status of insect resistance to neonicotinoid insecticides. *Pestic. Biochem. Physiol.* 121, 78–87. doi:10.1016/j.pestbp.2015.04.004

Bottrell, D. G., and Schoenly, K. G. (2012). Resurrecting the ghost of green revolutions past: The Brown planthopper as a recurring threat to high-yielding rice production in tropical Asia. *J. Asia-Pac Entomol.* 15 (1), 122–140. doi:10.1016/j.aspen.2011.09.004

Brentassi, M. E., Macia, A., de la Fuente, D., Cano, M. E., and Marino de Remes Lenicov, A. M. (2017). Food quality affects wing-form, demographic traits and number of yeast-like symbionts (YLS) in the planthopper vector, *delphacodes kuscheli* (Hemiptera: Delphacidae). *Stud. Neotropical Fauna Environ.* 52 (1), 25–36. doi:10.1080/01650521.2016.1258107

Chen, P., Zhang, M., and Wang, X. (2017). Cloning and characterization of a novel CRAL_TRIO domain gene *BPHsec1* from Brown planthopper. *Bull. Insectology* 70 (2), 251–259.

Chi, H., You, M., Atlıhan, R., Smith, C. L., Kavousi, A., Özgökçe, M. S., et al. (2020). Age-stage, two-sex life table: An introduction to theory, data analysis, and application. *Entomol. Gen.* 40 (2), 103–124. doi:10.1127/entomologia/2020/0936

Fan, H-W., Noda, H., Xie, H.-Q., Suetsugu, Y., Zhu, Q. H., and Zhang, C. X. (2015). Genomic analysis of an ascomycete fungus from the rice planthopper reveals how it adapts to an endosymbiotic lifestyle. *Genome Biol. Evol.* 7 (9), 2623–2634. doi:10.1093/gbe/evv169

Fujii, T., Sanada-Morimura, S., Matsukura, K., Van Chien, H., Cuong, L. Q., Loc, P. M., et al. (2020). Energy reserve compensating for trade-off between metabolic resistance and life history traits in the Brown planthopper (Hemiptera: Delphacidae). *J. Econ. Entomol.* 113 (4), 1963–1971. doi:10.1093/jee/toaa120

He, Y., Chen, L., Chen, J., Zhang, J., Chen, L., Shen, J., et al. (2011a). Electrical penetration graph evidence that pymetrozine toxicity to the rice Brown planthopper is by inhibition of phloem feeding: Effect of pymetrozine on planthopper feeding. *Pest Manag. Sci.* 67 (4), 483–491. doi:10.1002/ps.2098

He, Y., Hu, D., Lv, M., Jin, L., Wu, J., Zeng, S., et al. (2013). Synthesis, insecticidal, and antibacterial activities of novel neonicotinoid analogs with dihydropyridine. *Chem. Cent. J.* 7 (1), 76. doi:10.1186/1752-153X-7-76

He, Y., Zhang, J., Chen, J., Wu, Q., Chen, L., Xiao, P., et al. (2011b). Influence of pymetrozine on feeding behaviors of three rice planthoppers and a rice leafhopper using electrical penetration graphs. *J. Econ. Entomol.* 104 (6), 1877–1884. doi:10.1603/EC11180

Horgan, F. G., Penalver Cruz, A., Arida, A., Ferrater, J. B., and Bernal, C. C. (2021). Adaptation by the Brown planthopper to resistant rice: A test of female-derived virulence and the role of yeast-like symbionts. *Insects* 12 (10), 908. doi:10.3390/insects12100908

Kliot, A., and Ghanim, M. (2012). Fitness costs associated with insecticide resistance: Fitness cost and insecticide resistance. *Pest Manag. Sci.* 68 (11), 1431–1437. doi:10.1002/ps.3395

Laughton, A. M., Garcia, J. R., and Gerardo, N. M. (2016). Condition-dependent alteration of cellular immunity by secondary symbionts in the pea aphid, *Acyrthosiphon pisum*. *Acyrthosiphon Pisum. J. Insect Physiol.* 86, 17–24. doi:10.1016/j.jinsphys.2015.12.005

Li, W., Mao, K., Liu, C., Gong, P., Xu, P., Wu, G., et al. (2020). Resistance monitoring and assessment of the control failure likelihood of insecticides in field populations of the whitebacked planthopper *Sogatella Furcifera* (Horváth). *Crop Prot.* 127, 104973. doi:10.1016/j.cropro.2019.104973

Li, Y., Wei, X., Cui, Y., and Chou, J. (2021). Prevention of sucking mouthparts pests of pesticide products - pyrazole ketone of aphid. *J. Pestic.* 60 (7), 522–528. doi:10.16820/j.cnki.1006-0413.2021.07.013

Liao, X., Mao, K., Ali, E., Jin, R., Li, Z., Li, W., et al. (2019). Inheritance and fitness costs of sulfoxaflor resistance in *Nilaparvata lugens* (stål). *Pest Manag. Sci.* 75 (11), 2981–2988. doi:10.1002/ps.5412

Liu, J., Zhuang, J., Huang, W., Chi, H., Wang, C., Hua, H., et al. (2020). Different adaptability of the Brown planthopper, *Nilaparvata lugens* (Stål), to gradual and abrupt increases in atmospheric CO_2. *J. Pest Sci.* 93 (3), 979–991. doi:10.1007/s10340-020-01221-x

Liu, S. H., Yang, B. J., Wang, A. Y., Luo, J., and Tang, J. (2018). The white gene in *Nilaparvata Lugens* and its expression pattern under two different survival stresses. *J. Asia-Pac. Entomol.* 21 (2), 701–707. doi:10.1016/j.aspen.2018.04.010

Maroniche, G. A., Sagadin, M., Mongelli, V. C., Truol, G. A., and Vas, M. (2011). Reference gene selection for gene expression studies using RT-QPCR in virus-infected planthoppers. *Virol. J.* 8, 308. doi:10.1186/1743-422X-8-308

Pang, K., Dong, S. Z., Hou, Y., Bian, Y.-L., Yang, K., and Yu, X.-P. (2012). Identification and quantification of one species of yeast-like symbiotes, *Candida*, in the rice Brown planthopper, *Nilaparvata Lugens*. *Insect Sci.* 19 (4), 477–484. doi:10.1111/j.1744-7917.2011.01486.x

Qin, Y., Xu, P., Jin, R., Li, Z., Ma, K., Wan, H., et al. (2021). Resistance of *Nilaparvata lugens* (Hemiptera: Delphacidae) to triflumezopyrim: Inheritance and fitness costs. *Pest Manag. Sci.* 77 (12), 5566–5575. doi:10.1002/ps.6598

Shi, J., Song, Y., Shentu, X.-P., and Yu, X.-P. (2021). Antimicrobials affect the fat body microbiome and increase the Brown planthopper mortality. *Front. Physiol.* 12, 644897. doi:10.3389/fphys.2021.644897

Shi, X., Jiang, L., Wang, H., Qiao, K., Wang, D., and Wang, K. (2011). Toxicities and sublethal effects of seven neonicotinoid insecticides on survival, growth and reproduction of imidacloprid-resistant cotton aphid, Aphis gossypii. *Aphis Gossypii. Pest Manag. Sci.* 67 (12), 1528–1533. doi:10.1002/ps.2207

Song, Y., Shi, J., Xiong, Z., Shentu, X., and Yu, X.-P. (2021). Three antimicrobials alter gut microbial communities and causing different mortality of Brown planthopper, *Nilaparvata lugens* (Stål). *Pestic. Biochem. Physiol.* 174, 104806. doi:10.1016/j.pestbp.2021.104806

Ullah, F., Gul, H., Desneux, N., Said, F., Gao, X., and Song, D. (2020a). Fitness costs in chlorfenapyr-resistant populations of the chive maggot, *Bradysia Odoriphaga*. *Ecotoxicology* 29 (4), 407–416. doi:10.1007/s10646-020-02183-7

Ullah, F., Gul, H., Tariq, K., Desneux, N., Gao, X., and Song, D. (2020b). Fitness costs in clothianidin-resistant population of the melon aphid, *Aphis gossypii*. *PLOS ONE* 15 (9), e0238707. doi:10.1371/journal.pone.0238707

Vigneron, A., Charif, D., Vincent-Monégat, C., Vallier, A., Gavory, F., Wincker, P., et al. (2012). Host gene response to endosymbiont and pathogen in the *cereal weevil sitophilus oryzae*. *BMC Microbiol.* 12 (1), S14. doi:10.1186/1471-2180-12-S1-S14

Wang, J., Wei, D., Dou, W., Hu, F., Liu, W.-F., and Wang, J-J. (2013). Toxicities and synergistic effects of several insecticides against the oriental fruit fly (Diptera: Tephritidae). *J. Econ. Entomol.* 106 (2), 970–978. doi:10.1603/ec12434

Wang, L.-X., Niu, C.-D., Salgado, V. L., Lelito, K., Stam, L., Jia, Y.-L., et al. (2019). Pymetrozine activates TRPV channels of Brown planthopper *Nilaparvata lugens*. *Pestic. Biochem. Physiol.* 153, 77–86. doi:10.1016/j.pestbp.2018.11.005

Wang, L.-X., Zhang, Y.-C., Tao, S., Guo, D., Zhang, Y., Jia, Y.-L., et al. (2020). Pymetrozine Inhibits reproductive behavior of Brown planthopper *Nilaparvata lugens* and fruit fly *Drosophila Melanogaster*. *Pestic. Biochem. Physiol.* 165, 104548. doi:10.1016/j.pestbp.2020.02.014

Wang, L., Tao, S., Zhang, Y., Jia, Y., Wu, S., and Gao, C. (2021). Mechanism of metabolic resistance to pymetrozine in *Nilaparvata lugens* over-expression of cytochrome P450 *CYP6CS1* confers pymetrozine resistance. *Pest Manag. Sci.* 77 (9), 4128–4137. doi:10.1002/ps.6438

Wang, Q., Lin, M., Shen, P., and Guan, Y. (2021). Elevation of fatty acid biosynthesis metabolism contributes to zhongshengmycin resistance in *Xanthomonas Oryzae*. *Antibiotics* 10 (10), 1166. doi:10.3390/antibiotics10101166

Xue, J., Zhou, X., Zhang, C.-X., Yu, L.-L., Fan, H.-W., Wang, Z., et al. (2014). Genomes of the rice pest Brown planthopper and its endosymbionts reveal complex complementary contributions for host adaptation. *Genome Biol.* 15 (12), 521. doi:10.1186/s13059-014-0521-0

Yang, Y., Huang, L., Wang, Y., Zhang, Y., Fang, S., and Liu, Z. (2016). No cross-resistance between imidacloprid and pymetrozine in the Brown planthopper: Status and mechanisms. *Pestic. Biochem.* 130, 79–83. doi:10.1016/j.pestbp.2015.11.007

Zhang, Q. (2007). Strategies for developing green super rice. *Proc. Natl. Acad. Sci. U. S. A.* 104 (42), 16402–16409. doi:10.1073/pnas.0708013104

Zhang, X., Liu, X., Zhu, F., Li, J., You, H., and Lu, P. (2014). Field Evolution of insecticide resistance in the Brown planthopper *Nilaparvata lugens* (Stål) in China. *Crop Prot.* 58, 61–66. doi:10.1016/j.cropro.2013.12.026

Zhang, X., Mao, K., Liao, X., He, B., Jin, R., Tang, T., et al. (2018). Fitness cost of nitenpyram resistance in the Brown planthopper *Nilaparvata lugens*. *J. Pest Sci.* 91 (3), 1145–1151. doi:10.1007/s10340-018-0972-0982

Zheng, R., Ji, J., Wu, J., Zhang, R., Li, Y., Yu, X.-P., et al. (2021). *PCE3* plays a role in the reproduction of male *Nilaparvata lugens*. *Insects* 12 (2), 114. doi:10.3390/insects12020114

frontiers | Frontiers in Physiology

TYPE Original Research
PUBLISHED 03 May 2023
DOI 10.3389/fphys.2023.1174236

OPEN ACCESS

EDITED BY
Ting Li,
Alabama State University, United States

REVIEWED BY
Qingyun Diao,
Institute of Apiculture Research (CAAS), China
Ran Wang,
Beijing Academy of Agriculture and Forestry Sciences, China

*CORRESPONDENCE
Wensu Han,
hwswill8@126.com
Jinglin Gao,
jinglin.g@163.com

RECEIVED 26 February 2023
ACCEPTED 07 April 2023
PUBLISHED 03 May 2023

CITATION
Han W, Ye Z, Gu Y, Zhong Y, Gao J, Zhao S and Wang S (2023), Gut microbiota composition and gene expression changes induced in the *Apis cerana* exposed to acetamiprid and difenoconazole at environmentally realistic concentrations alone or combined.
Front. Physiol. 14:1174236.
doi: 10.3389/fphys.2023.1174236

Gut microbiota composition and gene expression changes induced in the *Apis cerana* exposed to acetamiprid and difenoconazole at environmentally realistic concentrations alone or combined

Wensu Han[1,2]*, Zheyuan Ye[1], Yifan Gu[1,3,4], Yihai Zhong[1,2], Jinglin Gao[1,2]*, Shan Zhao[1,2] and Shijie Wang[1,2]

[1]Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences, Haikou, China, [2]Bee Industry Technology Research Center, Chinese Academy of Tropical Agricultural Sciences, Haikou, China, [3]Sanya Institute of China Agricultural University, Sanya, China, [4]Department of Entomology, College of Plant Protection, China Agricultural University, Beijing, China

Apis cerana is an important pollinator of agricultural crops in China. In the agricultural environment, *A. cerana* may be exposed to acetamiprid (neonicotinoid insecticide) and difenoconazole (triazole fungicide), alone or in combination because they are commonly applied to various crops. At present, our understanding of the toxicological effects of acetamiprid and difenoconazole on honey bee gut microbiomes is limited. The primary objective of this study was to explore whether these two pesticides affect honey bees' gut microbiota and to analyze the transcriptional effects of these two pesticides on honey bees' head and gut. In this study, adults of *A. cerana* were exposed to acetamiprid and/or difenoconazole by contaminated syrup at field-realistic concentrations for 10 days. Results indicated that acetamiprid and/or difenoconazole chronic exposure did not affect honey bees' survival and food consumption, whereas difenoconazole decreased the weight of honey bees. 16S rRNA sequencing suggested that difenoconazole and the mixture of difenoconazole and acetamiprid decreased the diversity index and shaped the composition of gut bacteria microbiota, whereas acetamiprid did not impact the gut bacterial community. The ITS sequence data showed that neither of the two pesticides affected the fungal community structure. Meanwhile, we also observed that acetamiprid or difenoconazole significantly altered the expression of genes related to detoxification and immunity in honey bees' tissues. Furthermore, we observed that the adverse effect of the acetamiprid and difenoconazole mixture on honey bees' health was greater than that of a single mixture. Taken together, our study demonstrates that acetamiprid and/or difenoconazole exposure at field-realistic concentrations induced changes to the honey bee gut microbiome and gene expression.

KEYWORDS

***Apis cerana*, acetamiprid, difenoconazole, gut microbiota, gene expression**

Introduction

Apis cerana is an important indigenous species, and it was the keystone pollinator of agricultural crops in China before the introduction of European honeybees *Apis mellifera* in the late 19th century (Guo et al., 2015). Compared to *A. mellifera*, *A. cerana* has a strong ability to collect scattered floral resources and work in cold temperatures and defend against the ectoparasitic mite, *Varroa destructor* (Chen et al., 2017). The managed colony of *A. cerana* is the main pollinator released in the greenhouse, especially in parts of Southeast Asia (Osterman et al., 2021). Currently, despite the ecological and economic importance, *A. cerana* colonies have undergone substantial range contractions (Gemeda et al., 2017) and a population decline in recent years (Zhao et al., 2017). Previous reports from all over the world showed that exposure to pesticides, in particular, the class of insecticides known as neonicotinoids, is one of the reasons for the population decline of honey bees and wild bees (Pisa et al., 2015; Brandt et al., 2016; Mitchell et al., 2017; Mokkapati et al., 2021; Willis Chan and Raine, 2021).

Neonicotinoids are neurotoxins that act as nicotinic acetylcholine receptor agonists in the insect central nervous system and cause over-stimulations to nerve cells resulting in paralysis and death (Wang et al., 2020). Owing to their lower toxicity to mammals, neonicotinoids began to be widely used in agriculture starting during the 1990s (Matsuda et al., 2020). Global sales of neonicotinoids are worth US$1 billion/year (Tasman et al., 2020). Neonicotinoids are systemically incorporated into plant tissues, including pollen and nectar (David et al., 2016). Pollens and nectars are the major food source for honey bees. Thus, foragers are often directly exposed to neonicotinoid insecticides while visiting flowers, and hive bees can be exposed to contaminated pollens and nectars that are brought back to the hive. Mitchell et al. (2017) reported that 75% of honey bee colony samples from all over the world contain at least one neonicotinoid.

In addition to insecticides, pollen and nectar analyses showed a high incidence of fungicides (Mullin et al., 2010; David et al., 2016; Tong et al., 2016; Gaweł et al., 2019). In comparison, fungicide application exceeds that of both insecticides and herbicides on a global scale (Rondeau and Raine, 2022). Fungicides are commonly applied during the blooming period to control fungal disease, which are assumed to be without risks to pollinators (Mitchell et al., 2017; Favaro et al., 2019). However, much more recent research works have shown that fungicides can affect honey bees' food consumption (Liao et al., 2017), nest recognition (Artz and Pitts-Singer, 2015), metabolism (Mao et al., 2017; Christen et al., 2019), respiration (Han et al., 2018), and immune function (Degrandi-Hoffman et al., 2015). Moreover, fungicides may enhance the toxicity of insecticides to honey bees, in particular, triazole fungicides and neonicotinoids or pyrethroids in the mixture (Wade et al., 2019).

In agricultural environments, foragers are often exposed to neonicotinoid insecticides and triazole fungicides because these pesticides are frequently co-applied in a tank mix or commonly used in seed coating. All honey bee colony members are chronically exposed to these pesticides through the foraging of contaminated pollens and nectars that are brought back to the colony (Rouzé et al., 2019). Pesticide mixtures can have additive effects through the same or different modes of action or even synergism or antagonism in toxicity. Thus, more attention should be paid to the magnitude of specific mixture-induced effects.

Ingested pesticides are in contact with the honey bee's gut and may alter its physiology; so, in this study, we investigated the effects of acetamiprid and difenoconazole exposure on the gut microbiota composition and physiology of *A. cerana*, alone or in combination. We selected these compounds due to their systemic properties, which are intensively used throughout the crop growing season and simultaneously detected in pollen and honey samples (Tong et al., 2018; Gaweł et al., 2019).

Acetamiprid belongs to the group of cyano-substituted neonicotinoids, which are generally considered safe for bees, with oral and contact toxicities with two to three orders of magnitude lower than those of the nitro-substituted neonicotinoids, such as imidacloprid, clothianidin, and thiamethoxam (Feyereisen, 2018). Thus, due to the comparatively more "bee-friendly" properties of acetamiprid, it is permitted to be sprayed on flowering crops during daylight when honey bees are actively foraging (Godfray et al., 2014). Mitchell et al. (2017) reported that maximum and average concentrations among positive honey samples were the highest for acetamiprid and thiacloprid, but acetamiprid is present in Asia, whereas thiacloprid is present in Europe.

Difenoconazole belongs to triazole fungicides. This fungicide inhibits sterol biosynthesis, which is crucial for maintaining the cell membrane integrity of fungi (Figueirêdo et al., 2019). Aquatic organisms' long-term exposure to difenoconazole at low concentrations would result in the bioaccumulation of this compound and elicit estrogenic endocrine-disruption effects (Zhang et al., 2017; Teng et al., 2018). Stingless bee *Tetragonisca angustula* exposed to difenoconazole were less tolerant when it was applied via ingestion or on treated surfaces (Leite et al., 2021). Prado et al. (2020) confirmed that difenoconazole accumulates in tissues of an adult forager of stingless bee *Melipona scutellaris* Latreille, regardless of topical and oral exposure, and caused death. However, according to the PPDB (Pesticide Properties Database, 2019), this fungicide is not considered toxic for bees. Our previous research has shown that the acetamiprid and difenoconazole mixture has greater toxic effects on *A. cerana* than the individual compounds (Han et al., 2017). Hence, intensively studying the impact of these two pesticides on *A. cerana* is imperative.

The gut microbiota is a complex ecosystem of symbiotic bacteria that interacts with multiple organs and systems in the host via metabolites, proteins, and genes. Recently, the gut microbiota has emerged as a critical factor affecting bees' health and fitness (Ribière et al., 2019). The honey bee's gut microbiota is relatively simple and conservative; it provides several health benefits to bees such as promoting the digestion of food, stimulating the immune system, protecting against pathogens, increasing the weight in adult bees, and stimulating the expression of host detoxification genes (Zheng et al., 2018; Ribière et al., 2019; Wu et al., 2020). In *A. mellifera*, 95%–99% of gut microbiota is dominated by five to eight core bacterial species. These members belong to different taxa, including *Snodgrassella*, *Gilliamella*, *Bifidobacterium*, *Lactobacillus* Firm-4, *Lactobacillus* Firm-5, *Bartonella*, *Frischella*, and *Commensalibacter* (Motta et al., 2018; Ribière et al., 2019; Motta et al., 2020). In *A. cerana*, the microbial taxa are the same as *A. mellifera* and at a relatively low level, which mainly includes *Bifidobacterium*, *Snodgrassella*,

Gilliamella, and *Lactobacillus* (Guo et al., 2015). Although several studies have shown that bees' gut microbiota composition can be disrupted by pesticides (Kakumanu et al., 2016; Motta et al., 2018; Rouzé et al., 2019; Liu et al., 2020; Motta et al., 2020; Zhu et al., 2020) and antibiotic (Raymann et al., 2017) exposure, which have been linked to bees colony mortalities, little is known about the interactive effects of pesticides' cocktail on the gut, which is one of the main entrances for toxic molecules.

In addition, changes in gene expression were reported in larvae and worker honey bees fed with sublethal doses of neonicotinoid insecticides or fungicides, where genes are related to neurotoxicity, memory formation, stress reaction, metabolism, detoxification, immunity, and other pathways (Christen et al., 2016; Christen and Fent, 2017; Wu et al., 2017; Christen et al., 2019). To the best of our knowledge, little is known about the molecular effects of a mixture of acetamiprid and ergosterol biosynthesis inhibitor (EBI) fungicides (difenoconazole) on honey bee.

A key question in ecotoxicological studies is whether the test doses applied in the laboratory are field realistic (Sgolastra et al., 2018). Therefore, in the present study, we chronically exposed nurse honey bees of *A. cerana* to acetamiprid and difenoconazole via sucrose solution, alone and in combinations. In an attempt to mimic field-realistic conditions, we used the concentrations of acetamiprid and difenoconazole found in beebread (Kubik et al., 2000; Tong et al., 2018; Almasri et al., 2021). Our aim was to evaluate whether the nurse honey bee exposure to acetamiprid and difenoconazole separately and in binary mixtures impacts the abundance and composition of the gut microbiota and whether the transcriptional responses of genes are related to detoxification enzymes, antioxidative enzymes, immune system, neuronal signaling, and development. We focus on target genes that play an important role in the physiology of bees that were previously suggested to be involved in the stress response to pesticides (Christen and Fent, 2017; Christen et al., 2019).

Materials and methods

Chemicals and solutions

Standards with a high purity level (98.2% and 96% for acetamiprid and difenoconazole, respectively) were obtained from Hainan Boswell Agrichemical Co., Ltd. The stock solutions (1,000 mg/L) for each compound were prepared in acetone and diluted into 50% sucrose solution (w/v) and stored at −20°C. The final concentration of 0.32 mg/L acetamiprid and 0.27 mg/L difenoconazole used for nursing honey bees' exposure were diluted in sucrose solution from stock solutions (Kubik et al., 2000; Tong et al., 2018). All treatment solutions were freshly prepared daily.

Honey bee rearing

For the laboratory experiment, three healthy colonies of honey bees (*A. cerana*) were obtained from outside the hives kept in the front of the building of the Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences, Haikou (N19°59′9" and E110°19′30"), China. These colonies were maintained according to standard beekeeping practices, and they did not present visible symptoms of any known diseases. Prior to our study, these hives were not treated with any pesticides.

Experimental design of laboratory exposures

Frames of late-stage capped brood from the three colonies were collected and transferred to an artificial climate incubator (34°C, 60% relative humidity; darkness) in the laboratory to monitor the emergence of *A. cerana* workers. Approximately 2000 newly emerged bees within a period of 12 h were marked on the thorax with a marker pen and returned to a single hive so that they could develop into nursing bees under the same conditions. After 7 days, these marked bees were captured, transferred to the laboratory, and distributed into iron cages (13 cm × 6 cm × 10 cm), and they were fed *ad libitum* with 50% sucrose solution (w/v) for acclimation 24 h before the beginning of the exposure experiment.

Next, the marked bees were divided into four treatment groups: control (C), ingestion of 50% sucrose solution free of pesticides; acetamiprid (TA), exposed to the 50% sucrose solution containing acetamiprid at the concentration of 0.32 mg/L; difenoconazole (TD), exposed to the 50% sucrose solution containing difenoconazole at the concentration of 0.27 mg/L; difenoconazole + acetamiprid (TDA), exposed simultaneously to the 50% sucrose solution containing the combined concentrations of 0.27 mg/L difenoconazole and 0.32 mg/L acetamiprid. Each experimental group was assayed in six replicates (six iron cages) and each cage contained 30 worker bees. The treatment solutions were replaced every 24 h. The exposure experiment lasted 10 days, and the dead bees in each group were recorded daily. The amount of solutions in the feeders was weighed daily before they were placed in the cages and again after they were removed from the cages. The difference was equivalent to the total amount of food consumed by live bees on the previous day, and then, we calculated the amount of food consumed by each honey bee. At the end of the test, the surviving honey bees were collected from each treatment and weighed, and then, their heads were removed by cutting with a blade and their whole guts were carefully collected by pulling the sting from the end of the abdomen using sterile forceps. These guts and heads were transferred into separate 1.5 mL centrifuge tubes and immediately frozen in liquid nitrogen and stored at −80°C until further analysis.

DNA extraction and amplification

From each treatment group, 12 guts were pooled together as a biological sample, and three replicates were used (a total of 12 samples). Total genomic DNA from samples was extracted using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories) according to the manufacturer's protocol. DNA quality and quantity were assessed by the ratios of 260 nm/280 nm and 260 nm/230 nm, respectively. Then, DNA was stored at −80°C until further processing. The V3–V4 region of the bacterial 16S rRNA gene was amplified with the universal primer pair (forward primer, 5'-ACTCCTACGGGAGGCAGCA-3'; reverse primer, 5'-GGACTACHVGGGTWTCTAAT-3'), and the ITS region for fungi was amplified using barcoded primers (forward primer, 5'-CTTGGTCATTTAGAGGAAGTAA-3'; reverse primer, 5'-GCT GCGTTCTTCATCGATGC-3'). All PCR reactions were carried

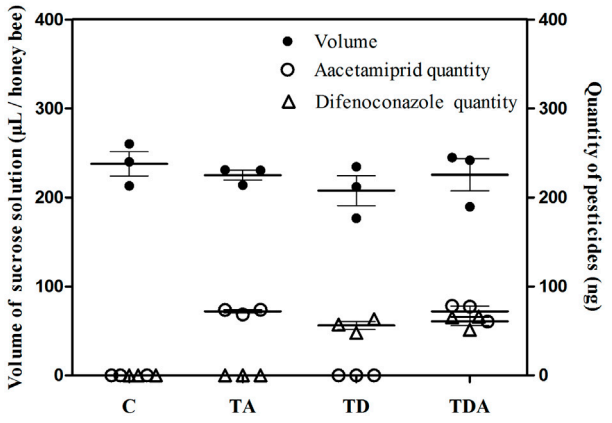


FIGURE 1
Mean uptakes of sucrose solution containing acetamiprid or/and difenoconazole and these two pesticides quantities per honey bee for 10 d feeding. The data (mean ± SEM) shown are representative of three colonies. Statistical analyses were performed using one-way ANOVA (volume) and Student's *t*-test (quantity). C: control; TA: acetamiprid treated; TD: difenoconazole treated; and TDA: difenoconazole + acetamiprid treated. The following figures are the same.

out with Phusion® High-Fidelity PCR Master Mix (New England Biolabs). Illumina MiSeq sequencing and bioinformatics analyses were performed by a commercial company (Biotree, Shanghai, China).

16S rRNA gene sequence and ITS sequence analysis

Operational taxonomic unit (OTU) data were summarized by using Uparse software (Uparse v7.0.1001). Sequences with ≥97% similarity were assigned to the same OTUs. Bacterial species annotation was performed by the SILVA database, and fungi species annotation was performed by the UNITE database. Alpha and beta diversities were calculated using QIIME software. Principal coordinate analysis (PCoA) was performed on Bray–Curtis distance matrices. Analysis of similarity (ANOSIM) was performed to determine the differences among groups. Linear discriminant analysis (LDA) of Effect Size (LEfSe) was assessed with the LEfSe tool. Flora's relative abundance between samples was compared by MetaStat analysis.

Gene expression analysis

The total RNA of three honey bee heads or three honey bee gut samples was pooled together as a biological sample and ground using a TissueLyser-64 instrument with a CoolPrep adapter. RNA was isolated following the manufacturer's instructions using the Eastep® Super Total RNA Extraction Kit (Promega, United States). Per each treatment, three biological replicates were isolated. 1,000 ng RNA was synthesized into cDNA using the GoScript Reverse Transcription system (Promega, A5001), according to the manufacturer's protocol. Primer sequences were taken from the literature or self-designed using the NCBI primer-blast tool. Sequences of used primers are shown in Supplementary Table S1. For all performed analyses, *β-actin* was used as a housekeeping gene for normalization. Relative abundance of the mRNA level was assessed using a QuantStudio 6 Flex real-time qPCR System (Applied Biosystems, United States) by using an SYBR Green PCR kit (Aidlab, Beijing, China). The relative expression of the target genes was calculated using $2^{-\Delta\Delta CT}$.

Statistical analysis

Statistical calculations were performed using SPSS software 19.0 (IBM, United States). Student's *t*-test was used for two-group comparisons. One-way ANOVA with an LSD test was used for four-group comparisons. The Kaplan–Meier survival curve and long-rank tests were used for survival analysis. The significance level used in all tests was $p \leq 0.05$. Prism version 8.0 software (GraphPad, San Diego, CA, United States) was used to make the statistical figures.

Results

Acetamiprid and difenoconazole uptake and effects on honey bees' survival and weight

The concentration of acetamiprid and difenoconazole, alone or in combination, in sucrose solution did not significantly influence the volume of solution taken up per honey bee during the 10 days of feeding (ANOVA; F = 0.743; d f = 3, 8; $p = 0.556$; Figure 1). The total doses of acetamiprid taken up per honey bee averaged 72.03 ± 1.78 and 72.11 ± 5.74 ng for TA- and TDA-treated groups, respectively, and there was no significant difference between the two groups (*t*-test, $t_4 = -0.015$ and $p = 0.989$). Likewise, the total doses of difenoconazole consumed per honey bee averaged 56.05 ± 4.56 and 60.85 ± 4.84 ng for TD- and TDA-treated groups, respectively, and there was no significant difference between the two groups (*t*-test, $t_4 = -0.721$ and $p = 0.511$).

After 10 days of exposure experiment, there was no significant difference in the percentage survival of honey bees among the four treatments (log-rank (Mantel–Cox) test: $\chi^2 = 1.432$; d f = 3; $p = 0.698$; Figure 2A). All the exposed honey bees behaved normally, and no honey bees that stopped moving were observed.

While the weights of honey bees were significantly reduced in TD- and TDA-treated groups when honey bee workers were exposed to an environmentally relevant concentration of acetamiprid and difenoconazole, alone or in combination for 10 days, there was no difference in the TA-treated group as compared with the control (F = 25.33; d f = 3, 334; $p < 0.0001$; Figure 2B).

Effects of acetamiprid and difenoconazole on the honey bee gut microbiota

In order to obtain the honey bees' gut microbes under natural conditions, these newly emerged honey bees were maintained in a single hive for 7 days because the newly emerged worker lacked gut

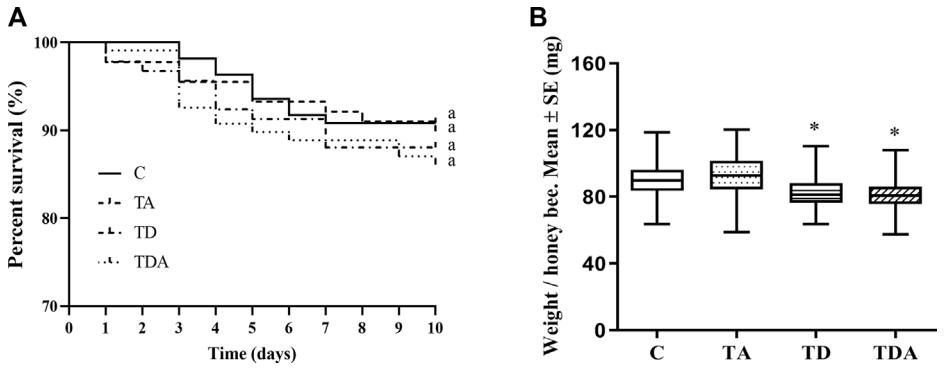


FIGURE 2
Effects of acetamiprid or/and difenoconazole 10 d on the honey bee workers' survival **(A)** and body weight **(B)**. **(A)** The percent survival of workers after pesticide exposure is shown as a Kaplan–Meier survival curve. The same letters behind the curves indicate no significant differences between treatments (log-rank (Mantel–Cox) test: $\chi^2 = 1.432$; d f = 3; $p = 0.698$). **(B)** The data (mean ± SEM) shown are representative of three colonies. Statistical analyses were performed using one-way ANOVA. * indicates a significant difference compared to the control ($p < 0.05$).

microbes and gained large characteristic communities in the ileum and rectum within 4–6 days within hives. A total of 920,091 16S rRNA genes and 873,261 ITS clean reads were obtained from pyrosequencing 12 samples [(3 treatments +1 control) × 3 replicates], each sample comprising pooled DNA from 12 guts of honey bee (Supplementary Tables S2, S3). For 16S data, 821,985 effective reads with an average length of 423 bp (range: 420–426 bp) were retained after stringent quality filtering (Supplementary Table S2). A maximum of 48 unique operational taxonomic units (OTUs were clustered based on a 97% similarity cut-off). Among them, 41 OTUs have been found in all samples (Figure 4A). Similarly, for fungi, 869,213 effective reads with an average length of 234 bp (range: 193–289 bp) were retained after stringent quality checking (Supplementary Table S3). Based on the 97% similarity level, all of the effective reads were clustered into 800 OTUs. Among them, 525 OTUs were detected in all samples (Figure 6A). The curves of OTU rank and rarefaction were calculated (Supplementary Figure S1). Rarefaction for both the 16S (Supplementary Figures S1A, B) and ITS (Supplementary Figures S1C, D) data showed a plateau supporting the estimates of richness.

Bacterial diversity and composition in response to acetamiprid and difenoconazole, alone and in combination

The results of cluster analysis are that the gut bacterial communities belonged to 6 phyla, 9 classes, 15 orders, 16 families, 24 genera, and 31 species, as shown in Figure 3. At the phylum level, Proteobacteria (48.67%), Firmicutes (35.04%), Bacteroidetes (10.58%), and Actinobacteria (5.32%) were found to be the most abundant phyla (>1%). The family Lactobacillaceae (19.29%) of class Bacilli (20.28%), Acetobacteraceae (18.03%) of class α-Proteobacteria (19.63%), and members of class γ-Proteobacteria (29.04%) were the most abundant community. The sequences from γ-Proteobacteria were predominantly dominated by members of the order Orbaceae (genera *Gilliamella*). Along with Orbaceae, reads assigned to Enterobacteriales (genera *Escherichia–Shigella*, *Serratia*, *Kosakonia*, *Klebsiella*, *Tatumella*, *Salmonella*, and *Enterobacter*), Aeromonadales (genera *Aeromonas*), and β-proteobacteriales (genera *Snodgrassella*) were also observed. In addition, members of Bacteroidia (10.58%) and Negativicutes (14.77%) were predominantly assigned to the family Weeksellaceae and Veillonellaceae, respectively.

Four alpha diversity parameters, namely, the Shannon, Simpson, ACE, and Chao1 indices, were selected for community diversity and richness comparisons. The alpha diversity of gut bacteria of the honey bee in TD and TDA groups had significant differences when compared with the control, as measured by the Shannon index (Figure 4B), while the Simpson index has no significance (Supplementary Figure S2A). In contrast to the diversity indices, there were no significant differences in the richness indices, as measured by the ACE and Chao1 indices (Supplementary Figures S2B, S2C).

The beta-diversity analysis was performed via a PCoA of Bray–Curtis distances to determine the similarity of bacterial communities. Results showed that the gut bacteria in the C and TA groups deviated from the TD and TDA groups (Figure 4C). The ANOSIM analysis revealed significant differences in the structure (ANOSIM, R = 0.355, $p = 0.013$) of gut bacteria among these treatments (Figure 4D). These data suggest that the gut bacterial community structures in honey bees were influenced by difenoconazole.

To identify the specific bacterial taxa associated with difenoconazole or/and acetamiprid exposure, we compared the gut bacteria of control and difenoconazole or/and acetamiprid treated honey bee using the linear discriminant analysis (LDA) Effect Size (LEfSe) and MetaStat method. LefSe analysis revealed that 3, 9, 8, and 2 bacterial taxa were enriched in C, TA, TD, and TDA groups, respectively (Figure 4E). The resulting cladogram showed that *Escherichia–Shigella* (genera), *Klebsiella oxytoca* (species), and *uncultured_bacterium_Fructobacillus* (species) were rich in control honey bees. The order Enterobacteriales and Rhizobiales, the family Enterobacteriaceae, Leuconostocaceae, and Rhizobiaceae, the genera *Bartonella* and *Fructobacillus*, and the species *uncultured_bacterium_*

10.3389/fphys.2023.1174236

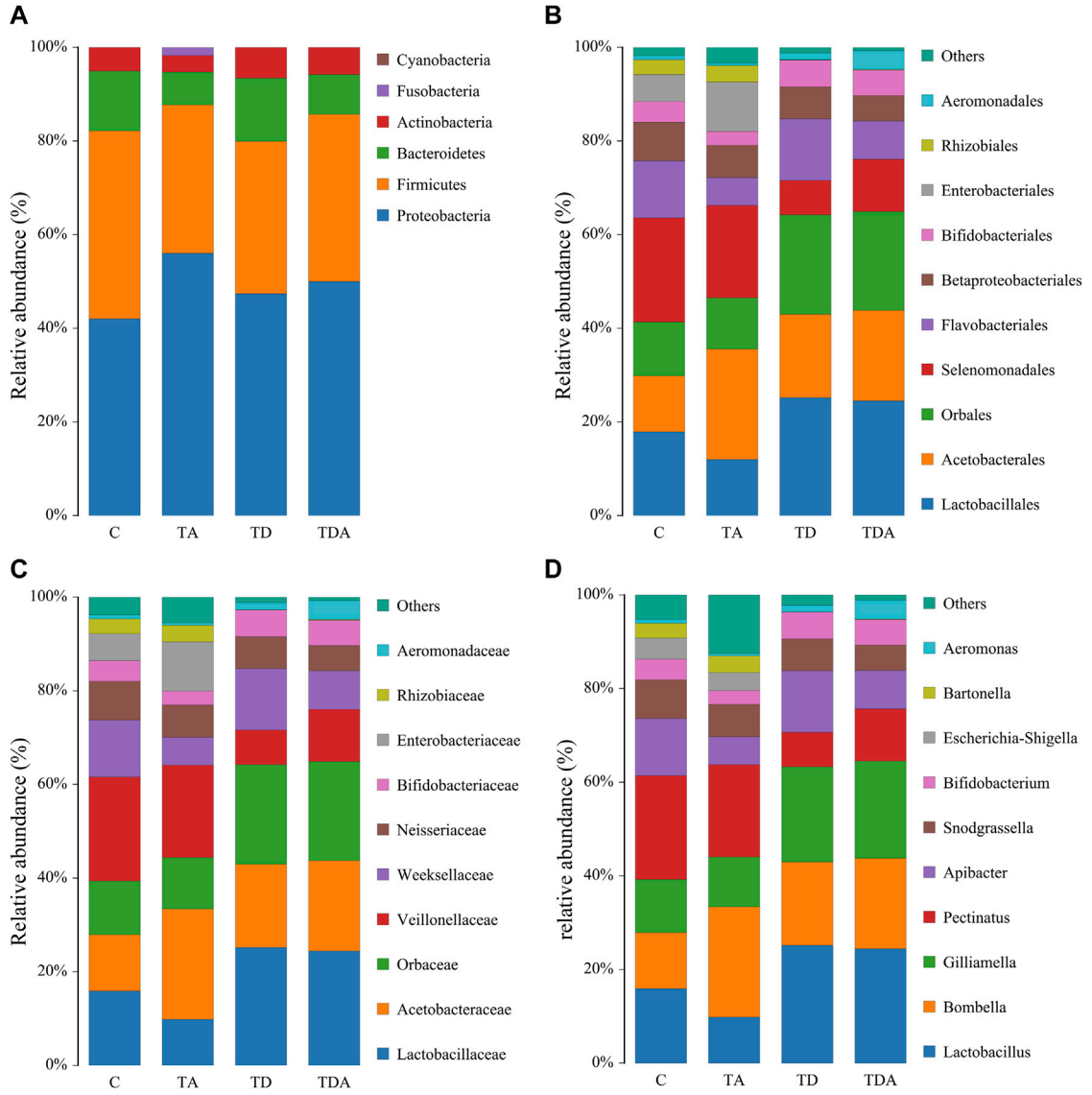


FIGURE 3
Relative abundance of the dominant gut bacterial communities in *Apis cerana* at the phylum **(A)**, order **(B)**, family **(C)**, and genus **(D)** levels. Each column represents the relative abundance of each bacterial taxon within a group.

Bartonella and *Fructobacillus fructosus* were predominant in the TA group. The order Lactobacillales and Orbales, the family Lactobacillaceae and Orbaceae, the genera *Lactobacillus*, and the species *uncultured_bacterium_Lactobacillus* and *Enterobacteriaceae bacterium Acj 204* were predominant in the TD group, while *Gilliamella* (genera) and *uncultured_bacterium_Gilliamella* (species) were rich in the TDA group (Figure 4F). At the genus level, using MetaStat analysis, we found *Lactobacillus* and *Gilliamella* in the TDA group were increased, while *Klebsiella* decreased when compared with the control ($p < 0.001$, $p = 0.027$, and $p = 0.045$) (Supplementary Table S4). *Lactobacillus*, *Candidatus_Schmidhempelia,* and *Gilliamella* in the TD group were higher than in the control ($p = 0.008$, $p = 0.021$, and $p = 0.038$), while *Pectinatus* was lower than in the control ($p = 0.047$) (Supplementary Table S5). *Lactobacillus* and *Apibacter* in the TA group were lower than in the control ($p = 0.007$ and $p = 0.049$) (Supplementary Table S6).

Fungal diversity and composition in response to acetamiprid and difenoconazole, alone and in combination

The results of cluster analysis are that the gut fungal communities belonged to 8 phyla, 24 classes, 56 orders, 121 families, 188 genera, and 192 species, and the results do not contain unclassified (Figure 5). At the phylum level, Ascomycota (67.11%), Basidiomycotav (17.91%), and mortierellomycota (1.18%) were found to be the most

10.3389/fphys.2023.1174236

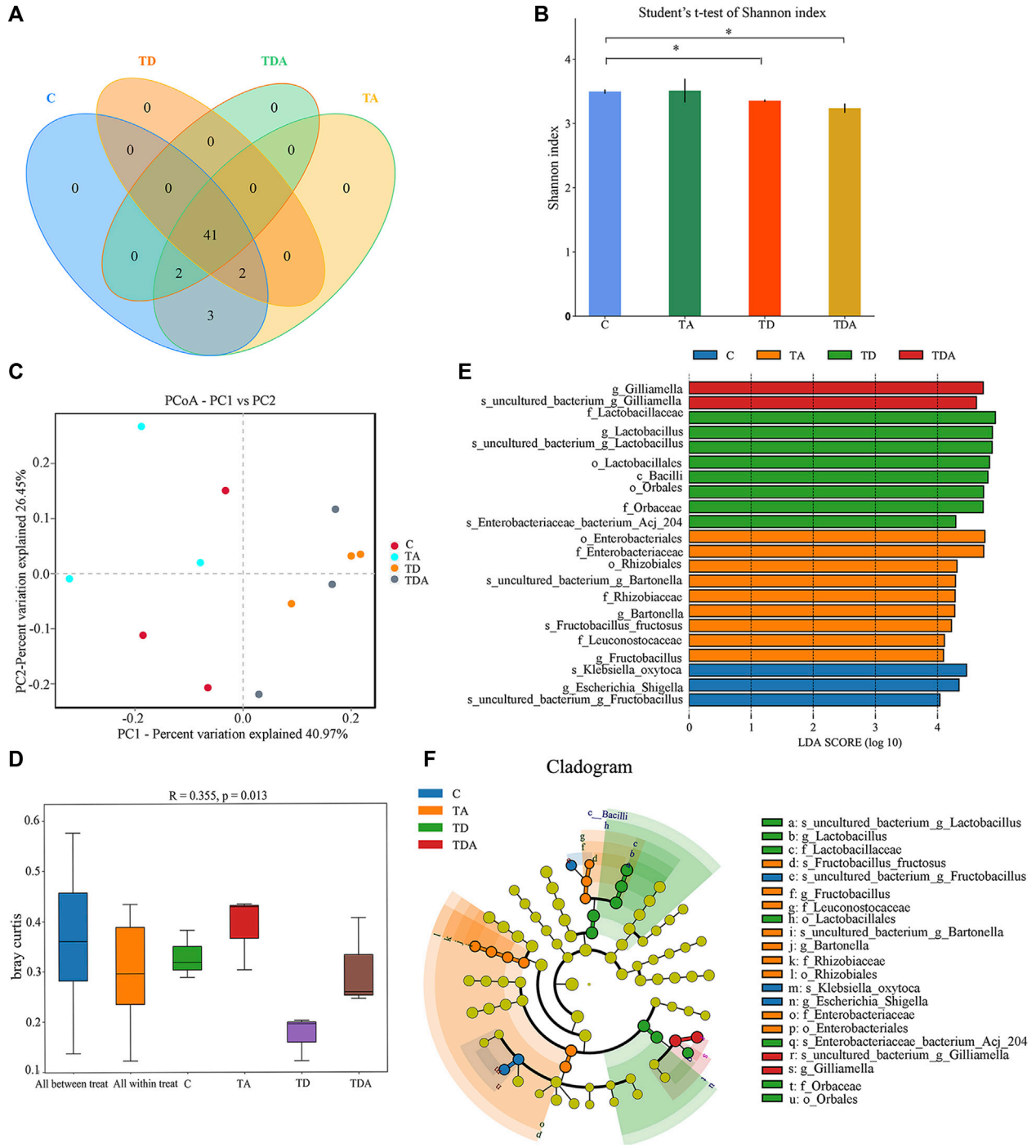


FIGURE 4
Gut bacterial microbiome alterations when *Apis cerana* exposed to acetamiprid or/and difenoconazole at environmentally relevant concentrations. **(A)** Venn diagram showing the numbers of unique and shared OTUs in the gut bacterial microbiota of *Apis cerana* between the pesticide-treated and the control groups. **(B)** Alpha diversity is measured by the Shannon index. Data (mean ± SEM) were analyzed by Student's *t*-test (*$p < 0.05$). **(C)** Beta diversity as shown on a PCoA plot. **(D)** The ANOSIM analysis revealed significant differences in the structure (ANOSIM, R = 0.355, p = 0.013) of gut bacteria among these treatments. **(E)** LefSe (LDA >4 logs) analysis showing differentially abundant gut bacteria among samples with different haze levels. **(F)** Cladogram showing phylotype differences between pesticide-treated *versus* control honey bees.

abundant phyla (>1%). The classes with the highest abundance (>5%) were Saccharomycetes (34.98%), Sordariomycetes (14.86%), Agaricomycetes (15.16%), and Eurotiomycetes (7.85%). Saccharomycetales (34.98%), Agaricales (9.06%), Eurotiales (6.14%), and Hypocreales (5.14%) were the most abundant orders (>5%). The families with the highest

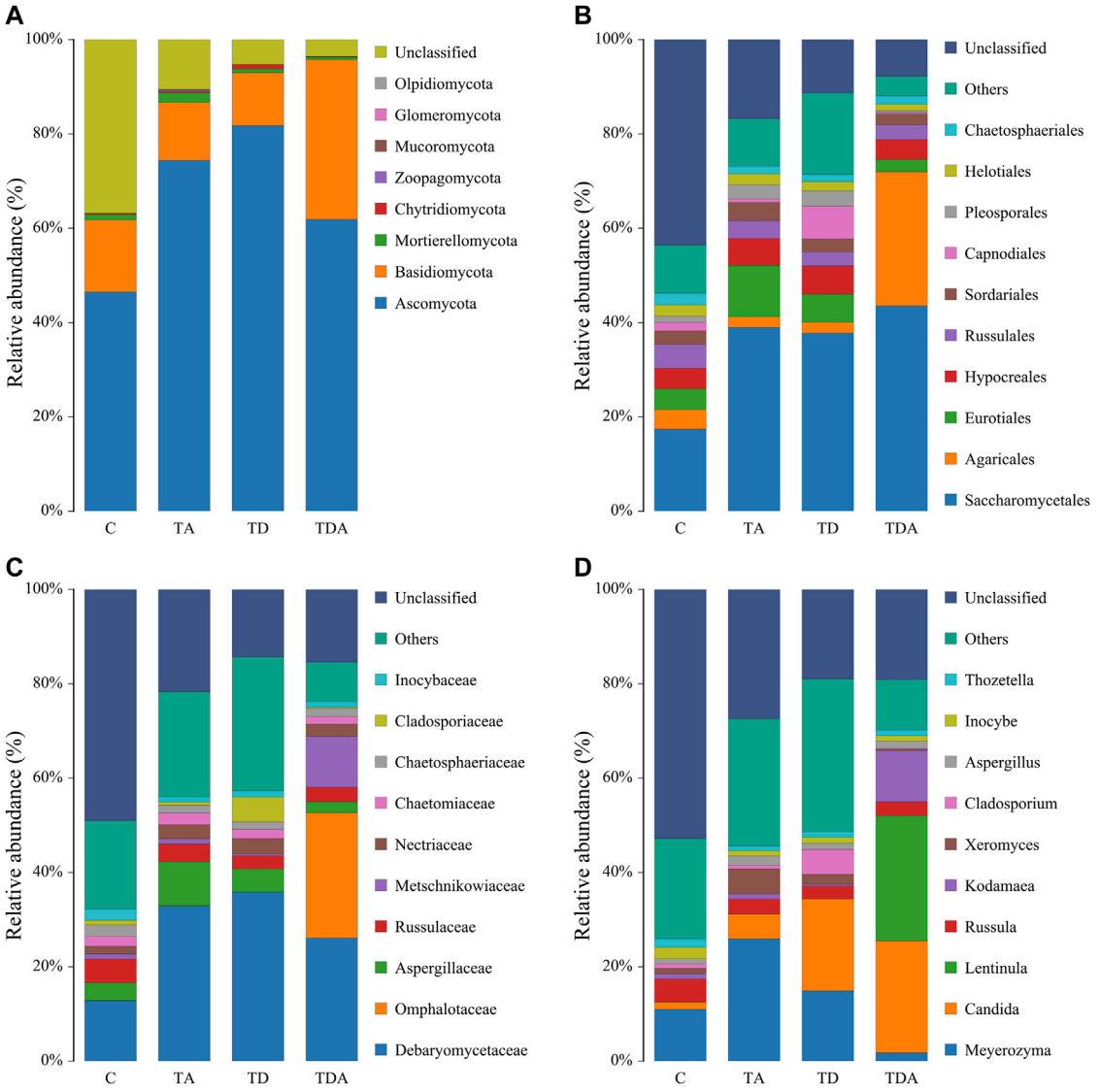


FIGURE 5
Relative abundance of the dominant gut fungal communities in *Apis cerana* at the phylum **(A)**, order **(B)**, family **(C)**, and genus **(D)** levels. Each column represents the relative abundance of each fungal taxon within a group.

abundance of fungi were Debaryomycetaceae (27.58%), Omphalotaceae (6.49%), Aspergillaceae (5.22%), and Russulaceae (3.62%). *Meyerozyma* (13.78%) and *Candida* (12.73%) of the family of Debaryomycetaceae were the most abundant genera.

The Shannon and Simpson indices showed that the fungal diversity had a significant difference between the TA and TDA groups (Figure 6B, Supplementary Figure S3A), the Chao1 and ACE indices showed that the fungal richness had a significant difference between the TD and TDA groups (Figure 6C, Supplementary Figure S3B); meanwhile, the Chao1 index also showed that the fungal richness had a significant difference between TA and TDA groups (Figure 6C), and there was no significant difference in all of the four alpha diversity indices when treated groups were compared with the control, but there may be a large variability among replicates in the control group. PCoA derived from the Bray–Curtis distance matrix of the fungal communities showed that samples from the control and the three treatment groups did not form separate clusters (Figure 6D). The ANOSIM analysis revealed that the acetamiprid or/and difenoconazole treatments did not have any significant impact on the fungal community structure (ANOSIM, R = 0.086, p = 0.223) (Figure 6E). LefSe analysis revealed that no fungal taxa were enriched in the control and the different treatment groups (Figure 6F). Using MetaStat analysis, we found two classes Saccharomycetes and Eurotiomycetes in the TA group were increased when compared with the control (p = 0.021 and p = 0.026) (Supplementary Table S7). Malasseziomycetes in the TDA group were decreased when compared with TA and TD groups (p = 0.005 and p = 0.039) (Supplementary Tables S8, S9). Leotiomycetes and Eurotiomycetes decreased, while Chytridiomycetes increased in the TDA group when compared with the TA group (p = 0.014, p = 0.024, and p = 0.010) (Supplementary Table S8), but Sordariomycetes and Dothideomycetes in the TDA

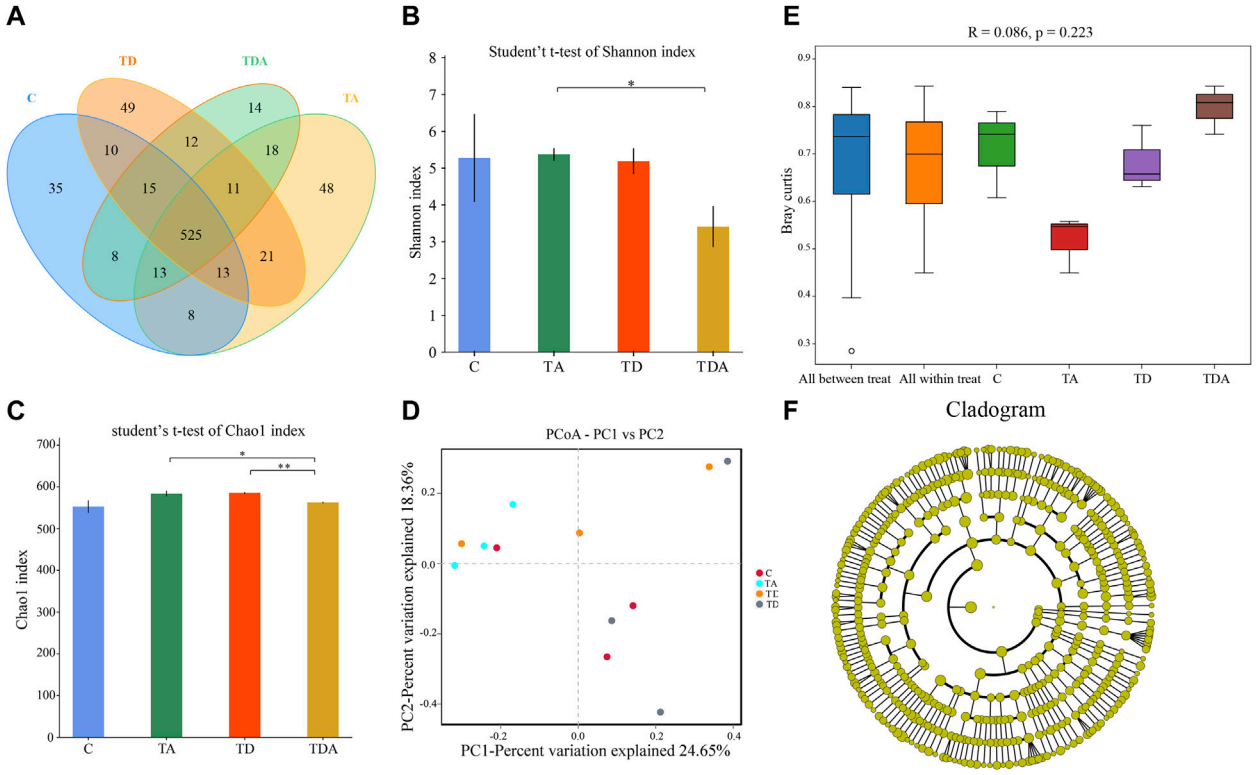


FIGURE 6
Gut fungal microbiome alterations when *Apis cerana* are exposed to acetamiprid or/and difenoconazole at environmentally relevant concentrations. **(A)** Venn diagram showing the numbers of unique and shared OTUs in the gut fungal microbiota of *Apis cerana* between the pesticide-treated and the control groups. **(B)** Alpha diversity is measured by the Shannon index. Data (mean ± SEM) were analyzed by Student's *t*-test (*$p < 0.05$; **$p < 0.01$). **(C)** Beta diversity as shown on a PCoA plot. **(D)** The ANOSIM analysis revealed no significant differences in the structure (ANOSIM, R = 0.086, $p = 0.223$) of gut fungus among these treatments. **(E)** LefSe analysis revealed that no fungal taxa were enriched in the control and the different treatment groups.

group were decreased when compared with the TD group ($p = 0.020$ and $p = 0.034$) (Supplementary Table S9).

Gene expression

To investigate the molecular effects of pesticide exposure, we assessed transcriptional alterations of selected genes (Figures 7, 8). These genes are involved in functions such as immunity, acetylcholine receptor, detoxification, antioxidant reactions, and hormonal regulation, which are activated in response to environmental stressors in honey bees.

Transcriptional alteration of immunity-related genes

Compared to control honey bees, the expression of *secapin* transcripts was significantly upregulated in the TA group, while the expression levels of *defensin*, *hymenoptaecin,* and *abaecin* were not significantly affected, whether in the head (Figure 7A) or gut (Figure 8A) ($p < 0.05$). In addition, the expression of *apidaecin* was significantly downregulated in the head of TA honey bees, while in the gut, it was not altered. The expression of *defensin* was significantly suppressed in the head of the TD group, while *abaecin*, *hymenoptaecin,* and *apidaecin* transcripts were not evidently altered ($p < 0.05$). In addition, difenoconazole significantly inhibited the expression of *defensin*, *hymenoptaecin,* and *apidaecin* in the gut of honey bees ($p < 0.05$). When honey bees were co-exposed to acetamiprid and difenoconazole, the *hymenoptaecin* transcript was significantly upregulated in the head, while the *apidaecin* transcript was significantly downregulated ($p < 0.05$). In contrast, the expression of *apidaecin* in the gut was significantly upregulated ($p < 0.05$).

Transcriptional alteration of detoxification-related genes

Significant differences were observed in the expression of detoxification genes between honey bees treated with pesticides and control honey bees (Figure 7B; Figure 8B). Acetamiprid and difenoconazole, alone or in combination, significantly decreased the expression levels of *CYP4G11* in the head and gut. It is worth noting that acetamiprid or difenoconazole, especially their mixture, showed the *CYP4G11* and *CYP336A1* transcripts with

10.3389/fphys.2023.1174236

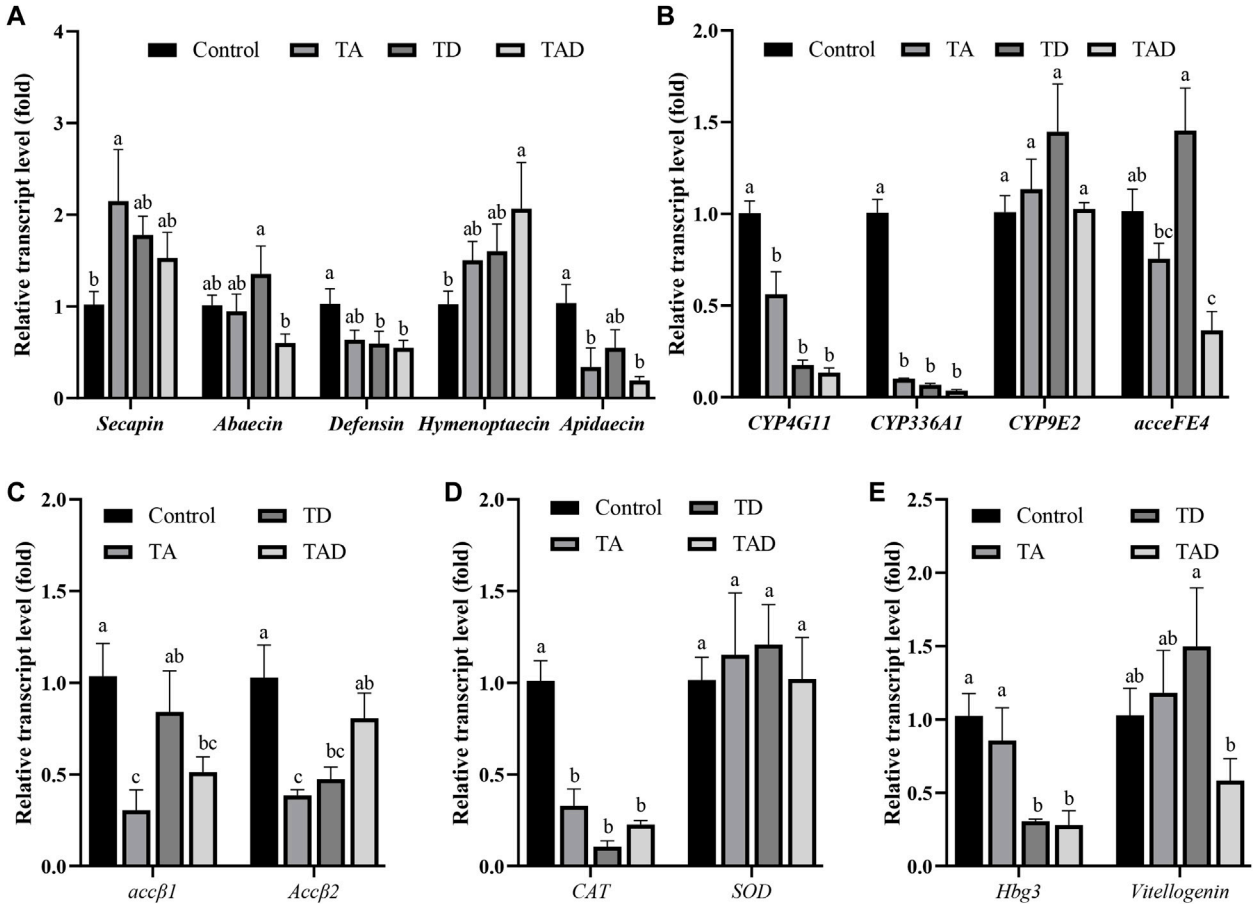


FIGURE 7
Normalized gene expression of immunity, *secapin*, *abaecin*, *defensin*, *hymenoptaecin*, and *apidaecin* **(A)**, detoxification, *CYP4G11*, *CYP9E2*, *CYP336A1*, and *acceFE4* **(B)**, acetylcholine receptor, *accβ1* and *accβ2* **(C)**, antioxidant reactions, *CAT* and *SOD* **(D)**, and hormonal regulation-related genes, *hbg-3* and *vitellogenin*, **(E)** in the head of *Apis cerana* exposed for 10 d to acetamiprid or/and difenoconazole at environmentally relevant concentrations. Data are means ± SEM. One-way ANOVA was performed for all treatments (LSD test), and bars topped with the same letters are not statistically different at $p = 0.05$.

the strongest significant downregulation in the head ($p < 0.01$). A similar trend was observed in the expression of *acceFE4*. Acetamiprid and difenoconazole mixtures significantly reduced the transcript of *acceFE4* in the honey bee head but strongly upregulated its expression in the gut ($p < 0.05$). A single exposure to acetamiprid or difenoconazole had no significant effect on the expression of *acceFE4* in the honey bee head; however in the gut, acetamiprid did not change its expression, but difenoconazole significantly induced it. Acetamiprid or/and difenoconazole did not change the *CYP9E2* transcript in the honey bee head, but they extremely induced the *CYP9E2* transcript in the gut ($p < 0.05$).

Transcriptional alteration of genes encoding acetylcholine receptors *Accβ1* and *Accβ2*

Acetamiprid significantly reduced the expression of *Accβ1* and *Accβ2* in the honey bee head ($p < 0.05$), while difenoconazole and acetamiprid–difenoconazole mixture led to only a weak suppression of *Accβ1* and *Accβ2* transcripts in the head (Figure 7C). In contrast, the acetamiprid and difenoconazole mixture significantly increased the expression of *Accβ1* and *Accβ2* in the honey bee gut, while single acetamiprid or difenoconazole led to only a weak induction, and the difference did not reach statistical significance (Figure 8C).

Transcriptional alteration of antioxidant reaction-related genes

The expression of *CAT* in the honey bee head was significantly suppressed by acetamiprid or/and difenoconazole exposure ($p < 0.05$) (Figure 7D), while in the gut, the expression of *CAT* was not altered (Figure 8D). Exposure to acetamiprid or/and difenoconazole had no significant effects on the expression of *SOD*, whether in the head or gut (Figures 7D, 8D).

10.3389/fphys.2023.1174236

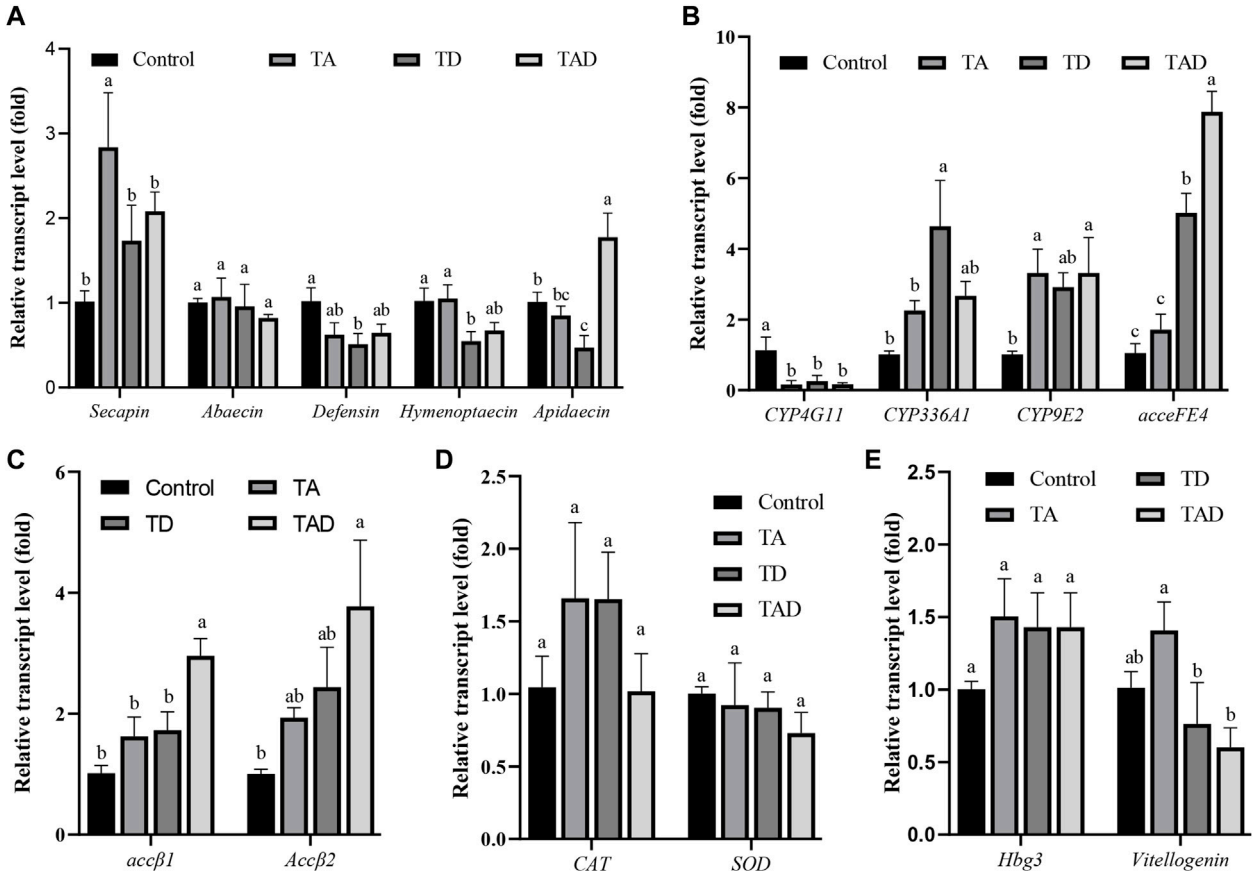


FIGURE 8
Normalized gene expression of immunity, *Secapin*, *abaecin*, *defensin*, *hymenoptaecin*, and *apidaecin* **(A)**, detoxification, *CYP4G11*, *CYP9E2*, *CYP336A1*, and *acceFE4* **(B)**, acetylcholine receptor, *accβ1* and *accβ2* **(C)**, antioxidant reactions, *CAT* and *SOD* **(D)**, and hormonal regulation-related genes, *hbg-3* and *vitellogenin*, **(E)** in the gut of *Apis cerana* exposed for 10 d to acetamiprid or/and difenoconazole at environmentally relevant concentrations. Data are means ± SEM. One-way ANOVA was performed for all treatments (LSD test), and bars topped with the same letters are not statistically different at $p = 0.05$.

Transcriptional alteration of hormonal regulation-related genes

Difenoconazole and acetamiprid–difenoconazole mixtures significantly downregulated the expression of *hbg-3* in the honey bee head ($p < 0.05$). In addition to this, the expression changes of *hbg-3* and *vitellogenin* in both the honey bee head and gut were observed when compared with the control, but the differences did not reach statistical significance (Figures 7E, 8E).

Discussion

There is a concern about the potential health risks of bee populations throughout the world that are exposed to a cocktail of pesticides, including neonicotinoids and fungicides (David et al., 2015; Mitchell et al., 2017). The focus of concern and controversy is the dose of pesticides, and whether the bees are likely to be exposed to them in the field (David et al., 2016). Thus, obtaining more information about field-realistic pesticide exposure on the effects on honey bees' health is vital to take this debate forward. In this study, we examined the effects of acetamiprid or/and difenoconazole on the survival, sucrose consumption, body weight, and gut microbiota composition of *A. cerana* at environmentally relevant concentrations and the molecular effects associated with them.

It is difficult to accurately estimate the concentrations of pesticides that honey bees encounter in the field. In this study, the doses (0.32 mg/kg acetamiprid and 0.27 mg/kg difenoconazole) administered to honey bees were detected from beebread, which is the main food for nurse workers. The total doses of acetamiprid uptakes in our experiment were 72.03 (alone) and 72.11 (combination) ng/honey bee for nursing honey bees. The dose corresponds to about 0.02 times the acetamiprid oral LD_{50} (3.208 μg/honey bee) obtained in a previous study for *A. cerana* (Han et al., 2017). The total doses of difenoconazole uptakes in our experiment were 56.05 (alone) and 60.85 (combination) ng/honey bee for nursing honey bees. These doses were far below those detected from the honey sample (1 μg/kg) (Herrera López et al., 2016) and much lower than the dose used by Iverson et al. (2019) (200 ng/honey bee). Considering the pesticide degradation in the environment and the honey bee feed on a mix of contaminated and uncontaminated plant pollen or nectar, we assume that honey bees

that received these low doses conform to the agricultural field's actual situation.

From the data presented, it has been indicated that acetamiprid or/and difenoconazole continuous exposure almost did not affect survival and food consumption, but difenoconazole decreased the weight of honey bees. Our previous study showed that the total mean doses of acetamiprid were 198.8 (alone) and 109.8 (combination) ng/honey bee for newly emerged bees and 1.39 (alone) and 1.36 (combination) μg/honey bee for forager bees, which severely affected *A. cernan* survival (Han et al., 2019). From these experimental results, we can infer that there was a dose-dependent effect of acetamiprid on honey bees' survival. In addition, one of the main reasons for the differences may be honey bees' age differences. Difenoconazole affected the weight of honey bees because this fungicide may be accumulated in honey bee tissues; honey bees need to increase more of their energetic investment in detoxification and immunity and promote the growth of beneficial microbiota.

Honey bees acquire their microbiota after emergence through interactions with their hive environment and social exchange (Guo et al., 2015). Our results demonstrated that the gut bacterial communities of *A. cerana* adult workers comprise four major phyla, Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria. This result is consistent with Luo et al.'s (2020) reports. At the genus level, previous studies showed that *Lactobacillus*, *Snodgrassella*, and *Gilliamella* were the major genera (Guo et al., 2015; Huang et al., 2018; Luo et al., 2020). In addition to this, we found *Bombella*, *Pectinatus,* and *Apibacter* also have very high abundance in our samples. Other genera of *Aeromonas*, *Atopobium*, *Bartonella*, *Bifidobacterium*, *Dysgonomonas*, *Enterobacter*, *Candidatus_Schmidhempelia*, *Escherichia–Shigella*, *Fructobacillus*, *Klebsiella*, *Kosakonia*, *Pseudomonas*, *Salmonella*, *Sebaldella*, *Serratia*, *Snodgrassella*, and *Tatumella* were observed. The occurrence of these genera in the gut of *A. cerana* and the exact role played by them will be investigated in future studies.

There is accumulating evidence indicating that gut microbiota is critical in the maintenance of physiological homeostasis, and perturbing it can induce detrimental effects (Sun et al., 2020; Xiong et al., 2020). Exposure to pesticides can influence the honey bee gut microbiota composition (Kakumanu et al., 2016; Motta et al., 2018; Liu et al., 2020). Our results showed that difenoconazole and the mixture of difenoconazole–acetamiprid significantly shaped the composition of the gut bacteria of *A. cerana* adult workers, but acetamiprid did not impact the gut bacterial community.

Agrochemical substances can result in microbial dysbiosis, with diversity and composition modifications that may increase a bee's vulnerability to other abiotic and biotic stressors (Muñoz-Colmenero et al., 2020). In this study, the bacterial diversity was significantly reduced, but the richness was not affected under difenoconazole exposure (TD and TDA groups). It is commonly considered that high microbiota diversity has a positive impact on the host's health due to more diverse microbial communities that are assumed to be more resistant and resilient to perturbations and external stressors (Shade et al., 2012), but the opposite argument is that higher richness of the core bacteria has positive effects on the bees' health, while a higher diversity in non-core taxa is considered deleterious (Ribière et al., 2019). In this study, difenoconazole-treated honey bees have lower diversity, but the core strain of *Lactobacillus* and *Gilliamella* was significantly higher than in the control. *Lactobacillus* and *Gilliamella* bacteria are important members of the intestinal tract of honey bees (Kwong and Moran, 2016). *Lactobacillus* can produce antibacterial and antiviral compounds, such as organic acids, diacetyl, benzoate, and bacteriocins (Luo et al., 2020). *Gilliamella* can metabolize a diverse array of plant-produced carbohydrates and utilize glucose, fructose, mannose, and so on (Zheng et al., 2016). *Lactobacillus* and *Gilliamella* play an important role in resisting pathogenic bacteria and the immune protection of honey bees. Our results also found that *Pectinatus* abundance is relatively reduced in response to difenoconazole exposure. *Pectinatus* is a recurrent brewing spoilage bacterium (Rodríguez-Saavedra et al., 2021). This bacterium in the honey bees' gut may come from the environment. There is little scientific information concerning the functional roles of this bacterium in the honey bees' gut. In addition, it is well known that *Escherichia–Shigella* is a known opportunistic pathogen (Zhou et al., 2018). In this study, the abundance of *Escherichia–Shigella* changed in TD and TDA groups, but this did not reach significance from a statistical standpoint. It is worth mentioning that the *Aeromonas* strains and *Aeromonas veronii* were much more abundant in those honey bees of the TDA group. *Aeromonas veronii* is a Gram-negative bacteria, which is a widely distributed novel pathogen that can affect humans and animals (Huang et al., 2020). Difenoconazole, as a fungicide, has bactericidal properties. Honey bees' recurrent consumption of food containing this fungicide may have caused the death of sensitive bacteria, thus disturbing gut physiological homeostasis. So, further research is needed to isolate these bacteria in honey bees' guts and determine their sensitivity to difenoconazole.

Furthermore, in the current study, the alpha-diversity and beta-diversity analysis indicated that acetamiprid does not significantly affect the gut bacteria composition of honey bees. PCoA also showed that the gut community compositions of exposed and control honey bees were similar between the two groups. Our result was similar to Liu et al. (2020), who reported thiacloprid (acetamiprid and thiacloprid both belongs to cyano-substituted neonicotinoids) exposure did not impact the abundance of honey bee gut microbiome in the low dose (0.2 mg/L), but significantly reduced abundance in the high dose (0.6 and 2.0 mg/L). One possible explanation as to why low-dose acetamiprid does not impact the honey bee gut microbiome could be because once acetamiprid entered the midgut (the primary place of metabolism), it was quickly eliminated from or metabolized by honey bee cells before it reaches the hindgut (the primary place of bacteria colonization). Although low-dose acetamiprid did not affect the gut bacteria composition, the core strain of *Lactobacillus* and *Apibacter* was significantly lower than in the control. *Lactobacillus* spp. are important probiotics, and they can secrete bacteriostatic substances (bacteriocins and lactic acid) to protect their hosts (Tang et al., 2021). *Apibacter* is prevalent in the gut of *A. cerana*; it may provide hosts with vital benefits (Zhang et al., 2021). The decrease in the abundance of *Lactobacillus* and *Apibacter* may lead to poor health of honey bees.

Similar to bacteria, fungi also play an important role in the maintenance of intestinal homeostasis, although fungi accounted for a small proportion microbiota of the alimentary canals of the bees

(Khan et al., 2020). Our results showed that the fungal communities of honey bee gut microbiota were dominated by members of *Ascomycota* and *Basidiomycota*, consistent with Kakumanu et al. (2016) and Paris et al.'s (2020) previous reports. Alpha diversity analysis showed that chronic exposure to difenoconazole or/and acetamiprid had no effect on the fungal community structure when compared to the control group. Of note, although the overall beta diversity does not differ remarkably among pesticide treatments, we found a significantly lower Chao1 index (a marker of species richness) in honey bees with co-exposure to difenoconazole and acetamiprid *versus* single exposure. The results suggest that the cocktail of pesticides may exacerbate the disturbance of the gut microbiota of honey bees. In addition, at the genus level, we observed 188 genera; among them, *Meyerozyma*, *Candida*, and *Aspergillus* are the main central taxa. It is reported that these fungi correlate with several bacterial taxa including *Firmicutes*, *Bacteroides*, and *Faecalibacterium* (Nagpal et al., 2020). It is worth mentioning here that the gut fungi that truly colonized the gut of these subjects *versus* transient fungi that came through diets remain unknown. The genus *Candida* comprises various opportunistic species implicated in various gut-related diseases in humans (Nagpal et al., 2020). At present, the fungal flora remains largely unexplored in honey bees' health, and this field needs further research.

It has been shown that gut microbiota composition correlates with altered gene expression in host tissues (Du et al., 2020). A previous study has shown that pesticides impacted the expression of immunity-related genes in honey bees' guts (Aufauvre et al., 2014). In the current study, we found acetamiprid induced the expression of *secapin*, which is an antimicrobial peptide with activity against bacteria and fungi in the innate immune response (Lee et al., 2016). Additionally, we also found the expression of *defensin*, *hymenoptaecin,* and *apidaecin* was significantly downregulated in the gut of honey bees following chronic exposure to difenoconazole. Acetamiprid had no effect on the expression of these four genes related to immunity in the honey bee gut. *Defensin*, *hymenoptaecin,* and *apidaecin* are key antimicrobial components in insect innate immunity against invading pathogens. The immune system of honey bees consists of three pathways, the TLR, Imd, and Jak/STAT pathways. Each pathway displays different functions (Christen and Fent, 2017). Therefore, acetamiprid and difenoconazole might have different models of action on honey bees' immune systems, which may lead to different compositions of gut microbiota.

Honey bees have detoxification systems that function in the metabolism of endogenous compounds and xenobiotics such as pesticides and plant toxins. It is well known that pesticides can change the expressions of detoxification-related genes in honey bees (Mao et al., 2017; Wu et al., 2017; Liu et al., 2021). In addition, honey bee gut dysbiosis leads to a change in P450 gene expression (Schwarz et al., 2016). The present study shows that the expression of P450 genes (*CYP4G11*, *CYP336A1*, and *CYP9E2*) and esterase gene (*acceFE4*) in the tissue of honey bee workers of *A. cerana* was altered after chronic exposure to acetamiprid or/and difenoconazole. *CYP4G11* belongs to the microsomal CYP4 family, which is involved in chemoreception, and its transcriptional alteration may induce chaotic behavior among honey bees (Mao et al., 2015; Wu et al., 2017). *CYP336A1* belongs to the microsomal CYP3 family, which plays an important role in protecting cells against oxidative damage (Zhu et al., 2016). *CYP9E2* belongs to the microsomal CYP9 family, which is involved in xenobiotic detoxification (Claudianos et al., 2006). In the head, we observed that the expression of *CYP4G11* and *CYP336A1* was significantly decreased in all pesticide treatment groups, and in the gut, the expression of *CYP4G11* was suppressed, while *CYP336A1* and *CYP9E2* were induced. Esterase *acceFE4* belongs to carboxylesterases, which are involved in xenobiotic metabolism (Ma et al., 2018). For the expression of *acceFE4*, the acetamiprid–difenoconazole mixture caused a significant decrease in the head and a significant increase in the gut. In the meanwhile, honey bees exposed to acetamiprid alone had no effect on *acceFE4* expression. These results demonstrated that low doses of acetamiprid and difenoconazole, especially the cocktail, seriously interrupted the detoxification gene expression in honey bees and enhances the pesticide risks for honey bees.

Nicotinic acetylcholine receptors (nAChRs) mediate fast cholinergic synaptic transmission in the insect nervous system and are important targets for insecticides. Alterations in neuronal signaling can have pronounced effects; for example, *A. mellifera* exposed to 3.8 ng/bee thiamethoxam caused locomotor deficits (Charreton et al., 2015). In this study, we found that acetamiprid triggered the downregulation of nAChR transcripts in the head, and the mixture of acetamiprid and difenoconazole triggered the upregulation of nAChR transcripts in the gut. Upregulation of nAChRs may represent a compensation reaction to the functional loss of the neuronal signaling upon exposure to neurotoxic pesticides (Christen et al., 2016).

In the case of the oxidative stress-related gene *CAT*, acetamiprid and difenoconazole significantly decreased their expression in the head, alone and in combination. *CAT* was involved in antioxidant reactions and xenobiotic detoxification (Aufauvre et al., 2014). In addition, we observed that difenoconazole and acetamiprid–difenoconazole mixtures led to the expressional downregulation of *hbg-3* in honey bees' heads. The gene product of *hbg-3* is involved in the transition of nurse bees to foragers. In foragers, the hypopharyngeal glands are shrinking, and at the same time, the expression of *hbg-3* is increasing (Christen et al., 2019). From the results, we can infer that difenoconazole may prolong the development of nurse bees to foragers by downregulating *hbg-3* in the heads of honey bees. At present, *vitellogenin* has become widely accepted as a marker of honey bees' overall health. It is an important regulator of life-span and foraging behavior, and changes in expression may have significant physiological effects (Christen et al., 2019). In the present study, acetamiprid and difenoconazole had no particularly pronounced effect on the expression of *vitellogenin* transcripts; thus, we think the transcripts showed only weak significance.

In conclusion, our results showed that acetamiprid or/and difenoconazole continuous exposure at concentrations that mimic environmental contamination almost did not affect the survival and food consumption of *A. cerana* under laboratory conditions. However, difenoconazole or acetamiprid–difenoconazole mixture-treated honey bees had structurally different bacterial communities compared to non-exposed colonies, but acetamiprid does not impact the gut bacterial community. Meanwhile, we also observed that acetamiprid or/and difenoconazole significantly altered the

expression of genes linked to detoxification in the honey bee tissues. Furthermore, it is worth mentioning that the toxic effects of acetamiprid and difenoconazole co-exposure on the molecular level were greater than those of the single exposure. However, there were still several limitations in our study. Future experiments should be designed to observe the toxicological effects of pesticide cocktail on honey bee gut microbiomes under a real exposure scenario (field condition), and the molecular mechanism of the toxic effect should be elucidated with the methods of multi-omics, so as to find new targets for protecting honey bees. Ultimately, our study provides a good reference for farmers to know the toxic effects of pesticides on honey bees and how to select the chemical mixture if they produce synergistic interactions at environmentally realistic concentrations.

Data availability statement

Publicly available datasets were analyzed in this study. The accession number is PRJNA956719. This data can be found here: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA956719.

Author contributions

WH conceptualized and designed the experiments and wrote the manuscript. ZY, SZ, YZ, and SW performed the experiments. FG and JG revised the manuscript. All authors provided their final approval for the publication. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the earmarked fund for CARS (No. CARS-44-SYZ11), Hainan Province Science and Technology Special Fund (ZDYF2021XDNY282), and Hainan Provincial Natural Science Foundation of China (2019RC273).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors, and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2023.1174236/full#supplementary-material

References

Almasri, H., Tavares, D. A., Tchamitchian, S., Pelissier, M., Sene, D., Cousin, M., et al. (2021). Toxicological status changes the susceptibility of the honey bee *Apis mellifera* to a single fungicidal spray application. *Environ. Sci. Pollut. Res.* 28, 42807–42820. doi:10.1007/s11356-021-13747-3

Artz, D. R., and Pitts-Singer, T. L. (2015). Effects of fungicide and adjuvant sprays on nesting behavior in two managed solitary bees, *Osmia lignaria* and *Megachile rotundata*. *PLoS ONE* 10 (8), e0135688. doi:10.1371/journal.pone.0135688

Aufauvre, J., Misme-Aucouturier, B., Viguès, B., Texier, C., Delbac, F., and Blot, N. (2014). Transcriptome analyses of the honey bee response to *Nosema ceranae* and insecticides. *PLoS ONE* 9 (3), e91686. doi:10.1371/journal.pone.0091686

Brandt, A., Gorenflo, A., Siede, R., Meixner, M., and Büchler, R. (2016). The neonicotinoids thiacloprid, imidacloprid, and clothianidin affect the immunocompetence of honey bees (*Apis mellifera* L.). *J. Insect. Physiol.* 86, 40–47. doi:10.1016/j.jinsphys.2016.01.001

Charreton, M., Decourtye, A., Henry, M., Rodet, G., Sandoz, J.-C., Charnet, P., et al. (2015). A locomotor deficit induced by sublethal doses of pyrethroid and neonicotinoid insecticides in the honey bee *Apis mellifera*. *Plos ONE* 10, e0144879. doi:10.1371/journal.pone.0144879

Chen, C., Liu, Z., Luo, Y., Xu, Z., Wang, S., Zhang, X., et al. (2017). Managed honey bee colony losses of the Eastern honey bee (*Apis cerana*) in China (2011-2014). *Apidologie* 48, 692–702. doi:10.1007/s13592-017-0514-6

Christen, V., and Fent, K. (2017). Exposure of honey bees (*Apis mellifera*) to different classes of insecticides exhibit distinct molecular effect patterns at concentrations that mimic environmental contamination. *Environ. Pollut.* 226, 48–59. doi:10.1016/j.envpol.2017.04.003

Christen, V., Krebs, J., and Fent, K. (2019). Fungicides chlorothanolin, azoxystrobin and folpet induce transcriptional alterations in genes encoding enzymes involved in oxidative phosphorylation and metabolism in honey bees (*Apis mellifera*) at sublethal concentrations. *J. Hazard. Mater.* 377, 215–226. doi:10.1016/j.jhazmat.2019.05.056

Christen, V., Mittner, F., and Fent, K. (2016). Molecular effects of neonicotinoids in honey bees (*Apis mellifera*). *Environ. Sci. Technol.* 50, 4071–4081. doi:10.1021/acs.est.6b00678

Claudianos, C., Ranson, H., Johnson, R. M., Biswas, S., Schuler, M. A., Berenbaum, M. R., et al. (2006). A deficit of detoxification enzymes: Pesticide sensitivity and environmental response in the honey bee. *Insect Mol. Biol.* 15, 615–636. doi:10.1111/j.1365-2583.2006.00672.x

David, A., Botías, C., Abdul-Sada, A., Goulson, D., and Hill, E. M. (2015). Sensitive determination of mixtures of neonicotinoid and fungicide residues in pollen and single bumblebees using a scaled down QuEChERS method for exposure assessment. *Anal. Bioanal. Chem.* 407, 8151–8162. doi:10.1007/s00216-015-8986-6

David, A., Botías, C., Abdul-Sada, A., Nicholls, E., Rotheray, E. L., Hill, E. M., et al. (2016). Widespread contamination of wildflower and bee-collected pollen with complex mixtures of neonicotinoids and fungicides commonly applied to crops. *Environ. Int.* 88, 169–178. doi:10.1016/j.envint.2015.12.011

Degrandi-Hoffman, G., Chen, Y., Dejong, E. W., Chambers, M. L., and Hidalgo, G. (2015). Effects of oral exposure to fungicides on honey bee nutrition and virus levels. *J. Econ. Entomol.* 108 (6), 2518–2528. doi:10.1093/jee/tov251

Du, Z., Wang, Q., Huang, X., Yi, S., Mei, S., Yuan, G., et al. (2020). Effect of berberine on spleen transcriptome and gut microbiota composition in experimental autoimmune uveitis. *Int. Immunopharmacol.* 81, 106270. doi:10.1016/j.intimp.2020.106270

Favaro, R., Bauer, L. M., Rossi, M., D'Ambrosio, L., Bucher, E., and Angeli, S. (2019). Botanical origin of pesticide residues in pollen loads collected by honey bees during and after apple bloom. *Front. Physiol.* 10, 1069. doi:10.3389/fphys.2019.01069

Feyereisen, R. (2018). Toxicology: Bee P450s take the sting out of cyanoamidine neonicotinoids. *Curr. Biol.* 28, R560–R562. doi:10.1016/j.cub.2018.03.013

Figueirêdo, L. P. d., Daam, M. A., Mainardi, G., Mariën, J., Espíndola, E. L. G., Gestel, C. A. M. v., et al. (2019). The use of gene expression to unravel the single and mixture toxicity of abamectin and difenoconazole on survival and reproduction of the springtail *Folsomia candida*. *Environ. Pollut.* 244, 342–350. doi:10.1016/j.envpol.2018.10.077

Gaweł, M., Kiljanek, T., Niewiadowska, A., Semeniuk, S., Goliszek, M., Burek, O., et al. (2019). Determination of neonicotinoids and 199 other pesticide residues in honey by liquid and gas chromatography coupled with tandem mass spectrometry. *Food Chem.* 282, 36–47. doi:10.1016/j.foodchem.2019.01.003

Gemeda, T. K., Shao, Y., Wu, W., Yang, H., Huang, J., and Wu, J. (2017). Native honey bees outperform adventive honey bees in increasing *Pyrus bretschneideri* (Rosales: Rosaceae) pollination. *J. Econ. Entomol.* 110, 2290–2294. doi:10.1093/jee/tox286

Godfray, H. C. J., Blacquière, T., Field, L. M., Hails, R. S., Potts, S. G., Raine, N. E., et al. (2014). A restatement of recent advances in the natural science evidence base concerning neonicotinoid insecticides and insect pollinators. *Proc. R. Soc. B* 282, 20151821. doi:10.1098/rspb.2015.1821

Guo, J., Wu, J., Chen, Y., Evans, J. D., Dai, R., Luo, W., et al. (2015). Characterization of gut bacteria at different developmental stages of Asian honey bees, *Apis cerana*. *J. Invertebr. Pathol.* 127, 110–114. doi:10.1016/j.jip.2015.03.010

Han, W., Ren, C., Gao, J., Qi, B., Wang, S., Zhao, S., et al. (2017). Potential synergistic effect of common fungicides on acetamiprid toxicity to Chinese honey bee (*Apis creana cerana*). *Asian J. Ecotoxicol.* 12, 273–280. doi:10.7524/AJE.1673-5897.20170930001

Han, W., Wang, Y., Gao, J., Wang, S., Zhao, S., Liu, J., et al. (2018). Acute toxicity and sublethal effects of myclobutanil on respiration, flight and detoxification enzymes in *Apis cerana cerana*. *Pestic. Biochem. Physiol.* 147, 133–138. doi:10.1016/j.pestbp.2017.11.001

Han, W., Yang, Y., Gao, J., Zhao, D., Ren, C., Wang, S., et al. (2019). Chronic toxicity and biochemical response of *Apis cerana cerana* (Hymenoptera: Apidae) exposed to acetamiprid and propiconazole alone or combined. *Ecotoxicology* 28 (4), 399–411. doi:10.1007/s10646-019-02030-4

Herrera López, S., Lozano, A., Sosa, A., Hernando, M. D., and Fernández-Alba, A. R. (2016). Screening of pesticide residues in honey bee wax comb by LC-ESI-MS/MS. A pilot study. *Chemosphere* 163, 44–53. doi:10.1016/j.chemosphere.2016.07.008

Huang, H., Zhou, P., Chen, P., Xia, L., Hu, S., Yi, G., et al. (2020). Alteration of the gut microbiome and immune factors of grass carp infected with *Aeromonas veronii* and screening of an antagonistic bacterial strain (Streptomyces flavotricini). *Microb. Pathog.* 143, 104092. doi:10.1016/j.micpath.2020.104092

Huang, S. K., Ye, K. T., Huang, W. F., Ying, B. H., Su, X., Lin, L. H., et al. (2018). Influence of feeding type and *Nosema ceranae* infection on the gut microbiota of *Apis cerana* workers. *mSystems* 3, e00177–e00118. doi:10.1128/mSystems.00177-18

Iverson, A., Hale, C., Richardson, L., Miller, O., and McArt, S. (2019). Synergistic effects of three sterol biosynthesis inhibiting fungicides on the toxicity of a pyrethroid and neonicotinoid insecticide to bumble bees. *Apidologie* 50 (5), 733–744. doi:10.1007/s13592-019-00681-0

Kakumanu, M. L., Reeves, A. M., Anderson, T. D., Rodrigues, R. R., and Williams, M. A. (2016). Honey bee gut microbiome is altered by in-hive pesticide exposures. *Front. Microbiol.* 7, 1255. doi:10.3389/fmicb.2016.01255

Khan, K. A., Al-Ghamdi, A. A., Ghramh, H. A., Ansari, M. J., Ali, H., Alamri, S. A., et al. (2020). Structural diversity and functional variability of gut microbial communities associated with honey bees. *Microb. Pathog.* 138, 103793. doi:10.1016/j.micpath.2019.103793

Kubik, M., Nowacki, J., Pidek, A., Warakomska, Z., Michalczuk, L., Goszczynski, W., et al. (2000). Residues of captan (contact) and difenoconazole (systemic) fungicides in bee products from an apple orchard. *Apidologie* 31, 531–541. doi:10.1051/apido:2000144

Kwong, W. K., and Moran, N. A. (2016). Gut microbial communities of social bees. *Nat. Rev. Microbiol.* 14, 374–384. doi:10.1038/nrmicro.2016.43

Lee, K. S., Kim, B. Y., Yoon, H. J., Choi, Y. S., and Jin, B. R. (2016). Secapin, a bee venom peptide, exhibits anti-fibrinolytic, anti-elastolytic, and anti-microbial activities. *Dev. Comp. Immunol.* 63, 27–35. doi:10.1016/j.dci.2016.05.011

Leite, D. T., Sampaio, R. B., Chambó, E. D., Aguiar, C. M. L., de Godoy, M. S., and de Carvalho, C. A. L. (2021). Toxicity of chlorpyrifos, cyflumetofen, and difenoconazole on *Tetragonisca angustula* (Latreille, 1811) under laboratory conditions. *Int. J. Trop. Insect. Sci.* 42 (1), 435–443. doi:10.1007/s42690-021-00560-1

Liao, L.-H., Wu, W.-Y., and Berenbaum, M. R. (2017). Behavioral responses of honey bees (*Apis mellifera*) to natural and synthetic xenobiotics in food. *Sci. Rep.* 7, 15924. doi:10.1038/s41598-017-15066-5

Liu, Y.-J., Qiao, N.-H., Diao, Q.-Y., Jing, Z., Vukanti, R., Dai, P.-L., et al. (2020). Thiacloprid exposure perturbs the gut microbiota and reduces the survival status in honey bees. *J. Hazard. Mater.* 389, 121818. doi:10.1016/j.jhazmat.2019.121818

Liu, Z., Li, S., and Li, H. (2021). Interactions between sublethal doses of thiamethoxam and *Nosema ceranae* in the honey bee, *Apis mellifera*. *J. Apic. Res.* 60, 717–725. doi:10.1080/00218839.2021.1880760

Luo, z., Dong, Z., Chen, Y., Li, H., Tang, Q., Li, J., et al. (2020). Comparative analysis of the gut microbiota of *Apis cerana* in Yunnan using high-throughput sequencing. *Arch. Microbiol.* 202, 2557–2567. doi:10.1007/s00203-020-01974-0

Ma, M., Jia, H., Cui, X., Zhai, N., Wang, H., Guo, X., et al. (2018). Isolation of carboxylesterase (esterase FE4) from *Apis cerana cerana* and its role in oxidative resistance during adverse environmental stress. *Biochimie* 144, 85–97. doi:10.1016/j.biochi.2017.10.022

Mao, W., Schuler, M. A., and Berenbaum, M. R. (2017). Disruption of quercetin metabolism by fungicide affects energy production in honey bees (*Apis mellifera*). *Proc. Natl. Acad. Sci. U.S.A.* 114, 2538–2543. doi:10.1073/pnas.1614864114

Mao, W., Schuler, M. A., and Berenbaum, M. R. (2015). Task-related differential expression of four cytochrome P450 genes in honey bee appendages. *Insect Mol. Biol.* 24, 582–588. doi:10.1111/imb.12183

Matsuda, K., Ihara, M., and Sattelle, D. B. (2020). Neonicotinoid insecticides: Molecular targets, resistance, and toxicity. *Annu. Rev. Pharmacol. Toxicol.* 60, 241–255. doi:10.1146/annurev-pharmtox-010818-021747

Mitchell, E. A. D., Mulhauser, B., Mulot, M., Mutabazi, A., Glauser, G., and Aebi, A. (2017). A worldwide survey of neonicotinoids in honey. *Science* 358, 109–111. doi:10.1126/science.aan3684

Mokkapati, J. S., Bednarska, A. J., and Laskowski, R. (2021). The development of the solitary bee *Osmia bicornis* is affected by some insecticide agrochemicals at environmentally relevant concentrations. *Sci. Total Environ.* 775, 145588. doi:10.1016/j.scitotenv.2021.145588

Motta, E. V. S., Mak, M., De Jong, T. K., Powell, J. E., O'Donnell, A., Suhr, K. J., et al. (2020). Oral or topical exposure to glyphosate in herbicide formulation impacts the gut microbiota and survival rates of honey bees. *Appl. Environ. Microbiol.* 86, 011500–e1220. doi:10.1128/AEM.01150-20

Motta, E. V. S., Raymann, K., and Moran, N. A. (2018). Glyphosate perturbs the gut microbiota of honey bees. *Proc. Natl. Acad. Sci. U.S.A.* 115, 10305–10310. doi:10.1073/pnas.1803880115

Mullin, C. A., Frazier, M., Frazier, J. L., Ashcraft, S., Simonds, R., vanEngelsdorp, D., et al. (2010). High levels of miticides and agrochemicals in north American apiaries: Implications for honey bee health. *PLoS ONE* 5 (3), e9754. doi:10.1371/journal.pone.0009754

Muñoz-Colmenero, M., Baroja-Careaga, I., Kovačić, M., Filipi, J., Puškadija, Z., Kezić, N., et al. (2020). Differences in honey bee bacterial diversity and composition in agricultural and pristine environments – A field study. *Apidologie* 51, 1018–1037. doi:10.1007/s13592-020-00779-w

Nagpal, R., Neth, B. J., Wang, S., Mishra, S. P., Craft, S., and Yadav, H. (2020). Gut mycobiome and its interaction with diet, gut bacteria and alzheimer's disease markers in subjects with mild cognitive impairment: A pilot study. *eBioMedicine* 59, 102950. doi:10.1016/j.ebiom.2020.102950

Osterman, J., Aizen, M. A., Biesmeijer, J. C., Bosch, J., Howlett, B. G., Inouye, D. W., et al. (2021). Global trends in the number and diversity of managed pollinator species. *Agric. Ecosyst. Environ.* 322, 107653. doi:10.1016/j.agee.2021.107653

Paris, L., Peghaire, E., Moné, A., Diogon, M., Debroas, D., Delbac, F., et al. (2020). Honey bee gut microbiota dysbiosis in pesticide/parasite co-exposures is mainly induced by *Nosema ceranae*. *J. Invertebr. Pathol.* 172, 107348. doi:10.1016/j.jip.2020.107348

Pisa, L. W., Amaral-Rogers, V., Belzunces, L. P., Bonmatin, J. M., Downs, C. A., Goulson, D., et al. (2015). Effects of neonicotinoids and fipronil on non-target invertebrates. *Environ. Sci. Pollut. Res.* 22, 68–102. doi:10.1007/s11356-014-3471-x

Prado, F. S. R., dos Santos, D. M., de Almeida Oliveira, T. M., Micheletti Burgarelli, J. A., Castele, J. B., and Vieira, E. M. (2020). Determination and uptake of abamectin and difenoconazole in the stingless bee *Melipona scutellaris* Latreille, 1811 via oral and topic acute exposure. *Environ. Pollut.* 265, 114313. doi:10.1016/j.envpol.2020.114313

Raymann, K., Shaffer, Z., and Moran, N. A. (2017). Antibiotic exposure perturbs the gut microbiota and elevates mortality in honey bees. *PLOS Biol.* 15 (3), e2001861. doi:10.1371/journal.pbio.2001861

Ribière, C., Hegarty, C., Stephenson, H., Whelan, P., and O'Toole, P. W. (2019). Gut and whole-body microbiota of the honey bee separate thriving and non-thriving hives. *Microb. Ecol.* 78, 195–205. doi:10.1007/s00248-018-1287-9

Rodríguez-Saavedra, M., González de Llano, D., Beltran, G., Torija, M.-J., and Moreno-Arribas, M. V. (2021). *Pectinatus* spp. – unpleasant and recurrent brewing spoilage bacteria. *Int. J. Food Microbiol.* 336, 108900. doi:10.1016/j.ijfoodmicro.2020.108900

Rondeau, S., and Raine, N. E. (2022). Fungicides and bees: A review of exposure and risk. *Environ. Int.* 165, 107311. doi:10.1016/j.envint.2022.107311

Rouzé, R., Moné, A., Delbac, F., Belzunces, L., and Blot, N. (2019). The honey bee gut microbiota is altered after chronic exposure to different families of insecticides and infection by *Nosema ceranae*. *Microbes Environ.* 34, 226–233. doi:10.1264/jsme2.ME18169

Schwarz, R. S., Moran, N. A., and Evans, J. D. (2016). Early gut colonizers shape parasite susceptibility and microbiota composition in honey bee workers. *Proc. Natl. Acad. Sci. U.S.A.* 113, 9345–9350. doi:10.1073/pnas.1606631113

Sgolastra, F., Arnan, X., Cabbri, R., Isani, G., Medrzycki, P., Teper, D., et al. (2018). Combined exposure to sublethal concentrations of an insecticide and a fungicide affect feeding, ovary development and longevity in a solitary bee. *Proc. R. Soc. B* 285, 20180887. doi:10.1098/rspb.2018.0887

Shade, A., Peter, H., Allison, S. D., Baho, D. L., Berga, M., Bürgmann, H., et al. (2012). Fundamentals of microbial community resistance and resilience. *Front. Microbiol.* 3, 417–419. doi:10.3389/fmicb.2012.00417

Sun, R., Xu, K., Ji, S., Pu, Y., Man, Z., Ji, J., et al. (2020). Benzene exposure induces gut microbiota dysbiosis and metabolic disorder in mice. *Sci. Total Environ.* 705, 135879. doi:10.1016/j.scitotenv.2019.135879

Tang, Q. H., Miao, C. H., Chen, Y. F., Dong, Z. X., Cao, Z., Liao, S. Q., et al. (2021). The composition of bacteria in gut and beebread of stingless bees (Apidae: Meliponini) from tropics Yunnan, China. *Ant. Van Leeuwenhoek* 114, 1293–1305. doi:10.1007/s10482-021-01602-x

Tasman, K., Rands, S. A., and Hodge, J. J. L. (2020). The neonicotinoid insecticide imidacloprid disrupts bumblebee foraging rhythms and sleep. *iScience* 23, 101827. doi:10.1016/j.isci.2020.101827

Teng, M., Qi, S., Zhu, W., Wang, Y., Wang, D., Dong, K., et al. (2018). Effects of the bioconcentration and parental transfer of environmentally relevant concentrations of difenoconazole on endocrine disruption in zebrafish (*Danio rerio*). *Environ. Pollut.* 233, 208–217. doi:10.1016/j.envpol.2017.10.063

Tong, Z., Duan, J., Wu, Y., Liu, Q., He, Q., Shi, Y., et al. (2018). A survey of multiple pesticide residues in pollen and beebread collected in China. *Sci. Total Environ.* 640-641, 1578–1586. doi:10.1016/j.scitotenv.2018.04.424

Tong, Z., Wu, Y.-C., Liu, Q.-Q., Shi, Y.-H., Zhou, L.-J., Liu, Z.-Y., et al. (2016). Multi-residue analysis of pesticide residues in crude pollens by UPLC-MS/MS. *Molecules* 21, 1652. doi:10.3390/molecules21121652

Wade, A., Lin, C.-H., Kurkul, C., Regan, E. R., and Johnson, R. M. (2019). Combined toxicity of insecticides and fungicides applied to California almond orchards to honey bee larvae and adults. *Insects* 10, 20. doi:10.3390/insects10010020

Wang, Y., Zhu, Y. C., and Li, W. (2020). Interaction patterns and combined toxic effects of acetamiprid in combination with seven pesticides on honey bee (*Apis mellifera* L.). *Ecotox. Environ. Safe.* 190, 110100. doi:10.1016/j.ecoenv.2019.110100

Willis Chan, D. S., and Raine, N. E. (2021). Population decline in a ground-nesting solitary squash bee (*Eucera pruinosa*) following exposure to a neonicotinoid insecticide treated crop (*Cucurbita pepo*). *Sci. Rep.* 11, 4241. doi:10.1038/s41598-021-83341-7

Wu, M.-C., Chang, Y.-W., Lu, K.-H., and Yang, E.-C. (2017). Gene expression changes in honey bees induced by sublethal imidacloprid exposure during the larval stage. *Insect biochem. Mol. Biol.* 88, 12–20. doi:10.1016/j.ibmb.2017.06.016

Wu, Y., Zheng, Y., Chen, Y., Wang, S., Chen, Y., Hu, F., et al. (2020). Honey bee (*Apis mellifera*) gut microbiota promotes host endogenous detoxification capability via regulation of P450 gene expression in the digestive tract. *Microb. Biotechnol.* 13, 1201–1212. doi:10.1111/1751-7915.13579

Xiong, F., Qin, L., Hao, Y.-t., Zhao, D., Li, W.-x., Zou, H., et al. (2020). Gut microbiota modulation and immunity response induced by *Citrobacter freundii* strain GC01 in grass carp (*Ctenopharyngodon idellus*). *Aquaculture* 521, 735015. doi:10.1016/j.aquaculture.2020.735015

Zhang, L., Dong, X., Wang, C., Zuo, Z., and Chen, M. (2017). Bioaccumulation and the expression of hepatic cytochrome P450 genes in marine medaka (*Oryzias melastigma*) exposed to difenoconazole. *J. Environ. Sci.* 52, 98–104. doi:10.1016/j.jes.2016.03.011

Zhang, W., Zhang, X., Su, Q., Tang, M., Zheng, H., and Zhou, X. (2021). Genomic features underlying the evolutionary transitions of *Apibacter* to honey bee gut symbionts. *Insect Sci.* 29, 259–275. doi:10.1111/1744-7917.12912

Zhao, W., Wang, M., Liu, Y., Gong, X., Dong, K., Zhou, D., et al. (2017). Phylogeography of *Apis cerana* populations on Hainan island and southern mainland China revealed by microsatellite polymorphism and mitochondrial DNA. *Apidologie* 48, 63–74. doi:10.1007/s13592-016-0450-x

Zheng, H., Nishida, A., Kwong, W. K., Koch, H., Engel, P., Steele, M. I., et al. (2016). Metabolism of toxic sugars by strains of the bee gut symbiont *Gilliamella apicola*. *mBio* 7, 013266–e1416. doi:10.1128/mBio.01326-16

Zheng, H., Steele, M. I., Leonard, S. P., Motta, E. V. S., and Moran, N. A. (2018). Honey bees as models for gut microbiota research. *Lab. Anim.* 47, 317–325. doi:10.1038/s41684-018-0173-x

Zhou, W., Yan, Y., Mi, J., Zhang, H., Lu, L., Luo, Q., et al. (2018). Simulated digestion and fermentation *in vitro* by human gut microbiota of polysaccharides from bee collected pollen of Chinese wolfberry. *J. Agric. Food. Chem.* 66, 898–907. doi:10.1021/acs.jafc.7b05546

Zhu, L., Qi, S., Xue, X., Niu, X., and Wu, L. (2020). Nitenpyram disturbs gut microbiota and influences metabolic homeostasis and immunity in honey bee (*Apis mellifera* L.). *Environ. Pollut.* 258, 113671. doi:10.1016/j.envpol.2019.113671

Zhu, M., Zhang, W., Liu, F., Chen, X., Li, H., and Xu, B. (2016). Characterization of an *Apis cerana cerana* cytochrome P450 gene (AccCYP336A1) and its roles in oxidative stresses responses. *Gene* 584, 120–128. doi:10.1016/j.gene.2016.02.016

frontiers | Frontiers in Physiology

TYPE Original Research
PUBLISHED 05 May 2023
DOI 10.3389/fphys.2023.1180655

OPEN ACCESS

EDITED BY
Qingli Shang,
Jilin University, China

REVIEWED BY
Kangsheng Ma,
Huazhong Agricultural University, China
Tao Tang,
Hunan Academy of Agricultural Sciences, China
Jianhong Li,
Huazhong Agricultural University, China

*CORRESPONDENCE
Lei Zhang,
zhanglei86@cau.edu.cn
Xue-Gui Wang,
wangxuegui@sicau.edu.cn

†These authors have contributed equally to this work and share first authorship

RECEIVED 06 March 2023
ACCEPTED 14 April 2023
PUBLISHED 05 May 2023

CITATION
Chen H-L, Hasnain A, Cheng Q-H, Xia L-J, Cai Y-H, Hu R, Gong C-W, Liu X-M, Pu J, Zhang L and Wang X-G (2023), Resistance monitoring and mechanism in the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) for chlorantraniliprole from Sichuan Province, China.
Front. Physiol. 14:1180655.
doi: 10.3389/fphys.2023.1180655

Resistance monitoring and mechanism in the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) for chlorantraniliprole from Sichuan Province, China

Hui-Lin Chen[1,2†], Ali Hasnain[1,2,3†], Qing-Hua Cheng[4], Li-Juan Xia[5], Yu-Hao Cai[6], Rong Hu[2], Chang-Wei Gong[1,2], Xue-Mei Liu[1,2], Jian Pu[1,2], Lei Zhang[6*] and Xue-Gui Wang[1,2*†]

[1]State Key Laboratory of Crop Gene Exploration and Utilization in Southwest China, Sichuan Agricultural University, Chengdu, China, [2]College of Agriculture, Sichuan Agricultural University, Chengdu, China, [3]College of Plant Protection, Nanjing Agricultural University, Nanjing, China, [4]Key Laboratory of Integrated Pest Management on Crops in Southwest, Sichuan Academy of Agricultural Sciences, Ministry of Agriculture, Institute of Plant Protection, Chengdu, China, [5]Talent Development Service Center, Sichuan Provincial Department of Agriculture and Rural Affairs, Chengdu, China, [6]Department of Entomology, China Agricultural University, Beijing, China

The fall armyworm, *Spodoptera frugiperda* (Noctuidae: Lepidoptera), is a wide-reaching notorious insect pest of important cereal crops, which has developed resistance to multiple classes of insecticides. It invaded the Sichuan Province of China in 2019. In this study, we performed resistance monitoring of insecticides for 11 field-collected populations from Sichuan, and all the populations were susceptible to emamectin benzoate and chlorpyrifos. The variations in resistance level to indoxacarb (resistance ratio (RR), 9.23–45.53-fold), spinetoram (RR, 4.32–18.05-fold), and chlorantraniliprole (RR, 2.02–10.39-fold) were observed among these populations. To investigate the resistance mechanism of chlorantraniliprole, synergism tests were performed and showed that piperonyl butoxide had a slight synergistic effect on chlorantraniliprole for the QJ-20 population (1.43-fold) in moderate resistance (RR, 10.39-fold) compared with the treatment group without synergist. Furthermore, the expression scanning for resistance-related genes showed that five P450 genes (*CYP6AE43, CYP321A8, CYP305A1, CYP49A1*, and *CYP306A1*) and the ryanodine receptor gene (*Ryr*, chlorantraniliprole target) were overexpressed in the QJ-20 population. These results indicated that the fall armyworm in Sichuan has exhibited diverse susceptibilities to several classes of insecticides, and the overexpression of *Ryr* and several P450 genes may contribute to the development of resistance in *S. frugiperda* to chlorantraniliprole.

KEYWORDS

Spodoptera frugiperda, chlorantraniliprole, cytochrome P450, qRT-PCR, resistance mechanism

1 Introduction

The fall armyworm (FAW), *Spodoptera frugiperda* (Noctuidae: Lepidoptera), is an instinctive pest of the tropical regions of the continents of North and South America, which has rapidly spread over a large area (Sparks et al., 1979). Being polyphagous, it is a notorious pest to more than 350 plant species, including maize, cotton, sugarcane, wheat, sorghum, rice, tomato, beet, and pasture grasses (Montezano et al., 2018). There are two host plant strains of this insect pest, the "corn-biotype" (C-biotype) mostly feeds on maize, sorghum, and cotton, while the "rice-biotype" (R-biotype) mostly targets rice and many other pasture grasses (Dumas et al., 2015).

Among the control methods for agricultural insect pests, chemical control is widely preferable in the farming community across the world. Common pesticides include organophosphorus, carbamate, pyrethroid, and benzoylurea insecticides, which have been supplemented by newer insecticides (indoxacarb, chlorantraniliprole, and emamectin benzoate) in recent years. Owing to the overuse of insecticide, the resistance ratios (RRs) of several FAW populations collected from Mexico and Puerto Rico to permethrin, chlorpyrifos, chlorantraniliprole, and flubendiamide were up to 500-fold in 2016 and were similar for methomyl, deltamethrin, and cypermethrin (Gutierrez et al., 2019). It was reported that the FAW populations invading China carried resistance to the pyrethroids and organophosphates insecticides (Zhang B. et al., 2020). However, most FAW populations in China were found to be susceptible to emamectin benzoate, chlorantraniliprole, spinetoram, indoxacarb, lambda-cyhalothrin, and acephate (Wang et al., 2022). The FAW had no previous record of being resistant to chlorantraniliprole during 2019–2021 (Liu et al., 2022; Wang et al., 2022). However, compared with the 2019 field-collected population, several FAW field populations (collected during 2020) showed slightly reduced toxicity in chlorantraniliprole (Wang et al., 2022), which is a broad spectrum, has high insecticidal efficacy, and is capable of controlling lepidopteran pests. Meanwhile, using less-harmful insecticides inappropriately for a brief period of time in the field would enhance the selection pressure on the target pests and raise the probability of resistance development (Li et al., 2021). The key method by which the FAW develops insecticide resistance is the enhanced detoxifying metabolism of pesticides, which is primarily caused by inadequate pesticide treatment. Previous studies on the FAW revealed that the overexpression of glutathione *S*-transferases (GSTs), cytochrome P450s (P450s), and esterases (ESTs) was mainly involved in developing resistance against pyrethroids, organophosphorus, and carbamate pesticides (Liu et al., 2022). In this regard, the application of different synergists can also be helpful to compare their activities under different conditions as these are crucial in the development of resistance against insecticides (Mohan and Gujar, 2003). There are numerous studies on general P450 functions in pesticide resistance. According to reports, insecticide adaptability is associated with known detoxification families, like P450 monooxygenases (Feyereisen et al., 2012). Comparative genomics analysis displayed that the cytochrome P450 gene family has vastly expanded to 425 members in the FAW, of which 283 genes are specific to the FAW (Gui et al., 2022). A comparison of the expression levels of several cytochrome P450 genes using quantitative real-time PCR (qRT-PCR) in the field-collected populations could also be helpful to understand the nature of the resistance mechanism (Lao et al., 2015). According to certain studies, the overexpression of some P450 genes may be the primary cause of the increase in P450 activity, which can contribute to the FAW's resistance to chlorantraniliprole (Gong et al., 2013; Elzaki et al., 2016). The transcriptomic and genomic studies of the FAW revealed that approximately 117–425 P450 genes in the FAW, including *CYP321A8*, *CYP321A9*, and *CYP321B1*, may play a critical role in insecticide detoxification and, thus, might be involved in pesticide resistance (Zhang D. D. et al., 2020; Chen and Palli, 2021).

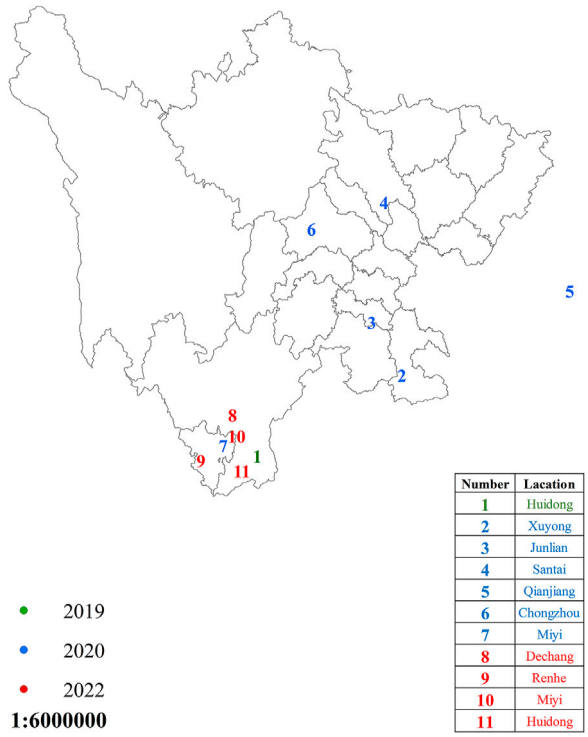


FIGURE 1
Spodoptera frugiperda sampling regions in Sichuan Province, China. The dots in green, blue, and red represent areas where *S. frugiperda* were sampled during the years 2019, 2020, and 2022, respectively.

There is currently limited literature on resistance monitoring in the FAW from China. It is urgently necessary to gain scientific and practical knowledge about resistance monitoring and to comprehend the underlying mechanisms of insecticide resistance in the FAW to design Integrated Pest Management (IPM) or Insect Resistance Management (IRM). In this study, we examined the resistance levels of 11 *S. frugiperda* field populations from Sichuan Province against emamectin benzoate, chlorpyrifos, spinetoram, chlorantraniliprole, and indoxacarb. In addition, we determined the synergistic effects of three different synergists and compared the detoxification enzymes' activities under different treatments. Moreover, we observed the relative expression levels for 26 resistance-related genes (one ryanodine receptor gene (*Ryr)* and 25 P450 genes) in the field population by qRT-PCR. Therefore, the results of our study may be useful in planning

appropriate management strategies for the resistant field populations of the FAW, mainly against chlorantraniliprole, and provide a theoretical basis for exploring the extent of resistance and its mechanism against different insecticides used for controlling *S. frugiperda*.

2 Materials and methods

2.1 Insects

The 11 populations of the FAW were collected from different places in Sichuan Province, China, in 2019–2022 (Figure 1). The larvae were reared on artificially prepared food. The newly hatched larvae were shifted into small boxes containing food, and when they reached the third instar, they were shifted individually into small glass tubes with artificial food to avoid cannibalism, which is highly reported in the FAW (Chapman et al., 2001). The adults were reared on a 10% honey water solution. The newly laid eggs and pupae were treated with sodium hypochlorite disinfection solution (0.2–0.3%) to avoid any contamination (Wang et al., 2018). All the developmental stages were kept under the controlled conditions of 70–80% relative humidity (RH), 26 ± 1 °C temperature, and a 16:8 h (L:D) photoperiod.

2.2 Insecticides and chemicals

Five different insecticides were used in this research, including 74% emamectin benzoate (Nanjing Red Sun Co., Ltd., Gaochun, China), 97% chlorpyrifos (Hubei Sharonda Co., Ltd., Jingzhou, China), 95% chlorantraniliprole (Corteva Agriscience, Indianapolis, United States), 95% indoxacarb (Jiangsu Flag Chemical Industry Co., Ltd., Nanjing, China), and 99% spinetoram (Wuhan Xiyu Biotechnology Co., Ltd., Hubei, China). The synergists included diethyl maleate (DEM), piperonyl butoxide (PBO), and triphenyl phosphate (TPP), which were bought from Aladdin Shanghai Co., Ltd. (Nanjing, China). α-Naphthyl acetate (α-NA), 1-chloro-2,4-dinitrobenzene (CDNB), and fast blue salt B were purchased from Chengdu Ai Keda Chemical Technology Co., Ltd. (Chengdu, China). Bovine serum albumin (BSA), NADPH, DTT, PMSF, and reduced glutathione (GSH) were purchased from Beijing Solarbio Science and Technology Co., Ltd. (Beijing, China). The Coomassie brilliant blue G 250, EDTA Na_2, and sodium dodecyl sulfate (SDS) were bought from Chengdu Kelong Chemical Reagent Co., Ltd. (Chengdu, China).

2.3 Bioassays

For the bioassays, the topical application method was used for 2 days after the third instar larva molting (Wang et al., 2021). There were three replications for each concentration with 12 larvae per replication. The stock solutions for all insecticides were prepared using acetone or dimethyl sulfoxide and then serially diluted to make five or six concentrations of each, to cover mortality from 0 to 100%. A 50 μL microsyringe (Hamilton company, Reno, NV) coupled with a microapplicator (PB600-1 Repeating Dispenser, Hamilton Company) was used to apply 1 μL of each prepared concentration over the dorsal side of the frontal thorax of the third instar larva, as reported in previous reports (Brewer and Trumble, 1989; Mccaffery et al., 1991). We selected the 23rd instar larvae in the same condition to weigh and finally figure out 0.006 g/larva. One microliter of acetone per larva was applied or dimethyl sulfoxide was used for the control treatment. The treated larvae were transferred in 12-compartment culture plates, containing a small quantity of food. Each culture plate was considered one replication. The mortality was assessed 24 h after treatment. If they showed severe intoxication symptoms, including feeding cessation, severe growth inhibition or slow movement, and twitching when touched with a small brush, the larvae were considered dead.

The toxicity of synergists including DEM, TPP, and PBO with chlorantraniliprole was determined for the QJ-20 population using topical application, as previously mentioned. All these synergists were dissolved in acetone at a concentration of 1000 mg/L (Sun et al., 2018). After this, a 1.0 μL droplet was applied over the thoracic dorsum of each third instar larva using a microsyringe coupled with a microapplicator (PB600-1 Repeating Dispenser, Hamilton Company). After an hour of synergist application, the different concentrations of insecticide were applied over larvae, as described previously, and mortality was observed 24 h after treatment.

2.4 Enzymatic assays

The activities of CarEs, GSTs, and P450s were determined for the three experimental groups including the QJ-20 population, the QJ-20 population treated only with a synergist, and the QJ-20 population treated with LD_{25} of chlorantraniliprole plus a synergist (TPP or DEM or PBO) (Liu et al., 2022). The fifth instar larvae were selected, and their midguts were dissected over dry ice packs (Wang et al., 2018). These midguts were first put in 1.15% ice-cold KCl solution to isolate the excessive fatty material and were then transferred to a 1.5 mL centrifuge tube, labeled, and stored at −80 °C as quickly as possible.

The determination of CarEs activity was made according to Van Asperen (1962). First, 1 mL of buffer solution (0.04 mol/L phosphate buffer, pH 7.0) was added in a 5 mL glass homogenizer to homogenize the midguts on ice, and then, centrifugation (10000 ×*g* for 10 min at 4 °C) was performed, using a 5417R centrifuge (Eppendorf, Germany). Later, the supernatant was transferred to a clean tube, considered an enzyme source, and then, 1.8 mL of α-NA solution (having 3×10^{-4} mol/L physostigmine), 0.45 mL of buffer solution (0.04 mol/L, pH 7.0), and 50 μL of enzyme source were put in the 4 mL centrifuge tube and incubated in a water bath for 15 min at 30 °C. Next, 0.9 mL of staining solution (with 0.2 g of fast blue salt B in 20 mL distilled water plus 50 mL of 5% SDS) was added to stop the reaction. The changes in absorbance values were recorded (after 5 min of calibration with blank) at a wavelength of 600 nm, using the UV 2000 spectrophotometer (Unic Instruments Incorporated, Shanghai, China). The CarEs activity was expressed as millimoles of naphthol per minute per milligram of protein and calculated from the production of α-naphthol by referring to an experimentally determined standard curve.

10.3389/fphys.2023.1180655

TABLE 1 Resistance levels of the field-collected populations of fall armyworm *Spodoptera frugiperda* to several insecticides in the topical bioassay method.

Insecticide	Population	Slope ±SE	LD_{50} (μg.g $^{-1}$)	95% CI (μg/g)[a]	χ^2 (df)[b]	p*	RR
Chlorantraniliprole	SUS **	1.139 ± 0.234	0.410	0.229–0.602	15.42 (18)	0.6320	1.00
	HD-19	1.335 ± 0.329	0.830	0.137–1.708	4.52 (10)	0.9208	2.02
	XY-20	1.503 ± 0.269	1.651	0.695–2.711	6.35 (13)	0.9324	4.03
	JL-20	1.506 ± 0.285	1.408	0.535–2.383	7.66 (13)	0.8650	3.43
	ST-20	1.813 ± 0.337	1.807	0.901–2.732	6.94 (10)	0.7310	4.41
	QJ-20	1.768 ± 0.275	4.260	2.755–5.906	7.02 (10)	0.7236	10.39
	CZ-20	1.609 ± 0.288	1.607	0.708–2.586	6.23 (13)	0.9374	3.92
	MIYI-20	1.780 ± 0.309	1.770	0.886–2.702	5.52 (13)	0.9619	4.32
	DC-22	0.813 ± 0.157	1.838	0.623–3.376	11.31 (16)	0.7313	4.48
	HD-22	0.837 ± 0.157	1.910	0.689–3.438	8.22 (16)	0.8376	4.66
	MIYI-22	0.818 ± 0.154	2.812	1.161–4.853	10.79 (16)	0.9658	6.86
	RH-22	1.518 ± 0.201	2.536	1.546–3.662	16.12 (16)	0.8243	6.19
Emamectin benzoate	SUS **	1.830 ± 0.273	0.355	0.275–0.465	12.20 (18)	0.8370	1.00
	HD-19	2.145 ± 0.348	0.242	0.148–0.325	7.11 (16)	0.9711	0.68
	XY-20	3.810 ± 0.507	0.760	0.635–0.876	9.27 (13)	0.7522	2.14
	JL-20	3.038 ± 0.472	0.646	0.496–0.774	9.20 (13)	0.7576	1.82
	ST-20	4.073 ± 0.547	0.688	0.572–0.793	6.59 (13)	0.9225	1.94
	QJ-20	3.528 ± 0.534	0.574	0.446–0.683	8.49 (13)	0.8103	1.62
	CZ-20	3.457 ± 0.481	0.781	0.616–0.932	15.39 (13)	0.2836	2.20
	MIYI-20	2.919 ± 0.474	0.603	0.448–0.732	12.19 (13)	0.5121	1.70
	DC-22	1.016 ± 0.180	0.327	0.162–0.528	8.83 (13)	0.9447	0.92
	HD-22	1.397 ± 0.117	0.543	0.36–0.777	6.29 (13)	0.9104	1.53
	MIYI-22	1.368 ± 0.183	0.886	0.614–1.275	8.47 (13)	0.9557	2.44
	RH-22	1.036 ± 0.168	0.659	0.392–1.028	7.44 (13)	0.9432	1.86
Chlorpyrifos	RH-22(SUS *)	4.604 ± 0.544	106.680	94.958–122.008	8.38 (16)	0.9367	1.00
	HD-19	3.429 ± 0.342	113.680	99.719–130.333	7.94 (19)	0.9872	1.07
	XY-20	5.111 ± 0.591	122.309	108.919–136.648	3.71 (16)	0.9993	1.15
	JL-20	4.587 ± 0.524	149.808	131.630–169.454	9.47 (16)	0.8928	1.40
	ST-20	3.027 ± 0.341	135.578	115.128–159.575	7.93 (16)	0.9509	1.27
	QJ-20	4.582 ± 0.590	108.769	95.778–125.099	8.50 (13)	0.8095	1.02
	CZ-20	4.175 ± 0.484	113.701	100.362–131.351	8.94 (16)	0.9158	1.07
	MIYI-20	1.77 ± 0.175	122.074	71.764–233.539	4.27 (16)	0.9141	1.14
	DC-22	0.852 ± 0.233	150.399	88.847–460.269	1.86 (13)	0.9120	1.41
	HD-22	1.722 ± 0.230	179.766	95.600–1109.614	1.35 (13)	0.7125	1.69
	MIYI-22	1.047 ± 0.258	339.518	186.341–1455.745	6.69 (13)	0.7053	3.18
Indoxacarb	SUS **	2.147 ± 0.300	0.238	0.186–0.304	11.22 (18)	0.9170	1.00
	HD-19	2.090 ± 0.300	4.292	2.967–5.748	6.64 (10)	0.7589	18.03
	XY-20	2.155 ± 0.325	3.221	2.159–4.364	8.51 (10)	0.5791	13.53

(Continued on following page)

TABLE 1 (*Continued*) Resistance levels of the field-collected populations of fall armyworm *Spodoptera frugiperda* to several insecticides in the topical bioassay method.

Insecticide	Population	Slope ±SE	LD_{50} (μg.g $^{-1}$)	95% CI (μg/g)[a]	χ^2 (df)[b]	p*	RR
	JL-20	1.889 ± 0.250	5.333	3.684–7.144	10.81 (13)	0.6267	22.41
	ST-20	2.000 ± 0.291	5.162	3.606–6.948	5.20 (10)	0.8774	21.69
	QJ-20	2.005 ± 0.288	5.492	3.873–7.316	8.68 (10)	0.5627	23.08
	CZ-20	1.608 ± 0.266	3.921	2.365–5.604	5.90 (10)	0.8235	16.47
	DC-22	1.965 ± 0.239	10.837	8.388–13.813	9.92 (16)	0.9535	45.53
	HD-22	1.174 ± 0.177	2.197	1.177–3.372	14.31 (16)	0.8529	9.23
	MIYI-22	1.111 ± 0.165	3.521	2.036–5.280	14.40 (16)	0.8091	14.79
	RH-22	1.506 ± 0.216	3.050	1.990–4.256	10.12 (13)	0.8220	12.82
Spinetoram	SUS **	2.162 ± 0.261	0.518	0.416–0.651	12.20 (22)	0.7950	1.00
	DC-22	1.112 ± 0.172	2.240	1.061–3.634	16.48 (16)	0.9006	4.32
	HD-22	1.181 ± 0.166	9.095	5.782–14.005	18.69 (16)	0.8955	17.56
	MIYI-22	1.483 ± 0.185	5.870	4.129–7.957	11.75 (16)	0.9088	11.33
	RH-22	1.324 ± 0.175	9.349	6.617–13.010	14.91 (16)	0.9502	18.05

The median lethal dose (LD_{50}) is expressed as micrograms of active ingredient per gram of insect.
SUS*: The most susceptible population among the field populations was considered the susceptible baseline.
SUS**: The susceptibility baseline data were referred from the work of Wang et al., 2022.
[a]RR, resistance ratio, calculated as LD_{50} of field population/LD_{50} susceptibility baseline.
[b]Chi-square value (χ^2) and degrees of freedom (df) as calculated using Probit analysis (Polo Plus 2.0).

TABLE 2 Synergism of DEM, TPP, and PBO with chlorantraniliprole in the QJ-20 population of *S. frugiperda*.

Treatment	Slope ±SE	LD_{50} (μg.g $^{-1}$)[a] (95% CI)	χ^2 (df)	p*	SR[b]
Chlorantraniliprole	2.240 ± 0.320	0.716 (0.533–0.933)	15.09 (10)	0.7236	1
+ DEM	2.678 ± 0.349	0.667 (0.533–0.800)	10.56 (13)	0.6467	1.08
+ TPP	2.720 ± 0.356	0.767 (0.633–0.917)	12.87 (13)	0.4579	0.93
+ PBO	2.529 ± 0.363	0.500 (0.367–0.617)	12.08 (13)	0.5210	1.43

[a]The median lethal dose (LD_{50}) expressed as micrograms of active ingredient per gram of insect.
[b]SR (synergist ratio) = LD_{50} of synergist plus insecticide/LD_{50} of the insecticide.
*Goodness-of-fit test is significant at $p > 0.05$.

The GST activity was determined according to Habig and Jakoby (1981), for which the midguts of the fifth instar larvae were first homogenized over ice using 1 mL of buffer (0.1 mol/L phosphate buffer, having 1 mmol/L EDTA, pH 6.5), and the supernatant was transferred to the new tube after centrifugation at 10800 ×g for 10 min at 4 °C, the supernatant was considered as an enzyme source. Next, 90 μL of CDNB (15 mmol/L), 90 μL of reduced GSH (30 mmol/L), 2470 μL of phosphate buffer (0.1 mol/L, pH 6.5), and 50 μL of enzyme source were mixed in a 4 mL tube. The OD value was recorded for 2 min at 340 nm and calculated as $\triangle A^{340}$/min.

The method of Rose et al. (1995) was used to determine the activity of cytochrome P450s ethoxycoumarin *O*-deethylase (ECOD), with some modifications. The enzyme source was the supernatant of the homogenized midguts of the fifth instar larvae, obtained by adding 1 mL of buffer (0.1 mol/L phosphate buffer, pH 7.5, having 20% glycerol, 0.1 mmol/L DTT, 0.1 mmol/L EDTA, and 0.4 mmol/L PMSF), in a 5 mL glass homogenizer over the ice, and centrifugation was conducted at 10000 ×*g* for 15 min at 4 °C. Later, 90 μL of enzyme source was mixed with 100 μL of 2 mmol/L 4-nitroanisole and put into the culture plate and incubated at 27 °C for 3 min in the water bath. After this, 10 μL of 9.6 mmol/L NADPH was added to initiate the reaction, and the absorbance values at 405 nm for 2 min with 20 s intervals at 27 °C were determined using the microplate reader (Model 680 Microplate Reader, Bio-Rad).

The method of Bradford (1976) was used to determine the total protein content, considering BSA as a standard. A test solution of 3 mL was prepared by adding a certain prepared concentration of BSA with 2.5 mL of Coomassie Brilliant blue to measure the OD value at a wavelength of 595 nm using the UV 2000 Spectrophotometer (Unic Instruments Incorporated, Shanghai, China) to get the standard curve.

TABLE 3 Detoxification enzyme activities of the QJ-20 strain.

Treatment	CarE activity, mmol/min.mg pro	IR[a]	GST activity, mmol/min.mg pro	IR[a]	Cytochrome P450 ECOD activity, nmol/min.mg pro	IR[a]
QJ-20	0.519 ± 0.13 a		0.166 ± 0.01 a		0.629 ± 0.09 a	
QJ-20 treated with a synergist	0.282 ± 0.04 a	0.46	0.106 ± 0.02 a	0.36	0.273 ± 0.03 b	0.57
QJ-20 treated with a synergist plus LD_{25} dosage of chlorantraniliprole	0.426 ± 0.06 a	0.18	0.157 ± 0.02 a	0.05	0.439 ± 0.04 ab	0.30
	F3, 8 = 1.984, $p < 0.05$		F3, 8 = 3.118, p < 0.05		F3, 8 = 8.825, p < 0.05	

The synergists that used CarE, GST, and P450 activities in the populations were TPP, DEM, and PBO, respectively.
[a]Inhibition ratio (IR) presents the detoxification enzyme activities of QJ-20 treated with a synergist or plus LD_{25} dosage of chlorantraniliprole/the detoxification enzyme activities of QJ-20 treated without a synergist.
[b]Different letters indicate significant differences ($p < 0.05$) using ANOVA followed by Tukey's test ($p < 0.05$) with the SPSS version 17.0 software package (IBM).

2.5 qRT-PCR

A total of 26 genes were chosen from Giraudo et al. (2014) and the NCBI database to determine which genes contribute to the development of the FAW's resistance to chlorantraniliprole, and we designed their corresponding primers using Primer-BLAST on the NCBI website (Supplementary Table S1). The midgut of the fifth instar larvae was obtained by dissection on dry ice packs (Wang et al., 2018). RNA was examined, and cDNA synthesis and sequencing of genes were carried out. Due to the lack of a susceptible strain, the relative expressions of all the genes were later discovered in the QJ-20 population, a moderately resistant population (among the 11 populations), using the comparatively sensitive population (HD-19) of *S. frugiperda* as the control. Six higher-expression genes were screened out and were further determined in the six field populations collected in 2020 using qRT-PCR.

The fifth instar larvae were selected for the extraction of RNA using RNase Easy Water (Vazyme Biotech Ltd., China) according to the manufacturer's instructions, and later, the concentrations of RNA were measured using the Micro-Drop ultra microspectrophotometer (Baoyide Scientific Ltd., Shanghai, China). The Novoscript® plus all-in-one reagent kit (Novoprotein Scientific Inc., Shanghai, China) was used to prepare cDNA from 1 μg total RNA. The Applied Biosystems 7500 RT-PCR system (Applied Biosystems, Foster City, CA, United States) was used to perform qRT-PCR using the 2 × NovoStart® SYBRqPCR SuperMix Plus kit (Novoprotein Scientific Inc.). The total volume of the reaction mixture was 20 μL with 10 μL of 2 ×SuperMix, 1.5 μL of each primer, 1 μL of cDNA template, and 6 μL of RNase-free water. The protocol program was as follows: 95 °C for 1 min, followed by 40 cycles of 95°C for 20 s, 60°C for 20 s, and 72°C for 30 s. The reference gene was the elongation factor 1 alpha (*EF1α*) for *S. frugiperda* (Shu et al., 2020), and the experiment was performed for three technical replicates with three independent biological replicates. The $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) was used for calculating the relative expression levels of target genes.

2.6 Data analysis

The POLO 2.0 program (Leora Software, www.leorasoftware.com) was used to calculate the slope, LD_{50}, 95% confidence intervals (CI), and chi-square (χ^2) value of each insecticide 24 h after treatment (Wang et al., 2018). RRs were calculated using the susceptible baseline values for indoxacarb, emamectin benzoate, chlorantraniliprole, and spinetoram as the factor divisor from the result by Wang et al. (2022), while for chlorpyrifos, the most susceptible population was selected as the reference baseline. The resistance levels for insecticides were classified as susceptible (RR < 5.0), low level of resistance (5.0 ≤ RR < 10.0), moderate level of resistance (10.0 ≤ RR < 100.0), and high level of resistance (RR ≥ 100.0) (Lu et al., 2017; Zhang et al., 2017). The activities of CarEs, GSTs, and P450s along with the relative normalized expression of resistance-related genes were expressed as the mean ± standard error (SE), compared using the analysis of variance (ANOVA) followed by Tukey's test ($p < 0.05$) with the SPSS version 17.0 software package (IBM), while the graphs were prepared using the mean with SE in the Sigma Plot 10.0 software. Correlation analysis between the relative expressions of *Ryr*, P450 genes, and chlorantraniliprole resistance in the field populations of *S. frugiperda* in 2020 was calculated according to the Pearson method using the SPSS Statistics software package (Lu et al., 2017). A *p*-value of $p < 0.05$ or $p < 0.01$ was considered to be statistically significant or extremely significant.

3 Results

3.1 Susceptibility status and variations in field populations

The toxicities of five insecticides toward the field populations are given in Table 1. Field populations displayed low levels of resistance to chlorpyrifos. For chlorpyrifos, their LD_{50} values ranged from 106.680 μg/g (RH-22) to 339.518 μg/g (MIYI-22). For chlorantraniliprole, there was a deviation among the field populations whose RR values ranged from 2.02-fold (HD-19) to 10.39-fold (QJ-20), and their resistance levels ranged from susceptible to moderate level, and the QJ-20 population had the highest LD_{50} value (4.260 μg/g). For spinetoram, their RR values were ranging from 4.32-fold (DC-22) to 18.05-fold (RH-22). The LD_{50} values of all the different populations to emamectin benzoate were distributed between 0.242 μg/g (HD-19) and 0.886 μg/g (MIYI-

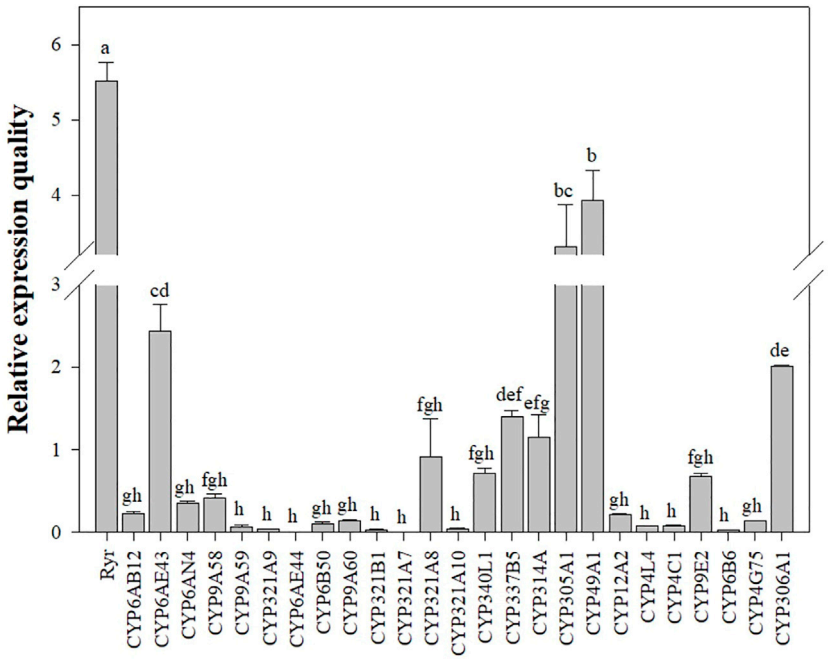


FIGURE 2
Relative expression levels of the *CYP450* and *Ryr* gene in the QJ-20 population of FAW. The relative expression levels were compared using one-way ANOVA followed by Tukey's test, at 0.05 level of significance, in SPSS version 17.0 software (IBM). Letters above the bars indicate significant differences ($p < 0.05$), and means followed by the same letters did not differ significantly ($p > 0.05$).

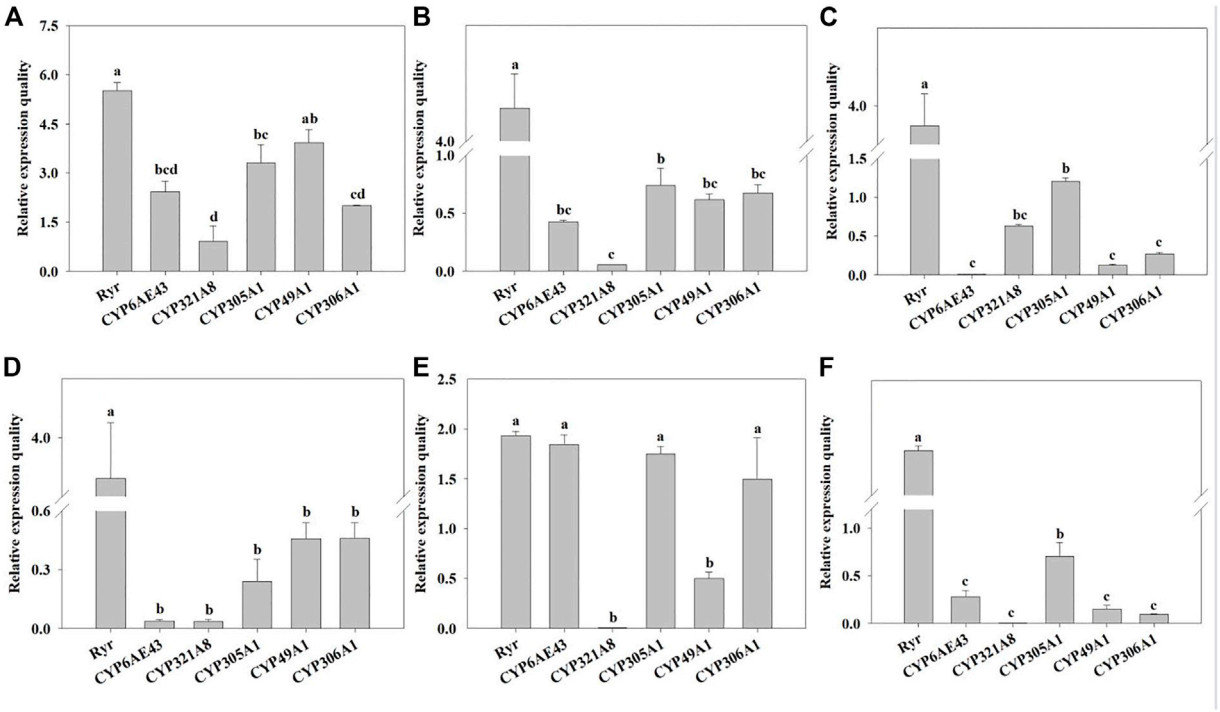


FIGURE 3
Relative normalized expressions of six resistance-related genes in all field populations. **(A)** QJ-20 field population, **(B)** XY-20 field population, **(C)** JL-20 field population, **(D)** ST-20 field population, **(E)** MIYI-20 field population, and **(F)** CZ-20 field population. Letters above the bars indicate significant differences ($p < 0.05$), and means followed by the same letters did not differ significantly ($p > 0.05$) according to Tukey's test with SPSS version 17.0 software package (IBM).

TABLE 4 Correlation analysis between the relative expressions of *Ryr*, P450 genes, and chlorantraniliprole resistance in the field populations of *S. frugiperda* in 2020.

	Resistance level	*Ryr*	*CYP6AE43*	*CYP321A8*	*CYP305A1*	*CYP49A1*	*CYP306A1*
Resistance level	1	0.810	0.792	0.245	0.857*	0.895*	0.770
Ryr	0.810	1	0.362	−0.143	0.557	0.534	0.401
CYP6AE43	0.792	0.362	1	0.658	0.912*	0.972**	0.963**
CYP321A8	0.245	−0.143	0.658	1	0.663	0.581	0.668
CYP305A1	0.857*	0.557	0.912*	0.663	1	0.930**	0.864*
CYP49A1	0.895*	0.534	0.972**	0.581	0.930**	1	0.967**
CYP306A1	0.770	0.401	0.963**	0.668	0.864*	0.967**	1

Pearson's correlation, the correlation coefficient > −1 and <1; the closer it is to −1 or 1, the stronger the correlation between two variables, and the closer it is to 0, the weaker the correlation. *$p < 0.05$; **$p < 0.01$.

22), and their RR values were ranging from 0.68-fold to 2.44-fold. According to insecticides of the sensitive baseline, the resistance of spinetoram was monitored in 2022, and only four populations were calculated. The LD_{50} values ranged from 2.197 μg/g to 10.837 μg/g with an RR of 9.23-fold (HD-22) to 45.53-fold (DC-22).

3.2 Synergism of TPP, PBO, and DEM

The effects of TPP, PBO, and DEM on the toxicity of chlorantraniliprole toward the QJ-20 population (RR = 10.39-fold) are given in Table 2. PBO had the highest synergistic of chlorantraniliprole for the QJ-20 population in moderate resistance (RR = 10.39-fold) compared with the treatment group without a synergist. The synergists PBO and DEM showed a slight increase in the toxicity of chlorantraniliprole, among which PBO enhanced the efficacy up to 1.43-fold and its LD_{50} treated by PBO decreased from 0.716 μg/g to 0.500 μg/g, while for DEM (LD_{50} = 0.667 μg/g) and TPP (LD_{50} = 0.767 μg/g), the synergistic ratios were only 1.08- and 0.93-fold, respectively.

3.3 Detoxification enzyme activities

To evaluate the role of these three detoxification enzymes in the development of resistance in *S. frugiperda* against chlorantraniliprole, the activities of CarEs, GSTs, and P450s were determined. As shown in Table 3, there was no significant difference among the activities of CarEs for all treatments (from 0.282 to 0.519 mmol/min. mg pro) ($p > 0.05$). Similarly, no significant difference was observed among the activities of GSTs with the values of 0.106–0.166 mmol/min. mg pro, respectively ($p > 0.05$). Meanwhile, for cytochrome P450s, there was significant difference in the activities between the QJ-20 population (0.629 nmol/min. mg pro) and PBO synergist application treatment (0.273 nmol/min. mg pro) ($p < 0.05$), but no significant difference with the treatment of PBO plus LD_{25} of chlorantraniliprole (0.439 nmol/min. mg pro) ($p > 0.05$).

3.4 Relative expressions of *Ryr* and *P450* genes

The mRNA expression levels of *Ryr* and P450 genes were detected in the QJ-20 population by qRT-PCR (Figure 2). Among these genes, six showed variation with the maximum relative expression for the *Ryr* gene (5.5-fold), followed by *CYP49A1*, *CYP305A1*, *CYP6AE43*, *CYP306A1*, and *CYP321A8*, with values of 3.9-, 3.3-, 2.4-, 2-, and 0.9-fold, respectively (Figure 2). Meanwhile, the relative expressions of these five *CYP450* genes and the *Ryr* gene were also calculated in the tested field populations, in which *CYP6AE43*, *CYP321A8*, *CYP305A1*, *CYP49A1*, and *CYP306A1* showed relative expression values ranging from 0.009- to 2.4-fold, 0.005- to 0.9-fold, 0.24- to 3.3-fold, 0.12- to 3.9-fold, and 0.9- to 2.0-fold, respectively (Figure 3).

Specifically, the *Ryr* gene showed significantly high relative expression levels, ranging from 1.93- to 5.5-fold, in all field populations ($p < 0.05$), compared with hd-19. Among them, QJ-20 showed the highest expression level (Figure 3). In the populations of XY-20, CZ-20, and JL-20, *Ryr* showed the highest relative expression, followed by *CYP305A1*, with the least significant relative expression of *CYP321A8* in XY-20 (Figure 3B) and CZ-20 (Figure 3F), but in the JL-20 population, the *CYP6AE43* expressed the least significant relative expression ($p < 0.05$) (Figure 3C). For the MIYI-20 population, *Ryr* showed the highest relative expression, followed by *CYP6AE43*, while *CYP321A8* expressed the lowest relative expression ($p < 0.05$) (Figure 3E). Moreover, in the ST-20 population, *Ryr* showed significant overexpression while *CYP321A8* expressed the lowest relative expression ($p < 0.05$) (Figure 3D).

To further verify the relationship between resistance to chlorantraniliprole and the relative gene expressions, we analyzed their correlation for six field populations in 2020. The *Ryr* expression had a correlation with chlorantraniliprole resistance but in a correlation value of 0.810 ($p > 0.05$), and the correlation coefficient of *CYP49A1* and *CYP305A1* reached 0.895 and 0.857 ($p < 0.05$), respectively, while for others, it was <0.8 ($p > 0.05$) (Table 4).

4 Discussion

Spodoptera frugiperda is an invasive pest, not only does the unscientific use of chemical insecticides in the field result in the outbreak of resistance but invasive pests themselves may also carry resistance to certain insecticides (Gao et al., 2013). In our study, most tested FAW populations were susceptible to emamectin benzoate, which was consistent with previous reports (Liu et al., 2022; Wang et al., 2022). However, indoxacarb and spinetoram showed medium toxicity and posed a high risk for the evolution of resistance, and the sensitivity to chlorpyrifos had dropped substantially. It might be that those insecticides had still been used in the field, and *S. frugiperda* was also exposed to such agents, resulting in a large LD_{50} value in the field population (Zhang et al., 2021; Zhang et al., 2022). Among them, three field populations of the FAW in Sichuan were in low or moderate resistance to chlorantraniliprole; the resistance level was also increasing with time, and there were potential resistance risks, as reported in previous reports (Wang et al., 2022). This might be due to using unreasonable pesticides, the field selection of the pest, or its migration from other places with high resistance levels.

The resistance mechanism of insects toward insecticides is comprised of two main aspects, including the detoxification enzyme activity upregulation and the target-induced decreased sensitivity (Zhang B. et al., 2020). Low-dose chlorantraniliprole treatment can increase resistance to the same class of insecticides, which suggests that this is the consequence of the induction of particular detoxification enzymes (Riaz et al., 2009). An increase in the detoxification enzymes' activities, such as CarEs, GSTs, and P450s, is one of the important reasons for the development of resistance toward insecticides (Pedersen et al., 2019), among which enhanced P450s activity plays an essential role (Liu et al., 2006; Mao et al., 2019). Our results also prove that the synergist PBO significantly increased the efficacy of chlorantraniliprole by reducing the P450 enzyme activities in our synergist experiment, and analogous results were also reported by Xu et al. (2019) and Tan et al. (2022), who suggested that P450 might play a primary role in the development of resistance toward chlorantraniliprole. Meanwhile, our result regarding enzyme activity determination also shows that the increase in P450 activity plays an important role in the resistance of *S. frugiperda* to chlorantraniliprole, which is consistent with the previous reports by Zhang D. D. et al. (2020), who reported that the increased activity of P450s enzymes might also be secondarily involved in the resistance mechanism of *S. frugiperda* against chlorantraniliprole.

The overexpression of some P450 genes may be the main reason for the increase in P450 activity, which can referee the resistance or induction of *S. frugiperda* to chlorantraniliprole (Gong et al., 2013; Elzaki et al., 2016). Our results show that four P450 genes *CYP49A1*, *CYP305A1, CYP6AE43,* and *CYP306A1* were upregulated in the chlorantraniliprole-resistant populations and when exposed to the sublethal concentrations of chlorantraniliprole, *CYP6AE43* in *S. frugiperda* was also upregulated (Xiao and Lu, 2022), while the downregulation of *CYP6AE43* would enhance the susceptibility of *S. frugiperda* to some insecticides (Zhang B. et al., 2020). However, the association analysis between gene expression and chlorantraniliprole resistance revealed that *CYP305A* and *CYP49A1* had the highest correlation with chlorantraniliprole resistance, reaching a significant level. Some studies have reported that pesticides, such as azadirachtin (Li et al., 2015) and phoxim (Yu et al., 2022), could induce the upregulation of *CYP305A* and *CYP49A1* expression. In addition to P450 genes, we also found that the *Ryr* expression was highly correlated with resistance to chlorantraniliprole, which was in support of reports published by Sun et al. (2012) and Qin et al. (2018), who considered that the diamide-resistant populations have higher mRNA expression levels of the *Ryr* gene in *Plutella xylostella*. However, it has also been reported that chlorantraniliprole-resistant populations have lower expression levels of the *Ryr* gene in *P. xylostella* and *Chilo suppressalis* (Gong et al., 2014; Wei et al., 2019). Thus, to investigate whether the aforementioned genes are associated with chlorantraniliprole resistance, in the future, we would design a suitable dsRNA for executing RNA interference (RNAi) or RNA knockout assays to fully elaborate their functions in the development of resistance toward chlorantraniliprole.

Our results show that the resistance of the field populations of *S. frugiperda* in Sichuan toward emamectin benzoate and chlorpyrifos was still at a sensitive level. However, they exhibited a low or moderate level of resistance to chlorantraniliprole. The synergist and detoxification enzyme activity experiment showed that the resistance of *S. frugiperda* against chlorantraniliprole might cause the upregulated activities of the detoxification enzymes. The result of qRT-PCR and the association analysis between gene expression and chlorantraniliprole resistance revealed that the upregulation of *CYP305A1*, *CYP49A1*, and *Ryr* might be related to the upregulated activities of the detoxification enzymes and the induction of particular detoxification enzymes. However, further investigation is imperative to provide functional evidence for a catalytic interaction of chlorantraniliprole, which could include the expression of the corresponding genes *in vivo* or *in vitro* and functional verification by molecular technologies such as RNAi or CRISP-cas9, and so on. Nonetheless, our results provide a foundation for subsequent efforts to control *S. frugiperda* with integrated pest management strategies.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding authors.

Author contributions

H-LC and AH: conceptualization, writing original draft, investigation, data curation, and formal analysis. Q-HC: project administration and resources. L-JX: funding acquisition and resources. Y-HC, RH, and C-WG: investigation. X-ML: supervision, funding, and acquisition. JP: supervision and project administration. LZ: formal analysis and investigation. X-GW:

conceptualization, methodology, review and editing, supervision, visualization, and funding acquisition.

Acknowledgments

The authors gratefully acknowledge the financial support of the Science and Technology Program of Sichuan, China (2022YFSY0034).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2023.1180655/full#supplementary-material

References

Asperen, K. V. (1962). A study of housefly esterase by means of a sensitive colorimetric method. *Insect Physiol.* 8, 401–414. doi:10.1016/0022-1910(62)90074-4

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254. doi:10.1006/abio.1976.9999

Brewer, M. J., and Trumble, J. T. (1989). Field monitoring for insecticide resistance in beet armyworm (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 82, 1520–1526. doi:10.1093/jee/82.6.1520

Chapman, J. W., Williams, T., Escribano, A., Caballero, P., Cave, R. D., and Goulson, D. E. (2001). Age-related cannibalism and horizontal transmission of a nuclear polyhedrosis virus in larval Spodoptera frugiperda. *Ecol. Entomol.* 24, 268–275. doi:10.1046/j.1365-2311.1999.00224.x

Chen, X., and Palli, S. R. (2021). Transgenic overexpression of P450 genes confers deltamethrin resistance in the fall armyworm, Spodoptera frugiperda. *Spodoptera frugiperda J. Pest Sci.* 95, 1197–1205. doi:10.1007/s10340-021-01452-6

Dumas, P., Legeai, F., Lemaitre, C., Scaon, E., Orsucci, M., Labadie, K., et al. (2015). Spodoptera frugiperda (Lepidoptera: Noctuidae) host-plant variants: Two host strains or two distinct species? *Genetica* 143, 305–316. doi:10.1007/s10709-015-9829-2

Elzaki, M. E. A., Zhang, W., Feng, A., Qiou, X., Zhao, W., and Han, Z. (2016). Constitutive overexpression of cytochrome P450 associated with imidacloprid resistance in Laodelphax striatellus (Fallén). *Pest Manag. Sci.* 72 (5), 1051–1058. doi:10.1002/ps.4155

Feyereisen, R., Dermauw, W., Wybouw, N., Rombauts, S., Menten, B., Vontas, J., et al. (2012). A link between host plant adaptation and pesticide resistance in the polyphagous spider mite Tetranychus urticae. *Tetranychus urticae P. Natl. Acad. Sci. USA.* 110 (2), E113–E122. doi:10.1073/pnas.1213214110

Gao, C., Yao, R., Zhang, Z., Wu, M., Zhang, S., and Su, J. (2013). Susceptibility baseline and chlorantraniliprole resistance monitoring in Chilo suppressalis (Lepidoptera: Pyralidae). *J. Econ. Entomol.* 106, 2190–2194. doi:10.1603/ec13058

Giraudo, M., Hilliou, F., Fricaux, T., Audant, P., Feyereisen, R., and Le Goff, G. (2015). Cytochrome P450s from the fall armyworm (Spodoptera frugiperda): Responses to plant allelochemicals and pesticides. *Insect Mol. Biol.* 24 (1), 115–128. doi:10.1111/imb.12140

Gong, W., Yan, H. H., Gao, L., Guo, Y. Y., and Xue, C. B. (2014). Chlorantraniliprole resistance in the diamondback moth (Lepidoptera: Plutellidae). *J. Econ. Entomol.* 107, 806–814. doi:10.1603/ec13456

Gong, Y., Li, T., Zhang, L., Gao, X., and Liu, N. (2013). Permethrin induction of multiple cytochrome P450 genes in insecticide resistant mosquitoes, *Culex quinquefasciatus*. *Int. J. Biol. Sci.* 9 (9), 863–871. doi:10.7150/ijbs.6744

Gui, F., Lan, T., Zhao, Y., Guo, W., Dong, Y., Fang, D., et al. (2022). Genomic and transcriptomic analysis unveils population evolution and development of pesticide resistance in fall armyworm Spodoptera frugiperda. *Protein Cell* 13 (7), 513–531. doi:10.1007/s13238-020-00795-7

Gutierrez, M. R., Mota, S. D., Blanco, C. A., Whalon, M. E., Teran, S. H., Rodriguez, J. C., et al. (2019). Field-evolved resistance of the fall armyworm (Lepidoptera: Noctuidae) to synthetic insecticides in Puerto Rico and Mexico. *J. Econ. Entomol.* 112, 792–802. doi:10.1093/jee/toy372

Habig, W. H., and Jakoby, W. B. (1981). Assays for differentiation of glutathione S-transferases. *Method. Enzymol.* 77, 398–405. doi:10.1016/s0076-6879(81)77053-8

Lao, S. H., Huang, X. H., Huang, H. J., Liu, C. W., Zhang, C. X., and Bao, Y. Y. (2015). Genomic and transcriptomic insights into the cytochrome P450 monooxygenase gene repertoire in the rice pest Brown planthopper, Nilaparvata lugens. *Nil. Lugens. BMC Genomics* 106, 301–309. doi:10.1016/j.ygeno.2015.07.010

Li, F. C., Ni, M., Zhang, H., Wang, B. B., Xu, K. Z., Tian, J. H., et al. (2015). Expression profile analysis of silkworm P450 family genes after phoxim induction. *Pestic. Biochem. Physiol.* 122, 103–109. doi:10.1016/j.pestbp.2014.12.013

Li, Y. H., Wang, Z. Y., and Romeis, J. (2021). Managing the invasive fall armyworm through biotech crops: A Chinese perspective. *Trends. Biotechnol.* 39, 105–107. doi:10.1016/j.tibtech.2020.07.001

Liu, J., Hao, Z., Yang, S., Lin, Y., Zhong, H., and Jin, T. (2022). Insecticide resistance and its underlying synergism in field populations of Spodoptera frugiperda (J. E. Smith) from Hainan Island, China. *Phytoparasitica* 50, 933–945. doi:10.1007/s12600-022-01004-3

Liu, X. N., Liang, P., Gao, W. X., and Shi, X. Y. (2006). Induction of the cytochrome P450 activity by plant allelochemicals in the cotton bollworm, Helicoverpa armigera (Hübner). *Pestic. Biochem. Physiol.* 84, 127–134. doi:10.1016/j.pestbp.2005.06.002

Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. *Methods* 25, 402–408. doi:10.1006/meth.2001.1262

Lu, Y. H., Wang, G. R., Zhong, L. Q., Zhang, F. C., Bai, Q., Zheng, S. R., et al. (2017). Resistance monitoring of Chilo suppressalis (Walker) (Lepidoptera: Crambidae) to chlorantraniliprole in eight field populations from east and central China. *Crop Prot.* 100, 196–202. doi:10.1016/j.cropro.2017.07.006

Mao, T. T., Li, F. C., Fang, Y. L., Wang, H., Chen, J., Li, M. X., et al. (2019). Effects of chlorantraniliprole exposure on detoxification enzyme activities and detoxification-related gene expression in the fat body of the silkworm, *Bombyx mori*. *Ecotox. Environ. Safe.* 176, 58–63. doi:10.1016/j.ecoenv.2019.03.074

McCaffery, A. R., Walker, A. J., and Topper, C. P. (1991). Insecticide resistance in the bollworm, Helicoverpa armigera from Indonesia. *Pest Manag. Sci.* 32, 85–90. doi:10.1002/ps.2780320109

Mohan, M., and Gujar, G. T. (2003). Local variation in susceptibility of the diamondback moth, Plutella xylostella (Linnaeus) to insecticides and role of detoxification enzymes. *Crop Prot.* 22, 495–504. doi:10.1016/S0261-2194(02)00201-6

Montezano, D. G., Specht, A., Sosa, D. R., Roque, V. F., Sousa, J. C., Paula, D., et al. (2018). Host plants of Spodoptera frugiperda (Lepidoptera: Noctuidae) in the americas. *Afr. Entomol.* 26, 286–300. doi:10.4001/003.026.0286

Pedersen, K. E., Pedersen, N. N., Meyling, N. V., Fredensborg, B. L., and Cedergreen, N. (2019). Differences in life stage sensitivity of the beetle *Tenebrio molitor* towards a pyrethroid insecticide explained by stage-specific variations in uptake, elimination, and activity of detoxifying enzymes. *Pestic. Biochem. Physiol.* 162, 113–121. doi:10.1016/j.pestbp.2019.09.009

Qin, C., Wang, C. H., Wang, Y. Y., Sun, S. Q., Wang, H. H., and Xue, C. B. (2018). Resistance to diamide insecticides in Plutella xylostella (Lepidoptera: Plutellidae): Comparison between lab-selected strains and field-collected populations. *J. Econ. Entomol.* 111, 853–859. doi:10.1093/jee/toy043

Riaz, M. A., Strode, C., Ranson, H., David, J. P., Poupardin, R., Reynaud, S., et al. (2009). Impact of glyphosate and benzo[a]pyrene on the tolerance of mosquito larvae to chemical insecticides. Role of detoxification genes in response to xenobiotics. *Aquat. Toxicol.* 93 (1), 61–69. doi:10.1016/j.aquatox.2009.03.005

Rose, R. L., Barbhaiya, L., Roe, R. M., Rock, G. C., and Hodgson, E. (1995). Cytochrome P450-associated insecticide resistance and the development of biochemical diagnostic assays in *Heliothis virescens*. *Pestic. Biochem. Physiol.* 51, 178–191. doi:10.1006/pest.1995.1018

Shu, B. S., Yu, H. K., Dai, J. H., Xie, Z. G., Qian, W. Q., and Lin, J. T. (2020). Stability evaluation of reference genes for real-time quantitative PCR normalization in S. frugiperda (Lepidoptera; Noctuidae). *J. Integr. Agr.* 19, 2471–2482. doi:10.1016/S2095-3119(20)63298-1

Sun, L., Yang, D., Rui, C., Cui, L., and Yuan, H. (2012). The effect of chlorantraniliprole to the mRNA expression of ryanodine receptor gene in. *Diamondback Moth. J. Pest Sci.* 14, 110–116. doi:10.1016/j.pestbp.2018.03.012

Sun, Y., Xu, L., Chen, Q., Qin, W. J., Huang, S. J., Qin, H. G., et al. (2018). Chlorantraniliprole resistance and its biochemical and new molecular target mechanisms in laboratory and field strains of Chilo suppressalis (Walker). *Pest Manage. Sci.* 123, 1416–1423. doi:10.1002/ps.4824

Tan, Q. M., Chen, W. W., Li, H. H., Liao, S. C., Yi, G. Q., Mei, Y., et al. (2022). Adipokinetic hormone signaling regulates cytochrome P450mediated chlorantraniliprole sensitivity in Spodoptera frugiperda (Lepidoptera: Noctuidae). *Pest Manage. Sci.* 78 (6), 2618–2628. doi:10.1002/ps.6896

Wang, H. H., Lv, S. L., Zhao, R., Liang, P., Zhang, S., and Gao, X. W. (2021). Establishment of the baseline for the relative sensitivity of Spodoptera frugiperda larvae to commonly used insecticides. *J. Insect Physiol.* 64 (12), 1427–1432. doi:10.16380/j.kcxb.2021.12.08

Wang, H. H., Zhao, R., Gao, J., Zhang, L., Zhang, S., Liang, P., et al. (2022). Genetic architecture and insecticide resistance in Chinese populations of Spodoptera frugiperda. *J. Pest Sci.* 2022. doi:10.1007/s10340-022-01569-2

Wang, X. G., Huang, Q., Hao, Q., Ran, S., Wu, Y. Q., Cui, P., et al. (2018). Resistance and enhanced cytochrome P450 monooxygenase activity in field populations of Spodoptera litura from Sichuan, China. *Crop Prot.* 106, 110–116. doi:10.1016/j.cropro.2017.12.020

Wei, Y. B., Yan, R., Zhou, Q. L., Qiao, L. Y., Zhu, G. N., Chen, M. L., et al. (2019). Monitoring and mechanisms of chlorantraniliprole resistance in Chilo suppressalis (Lepidoptera: Crambidae) in China. *J. Econ. Entomol.* 112, 1348–1353. doi:10.1093/jee/toz001

Xiao, T., and Lu, K. (2022). Functional characterization of CYP6AE subfamily P450s associated with pyrethroid detoxification in Spodoptera litura. *Int. J. Biol. Macromol.* 219, 452–462. doi:10.1016/j.ijbiomac.2022.08.014

Xu, L., Zhao, J., YangSun, Y., Xu, D. J., Xu, G. C., Xu, X. L., et al. (2019). Constitutive overexpression of cytochrome P450 monooxygenase genes contribute to chlorantraniliprole resistance in Chilo suppressalis (Walker). *Pest Manage. Sci.* 75, 718–725. doi:10.1002/ps.5171

Yu, H., Yang, X., Dai, J., Li, Y., Veeran, S., Lin, J., et al. (2022). Effects of azadirachtin on detoxification-related gene expression in the fat bodies of the fall armyworm, Spodoptera frugiperda. *Spodoptera frugiperda Environ. Sci. Pollut. Res. Int.* 30, 42587–42595. doi:10.1007/s11356-022-19661-6

Zhang, B., Su, X., Lu, L., Zhen, C., Zhu, B., Li, Y., et al. (2020a). Effects of three insecticides at the sublethal dose on the expression of cytochrome P450 genes in Spodoptera frugiperda (Lepidoptera: Noctuidae). *Acta. Entomol. sini.* 63 (5), 565–573. doi:10.16380/j.kcxb.2020.05.005

Zhang, B. Z., Su, X., Zhen, G. A., Lu, L. Y., Li, Y. S., Ge, X., et al. (2021). Silencing of cytochrome P450 in Spodoptera frugiperda (Lepidoptera: Noctuidae) by RNA interference enhances susceptibility to chlorantraniliprole. *J. Insect. Sci.* 2021. doi:10.1093/jisesa/ieaa047

Zhang, D. D., Xiao, Y. T., Xu, P. J., Yang, X. M., Wu, Q. L., Wu, K. M., et al. (2020b). Insecticide resistance monitoring for the invasive populations of Fall Armyworm, Spodoptera frugiperda in China. *J. Integr. Agr.* 19, 783–791. doi:10.1016/S2095-3119(20)63392-5

Zhang, M. Y., Zhang, P., Su, X., Guo, T. X., Zhou, J. L., Zhang, B. Z., et al. (2022). MicroRNA-190-5p confers chlorantraniliprole resistance by regulating CYP6K2 in Spodoptera frugiperda (Smith). *Pestic. Biochem. Physiol.* 184, 105133–133. doi:10.1016/j.pestbp.2022.105133

Zhang, X. L., Liao, X., Mao, K. K., Yang, P., Li, D. Y., Ali, E., et al. (2017). Neonicotinoid insecticide resistance in the field populations of Sogatella furcifera (Horváth) in Central China from 2011 to 2015. *J. Asia Pac. Entomol.* 20, 955–958. doi:10.1016/j.aspen.2017.07.004

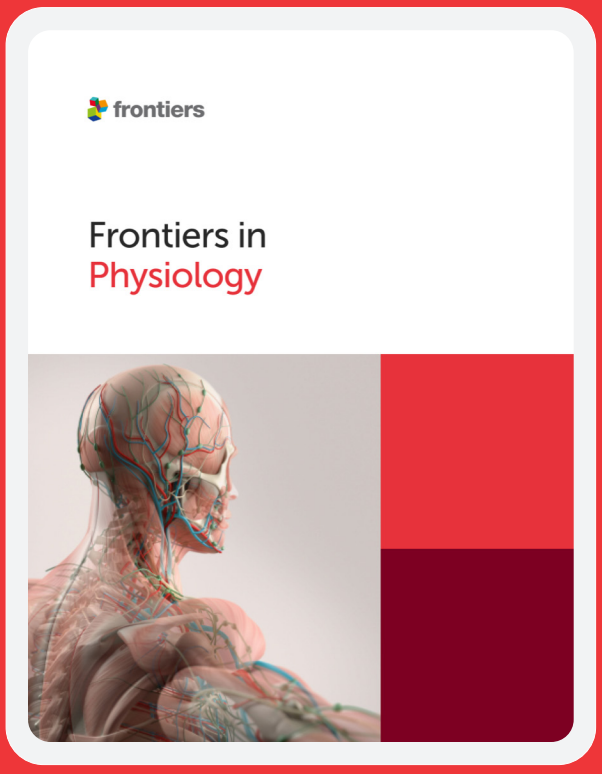
Frontiers in Physiology

Understanding how an organism's components work together to maintain a healthy state

The second most-cited physiology journal, promoting a multidisciplinary approach to the physiology of living systems - from the subcellular and molecular domains to the intact organism and its interaction with the environment.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact