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Tridax coronopifolia var. alboradiata (Asteraceae) essential oil: chemical characterization and repellent potential against Sitophilus zeamais (Coleoptera: Curculionidae)

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Currently, on a global level, there are major difficulties in pest control during the storage of grains and cereals. The most widely used method is the use of synthetic insecticides, a practice that pollutes the environment and causes health problems for consumers. As a result of this problem, this study evaluated the repellent effect of essential oil from Tridax coronopifolia var. alboradiata against Sitophilus zeamais. The oil was extracted via microwave-assisted hydrodistillation and characterized using gas chromatography. Repellency bioassays were conducted at concentrations of 50, 100, 200, 400, 600, and 800 ppm, with both positive and negative controls. The essential oil exhibited significant repellent activity against S. zeamais. At 800 ppm, the selection index was 0.27, indicating high repellency and a clear preference of insects for untreated maize grains. Chemical analysis revealed sixteen terpenes, accounting for 98.43% of the total volatile content. The predominant compounds were 2-carene (24.1%), camphene (12.6%), α -pinene (10.23%), and hexadecanal (9.11%). These results confirm the efficacy of T. coronopifolia var. alboradiata essential oil as a repellent against S. zeamais and underscore its potential as a botanical insecticide. The chemical profile provides valuable information to support its development as a sustainable alternative for managing stored grain pests.

KEYWORDS

Tridax coronopifolia var. alboradiata, essential oils, repellency, maize weevils, IPM (integrated pest management)

1 Introduction

Due to population growth, securing the global food supply has become a pressing challenge; according to Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO) (2024), agricultural production will need to increase by nearly 50% compared with ten years ago. One major threat to crop yields is the damage caused by insect pests, particularly during grain storage, which leads to significant economic losses. Among these pests, Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae), commonly known as the maize weevil, is considered the most serious pest of maize worldwide (Silva et al., 2003; Bohinc et al., 2018; Khandagale et al., 2019; Tangadi et al., 2021). Infestations can reduce both grain weight and quality (Abdullahi et al., 2014; Mbata et al., 2018), decrease moisture content, and promote fungal colonization, resulting in germ browning, heating, and overall deterioration of stored maize.

Synthetic chemical insecticides remain a common control method because of their effectiveness and rapid action (Oerke, 2006). Products frequently used against *S. zeamais* include malathion, deltamethrin, and fumigants such as methyl bromide, aluminum phosphide, sulfur fluoride, and propylene oxide (Boyer et al., 2012). However, these chemicals present several drawbacks, including grain contamination with residues, insect resistance—which often necessitates higher doses or product mixtures to maintain efficacy (Vilaseca et al., 2008)—as well as risks to the environment, soil, and water (Reyes et al., 2010) and adverse health effects (Hassaan et al., 2020; Schmidt et al., 2023). Their toxicity and persistence have led to increasing demand for safer, more sustainable pest management strategies.

Plant-derived natural products offer a promising alternative to synthetic insecticides (Silva et al., 2002; Ordoñez-Beltrán et al., 2019). Plants produce diverse secondary metabolites that serve as natural defenses against herbivores, insects, and microbial pathogens (Tofel et al., 2017). These compounds fall into three main chemical groups: phenolics (e.g., coumarins, flavonoids, tannins), nitrogen-containing compounds (e.g., alkaloids), and hydrocarbons such as terpenoids (Ferrero et al., 2006; Duplais et al., 2020). Many secondary metabolites affect insect behavior and physiology through multiple mechanisms, including toxicity, inhibition of growth, reproduction, and oviposition, as well as feeding deterrence or repellency (Rioja-Soto, 2020). They may also disrupt nervous system function by targeting essential enzymes, block metabolic pathways, and contribute to long-term pest suppression, while offering reduced environmental impact and greater target selectivity (Rioja-Soto, 2020; Lustre-Sánchez, 2022; Tangadi et al., 2021; Soto-Cáceres et al., 2022).

The push toward sustainable agriculture has driven the search for alternative pest control methods, including the use of biocontrol agents (BCAs) or biopesticides, which provide ecological benefits and reduced reliance on synthetic pesticides (Regnault-Roger, 2020). Essential oils from aromatic plants have shown strong repellent activity against various insect pests (Duplais et al., 2020), including *S. zeamais* in both laboratory and field studies (Granados-Echegoyen et al., 2017; Ferreira et al., 2020; Jayasundara

and Arampath, 2021). *Tridax coronopifolia* var. *alboradiata* (A. Gray) B.L. Rob. & Greenm. (Asterales: Asteraceae), commonly known as "rabbit grass" in southeastern Mexico, is an aromatic and edible plant traditionally used to flavor beans, broths, stews, tamales, sauces, and moles (Rosado-Aguilar et al., 2017). It can grow as an annual or perennial and is often found along roadsides and in irrigated areas (Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO), 2023). Several species of the *Tridax* genus have been reported to possess antiseptic, insecticidal, parasiticidal, and antimicrobial properties (Manzanero-Medina et al., 2020; Gomez et al., 2021).

The present study aimed to (i) evaluate the repellent efficacy of *T. coronopifolia* var. *alboradiata* essential oil against the maize weevil *S. zeamais* using a selection index bioassay, and (ii) identify its chemical constituents through gas chromatography–mass spectrometry (GC–MS).

2 Materials and methods

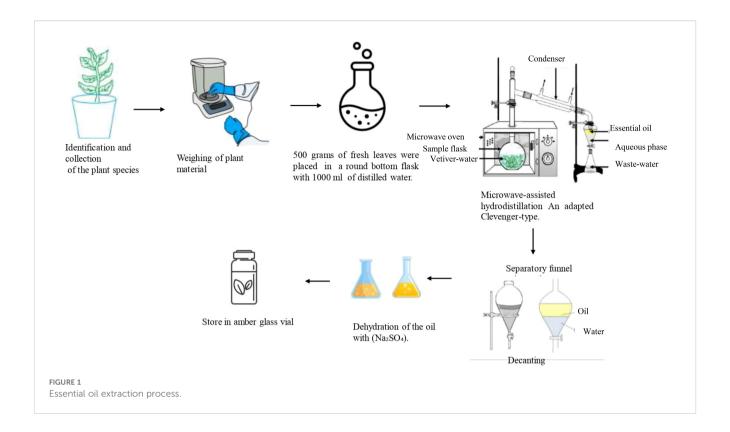
2.1 Insect rearing

Infested 'Criollo' maize grains were collected from farmers' fields in Villa de Zaachila, Oaxaca, Mexico ($16^{\circ}57'03''$ N, $96^{\circ}44'57''$ W) and transported to the Centro de Investigación para el Desarrollo Regional Integral (CIIDIR-OAX). Insects were identified using the taxonomic keys of Haines (1991) and Munyaneza and Henne (2013). To establish a laboratory colony, 100 pairs of adult *S. zeamais* were placed into 20 L plastic containers, each containing 2,500 g of maize grains. Containers were kept in a well-ventilated area and covered with anti-aphid mesh to ensure adequate gas exchange and insect respiration. After a 14-day oviposition period, all adult insects were removed using a fine sieve. The containers were maintained under controlled laboratory conditions (25 ± 2 °C; $70 \pm 10\%$ RH) until the F_1 generation emerged. Individuals from this generation were used in experimental bioassays (Juárez-Flores et al., 2010).

2.2 Essential oil extraction

Mature leaves of *T. coronopifolia* var. *alboradiata* were collected during the flowering stage in Villa de Zaachila, Oaxaca, Mexico. Plants were selected based on their aromatic properties, local availability, and traditional use in the region (Haines, 1991; Juárez-Flores et al., 2010). Taxonomic identification was confirmed by staff at the CIIDIR Oaxaca herbarium, where voucher specimens were deposited under reference number OAX-FLO-129–0402 to ensure traceability for future studies. Collected leaves were thoroughly washed and prepared for essential oil extraction.

Essential oil was obtained via microwave-assisted hydrodistillation with saturated steam at atmospheric pressure, following Dezfooli et al. (2012). An adapted Clevenger-type apparatus was used in conjunction with a standard household



microwave oven (Samsung MW1235WB, 2450 MHz) set at 70% power. A weight-to-volume ratio of 1:2 was applied, with 500 g of fresh leaves placed in a round-bottom flask containing 1,000 ml of distilled water. The extraction was carried out for 120 min, yielding 0.0095% (w/w). The oil was separated by decantation, dehydrated with anhydrous sodium sulfate (Na_2SO_4), and stored in amber glass vials at 4 °C until further analysis (Figure 1).

2.3 Volatile compound identification

Chemical analysis of the essential oil was performed using gas chromatography–mass spectrometry (GC–MS) on a Model 7890B gas chromatograph coupled to a Model 5977B quadrupole mass selective detector (electron impact ionization, EI) and a Model 7697A headspace sampler. Volatile compounds were separated on an HP-5MS UI capillary column (30 m \times 0.25 mm \times 0.25 µm). A 1 µL aliquot of the essential oil, diluted in isopropanol, was injected for compound identification. The oven temperature was programmed from 80°C to 300°C at a rate of 10°C/min, with a total run time of ~10 min. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. Compounds were identified by comparing retention times and mass spectra with those in the NIST HP-5MS library (Keffeous and Lynda, 2022). The relative abundance of each component was expressed as a percentage of the total ion current.

2.4 Selection index test

Repellent activity of *T. coronopifolia* var. *alboradiata* essential oil was assessed using maize grains as the substrate. Fifty grams of maize were placed in 20 cm porcelain plates, and the essential oil was diluted in 10 mL ethanol to prepare solutions of 50, 100, 200, 400, 600, and 800 ppm. One milliliter of each solution was applied to the maize using a micropipette to ensure uniform coverage. Plates were left for 20 min to allow complete ethanol evaporation before testing.

The bioassay apparatus consisted of three plastic containers connected by tubes (10 cm long \times 1 cm diameter). The two side containers each contained 50 g of maize: one treated with essential oil and one untreated. The central container housed 20 unsexed adult *S. zeamais* that had been starved for 24 h. Piperonyl butoxide with commercial deltamethrin (K-Obiol 2.5, Bayer Mexico) at 10 $\mu\text{L}/\text{cm}^2$ served as the positive control. After 24 h, the number of insects in each container was recorded, and the selection index (Si) was calculated following Mazzonetto and Vendramin (2003):

$$Si = (2 \times G)/(G + C)$$

where G is the percentage of insects in the treated container and C is the percentage in the untreated container. The repellent/ attractant effect of the essential oil was classified according to Arivoli and Tennyson (2013) as follows: neutral (Si = 1.00), non-repellent (Si > 1.00), low repellency (0.75–0.99), medium repellency

TABLE 1 Compounds from the essential oil of Tridax coronopifolia var. alboradiata analyzed by gas chromatography—mass spectrometry (GC-MS).

Peak	Compounds	(%)	Retention Time	Kovats Retention Index	Formula
1	1-hexanol	2.73	4.82	867	C ₆ H ₁₄ O
2	γ-terpinene	0.62	6.55	1060	C ₁₀ H ₁₆
3	Hexyl butanoate	0.05	8.07	1184	C ₁₀ H ₂₀ O
4	Nerolidol (E)	0.34	9.65	1562	C ₁₅ H ₂₆ O
5	Calarenepoxide	0.87	11.40	1592	C ₁₅ H ₂₄ O
6	Iso-sphatulenol	6.06	12.29	1631	C ₁₅ H ₂₄ O
7	α-pinene	10.23	13.30	917	C ₁₀ H ₁₆
8	2-carene	24.10	13.70	1001	C ₁₀ H ₁₆
9	Camphene	12.60	14.01	933	C ₁₀ H ₁₆
10	Hexadecanal	9.11	14.50	1822	C ₁₆ H ₃₂ O
11	2-bornanone	6.29	16.31	1145	C ₁₀ H ₁₆ O
12	Phytol	5.57	16.39	2122	C ₂₀ H ₄₀ O
13	β-caryophyllene	8.42	19.05	1598	C ₁₅ H ₂₄ 4
14	Safrole	1.62	20.62	1291.4	$C_{10}H_{10}O_2$
15	Caryophyllene oxide	2.80	22.73	1578	C ₁₅ H ₂₄ O
16	iso-methone	2.64	25.18	1164	C ₁₀ H ₁₈ O
17	Cyclosativene	1.68	26.77	1373.6	C ₁₅ H ₂₄
Total		98.43		_	

Peak: Highest retention point. Compounds: Identified compounds. (%): Presence of the compound in the essential oil. Retention time: Time each analyte needs to travel from the injector to the detector. Kovats retention index: Quantification of the relative elution times of the identified compounds. Formula: Chemical representation of the identified compound.

(0.50-0.74), high repellency (0.25-0.49), and very high repellency (0.00-0.24).

2.5 Experimental design and data analysis

Bioassays were conducted individually in a completely randomized design. G*Power software was used to calculate statistical power, determine sample size, and ensure result reliability. Data were analyzed by one-way ANOVA, and treatment means were compared using Tukey's test at p < 0.05. Statistical analyses were performed in Minitab (version 20.3). Selection index results are presented as means \pm standard deviation. All experiments were replicated four times to ensure statistical robustness (Alonso-Hernández et al., 2023; Pérez-Hernández et al., 2023).

3 Results

3.1 Identification of volatile compounds

Gas chromatography-mass spectrometry analysis of *T. coronopifolia* var. *alboradiata* essential oil identified 16 terpenes, representing 98.43% of the total composition (Table 1). The extraction yielded 800 µL of oil. The predominant constituents were 2-carene (24.1%), camphene

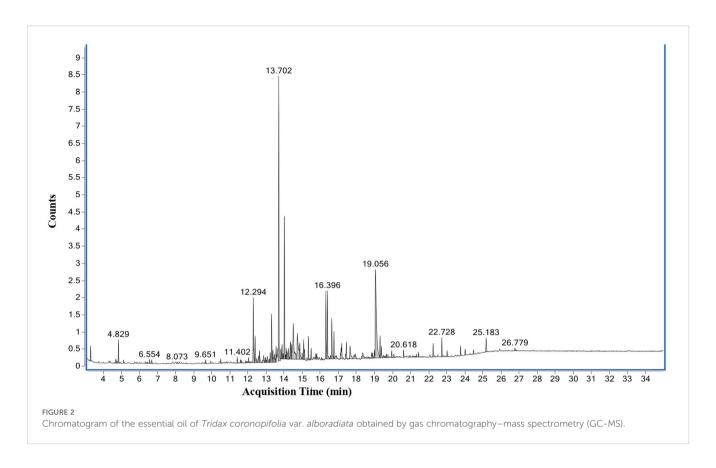
(12.6%), α -pinene (10.23%), hexadecanal (9.11%), β -caryophyllene (8.42%), 2-bornanone (6.29%), iso-sphatulenol (6.06%), phytol (5.57%), and caryophyllene oxide (2.80%) (Figure 2).

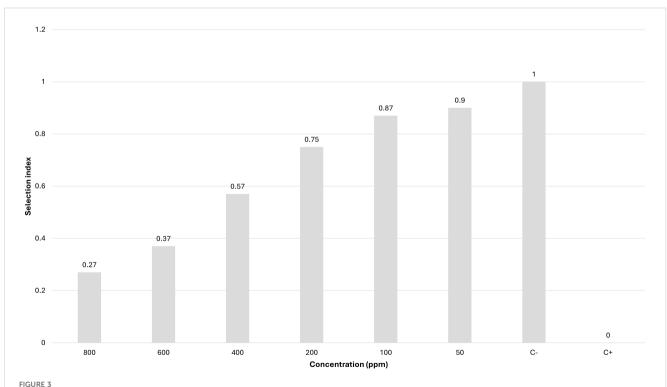
3.2 Selection index test

Repellency increased with oil concentration, as indicated by the decreasing number of weevils in treated maize containers. At the highest concentration (800 ppm), the SI was 0.27 (F = 197.64, df = 7.24, p < 0.001, $r^2 = 0.9829$), with 86.25% of weevils found in untreated maize. Concentrations of 600 and 800 ppm (SI = 0.37 and 0.27, respectively) were classified as having high repellent activity (0.25 \leq SI \leq 0.49). At the lowest concentration (50 ppm), the SI was 0.90, indicating low repellency (0.75 \leq SI \leq 0.99), with 55.00% of weevils in untreated maize. All concentrations of T. Coronopifolia var. Coronopifolia

4 Discussion

This study is the first to report the chemical characterization of the essential oil extracted from *T. coronopifolia* var. *alboradiata*.





Repellency index of the essential oil of Tridax coronopifolia var. alboradiata on Sitophillus zeamais. C-: negative control, without the addition of essential oil; C+: is the positive control, in which piperonyl butoxide with deltamethrin was used as a pyrethroid insecticide, applying 10 μ L per cm²

(K-Obiol 2.5, Bayer Mexico).

TABLE 2 Selection (preference) index of the grain maize weevil (Sitophilus zeamais) exposed to Tridax coronopifolia var. alboradiata essential oil.

Concentration (ppm)	Treated grain	Untreated grain	Selection index	Rating
800	13.75 ± 2.50 e	86.25 ± 2.50 b	0.27 ± 0.05 e	+++
600	18.75 ± 2.50 e	81.25± 2.50 b	0.37 ± 0.05 e	+++
400	28.75 ± 4.79 d	71.25 ± 4.79 c	0.57 ± 0.09 d	++
200	37.50 ± 2.89 c	62.50 ± 2.89 d	0.75 ± 0.05 c	+
100	43.75 ± 2.50 b	56.25 ± 2.50 e	0.87 ± 0.05 b	+
50	45.00 ± 0.00 ab	55.00 ± 0.00 ef	0.90 ± 0.00 ab	+
C-	50.00 ± 0.00 a	50.00 ± 0.00 f	1.00 ± 0.00 a	0
C+	0.00 ± 0.00 f	100 ± 0.00 a	0.00 ± 0.00 f	++++

Data in columns with different letters are significantly different p<0.05. Neutral activity (Si = 1; rating '0'), non-repellent activity (Si > 1.00), low repellent activity (0.75 \ge Si \ge 0.99; category '+'), medium repellent activity (0.50 \ge Si \ge 0.74; rating '++'), high repellent activity (0.25 \ge Si \ge 0.49; rating '+++'), very high repellent activity (0.00 \ge Si \ge 0.24; rating '++++'). Data in columns with different letters are significantly different p<0.05; C- is the negative control, without the addition of essential oil; C+ is the positive control, in which piperonyl butoxide with deltamethrin was used as a pyrethroid insecticide, applying 10 μ L per cm² (K-Obiol 2.5, Bayer Mexico).

Analysis identified several metabolites with known biological activities, with 2-carene being the most abundant compound (24.1%). This bicyclic monoterpene occurs naturally in the essential oils of several Lamiaceae species, including oregano (Origanum vulgare L.) and rosemary (Rosmarinus officinalis L.), both of which have demonstrated 100% mortality against S. zeamais in pest management studies (Costa-Becheleni et al., 2020). Camphene, another major compound, possesses a pungent camphor-like aroma and is present in plants such as citronella (Cymbopogon nardus (L.) Rendle), ginger (Zingiber officinale Roscoe), and several Lamiales species, with documented insecticidal, antifungal, antibacterial, and antioxidant properties (Wang et al., 2008; Flores-Villa et al., 2020). Additionally, αpinene, found in thyme (Thymus vulgaris L.) and various Salvia species, has shown insecticidal activity against the bean weevil (Sitophilus oryzae L.), achieving up to 98.9% efficacy (Tapia-Borja et al., 2020). Hexadecanal, a fatty aldehyde identified as a major compound in the oil, is known for its distinctive odor and may function as a chemical signal to attract or repel specific insects, enhancing the plant's defense against herbivores and pathogens. Since these identifications are based solely on HP-5MS library matches, further confirmation using retention indices or authentic standards is recommended.

The quality and efficacy of essential oils can be influenced by multiple factors, including the timing of leaf harvest, the plant part used, extraction method, plant origin, and variability in chemical composition. Genetic, environmental, and phenological factors, as well as post-harvest handling, can affect concentrations of bioactive compounds. Storage conditions also influence the stability of essential oil constituents. Such variability can lead to different chemotypes, which may affect the efficacy of oils in integrated pest management strategies (Granados-Echegoyen et al., 2017; Ferreira et al., 2020; Jayasundara and Arampath, 2021). Similar patterns have been observed in *Porophyllum linaria* Cav., where higher concentrations of active compounds correlated with enhanced bioactivity (Hernández-Cruz et al., 2019; Landero-Valenzuela et al., 2022). These observations support our finding

that pest repellency improves with increasing essential oil concentration and prolonged exposure.

Previous studies have evaluated the repellent effectiveness of other essential oils against S. zeamais, including oils from boldo (Peumus boldus Molina), Chilean laurel (Laurelia sempervirens (Ruiz & Pav.) Tul.), and tepa (Laureliopsis philippiana (Looser) R. Schodde), which contain safrole, a terpene also present in T. coronopifolia var. alboradiata (Bustos et al., 2017). Other natural products, such as powders and extracts of neem (Azadirachta indica A. Juss), have demonstrated low biocidal activity but higher repellent effects (Palomino-Reves et al., 2022). Preliminary studies on phytol and humulene I and II indicate that these compounds contribute to insecticidal activity (Arceo-Medina et al., 2016; Rizvi et al., 2018; Perdomo-Cedeño, 2020). In the present study, the observed repellent effect (SI = 0.27) at concentrations of 600 and 800 ppm qualifies as high repellency (0.25 \leq SI \leq 0.49) according to Mazzonetto and Vendramin (2003), with all tested concentrations showing repellency compared to the negative control. Similar results have been reported for Chenopodium ambrosioides L. (SI = 0.31) and Schinus molle L. (SI = 0.20) (Aros et al., 2019; Arias et al., 2017). The mode of action of terpenoids includes repellency and deterrence, interference with juvenile hormone and molting hormone, inhibition of chitin synthesis and digestive enzymes, reduced feeding, and prevention of oviposition (Pavela et al., 2020). The repellent properties of essential oils are strongly associated with the presence of monoterpenes and sesquiterpenes in their volatile profiles (Albarracin-Gomez et al., 2022; Opiyo et al., 2022).

Essential oils represent a sustainable and environmentally friendly alternative to synthetic insecticides. They reduce exposure to toxic chemicals, protect human health, minimize soil and water contamination, and are biodegradable, avoiding long-term ecological damage (Opiyo et al., 2022). Although synthetic insecticides act rapidly, essential oils offer a viable option when environmental safety, human health, and sustainability are priorities. Cost-effectiveness depends on factors such as equipment type, extraction parameters, and plant material. Hydrodistillation is both efficient and economical, ensuring

optimal utilization of natural resources (Albarracín-Montoyo and Gallo-Palma, 2003). Advances in sustainable production technologies are reducing costs while maintaining or improving essential oil quality, which is particularly relevant for stored grain preservation. The organic origin of essential oils also enhances marketability and supports food safety and security objectives (González-Puetate et al., 2023).

5 Conclusions and future directions

The results confirm that *T. coronopifolia* var. *alboradiata* essential oil is an effective repellent against the maize weevil (*S. zeamais*) in stored grain. These findings indicate that this essential oil is a promising natural alternative to conventional synthetic insecticides and could be integrated into pest management strategies for stored products. As a natural repellent, it provides an environmentally friendly and efficient option for crop protection. Future research should evaluate the oil's effects on seed viability, ensuring that concentrations up to 800 ppm do not compromise maize germination, sensory quality, or increase the risk of mycotoxin contamination.

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

LG-S: Investigation, Formal Analysis, Methodology, Writing original draft, Writing - review & editing. RP-P: Funding acquisition, Methodology, Writing - original draft, Supervision, Resources, Conceptualization, Writing - review & editing. BH: Project administration, Software, Writing - original draft, Methodology, Resources, Writing - review & editing. YO-H: Formal Analysis, Data curation, Writing - original draft, Writing - review & editing, Funding acquisition. AV-L: Methodology, Project administration, Investigation, Writing - original draft, Writing review & editing. TA-B: Validation, Writing - review & editing, Writing - original draft, Software, Supervision. BQ-G: Resources, Investigation, Project administration, Writing - review & editing, Methodology, Writing - original draft. SM-T: Funding acquisition, Writing - original draft, Formal Analysis, Writing - review & editing, Data curation. FA-B: Writing - review & editing, Software, Writing original draft, Supervision, Visualization, Validation. AF-B: Project administration, Resources, Writing - review & editing, Writing - original draft, Methodology. CG-E: Writing – original draft, Formal Analysis, Supervision, Software, Conceptualization, Resources, Methodology, Validation, Writing – review & editing, Data curation, Investigation, Visualization.

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Conflict of interest

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