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Evaluation of biochemical, nutrient content and productivity of oyster mushrooms biofortified with Zinc Oxide Nanoparticles

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In the current investigation, a successful effort has been implemented to bio-fortify *Pleurotus djamor* and *Pleurotus florida* with ZnONPs (Zinc Oxide Nanoparticles) for nutritional enrichment trial during two consecutive years 2020–2021. Two concentrations of ZnONPs, 20 and 40 ppm were added into the substrate at the spawning stage and their effect was monitored by measuring the physical attributes like mycelium radial growth, morphology (pilus length, pilus diameter, stipe length and stipe width) with biochemical analysis (anti-oxidative enzymes, phenols, flavonoids and total antioxidants) and nutritional analysis (macronutrients, micronutrients, protein content and total soluble sugar) of extracts obtained from the Zn (zinc) fortified Oyster mushrooms. Results have shown an improvement in the mycelial growth and yield of two *Pleurotus* spp. studied here, while the effect of ZnONPs on morphology varied positively with the concentrations used and the type of oyster mushroom. The anti-oxidative enzymes were found to be triggered upon application of ZnONPs reflecting the role of ZnONPs in activating the enzymes and thereby enhanced potential in free radical scavenging activity. Along with these the phenols, flavanoids and anti-oxidants were also found to be elevated upon application of ZnONPs. An enhancement in macro- and micro-nutrient concentration has justified the utilization of ZnONPs in biofortification. In near future, more emphasis should be laid on ZnONPs enriched biofortified mushrooms to boost nutritive potential of edible mushrooms and to encounter the ever growing metabolic deficiency problems with the increasing human population in the world.

KEYWORDS

biofortification, anti-oxidative enzymes, mycelial growth, oyster mushrooms, *Pleurotus* spp.

1 Introduction

Growing and producing staple food crops to have more micronutrient levels in their edible portions is the highly developing, agricultural-based method known as biofortification (Dhaliwal et al., 2022). It is a relatively economical and long-term approach to boost micronutrient supply which relies on the practice of breeding nutrients into food crops (Olson et al., 2021). However, at industrial level, fortification of products occurs during manufacturing

of food that raises the cost of the finished goods. For these reasons, the poorest people living in rural areas cannot afford fortified meals (Bourassa et al., 2023). A long-term solution for mineral replenishment advised as biofortification. Biofortification techniques, raises the bioavailability of minerals as well as their concentrations (Chadare et al., 2019).

The bio-fortification approach seeks to help all family members to consume adequate quantities of food every day, especially mothers and children, most vulnerable to micronutrient shortages (Naik et al., 2024). Mushrooms are being used in the pharmaceuticals, cosmetics, and food nutrition industries. In this industry, cosmetic products, nutrition food products, pharmaceutical products of mushroom and mushroom extracts are used to make medicines for various diseases (Rai et al., 2021). After *Agaricus bisporus,* the species of *Pleurotus* is regarded as the second most widely grown and disseminated edible fungus in the world. *Pleurotus* mushrooms have several advantageous in prospects of health and nutritional characteristics. Numerous research have documented the *Pleurotus* genus's wide range of pertinent traits, confirming that they act as desirable low-cost industrial tools that relieve the burden of ecological problems (Sánchez, 2010; Effiong et al., 2024). Oyster mushroom is a large and diversified genus of farmed mushrooms with significant commercial and therapeutic value. Oyster fungus can be found all over the world, in both tropical and temperate regions (Jayasuriya et al., 2020).

Zn deficiency alone cast a long shadow over millions of lives. The numbers were staggering, 800,000 deaths annually, nearly 450,000 of whom were children under five (Prasad, 2013; Hawrysz and Woźniacka, 2023). The world needed a solution, one that was accessible, sustainable, and capable of bridging the nutritional gap for millions in impoverished regions. Biofortification, a groundbreaking agricultural innovation that sought to infuse staple food crops with essential micronutrients. Unlike conventional food fortification, which enhanced nutrition during food processing but often remained out of reach for rural populations, biofortification worked from the ground up. It ensured that crops naturally contained higher nutrient levels, making them both cost-effective and sustainable (Ofori et al., 2022). In this landscape, the Pleurotus species emerged as key edible mushroom species. Second only to *Agaricus bisporus* in global cultivation, these mushrooms were more than just a culinary delight. They possessed extraordinary health benefits, their biochemical richness making them an industrial asset in environmental management and nutrition. Researchers marveled at the *Pleurotus* genus, hailing its potential to address both food security and ecological concerns (Mihai et al., 2022).

Meanwhile, in the realm of material science, another revolution was underway that promised to reshape the way agriculture tackled nutrient deficiencies. Zinc oxide (ZnO), a widely used metal oxide, had become a cornerstone in nanotechnology. Scientists discovered that nanoparticles, tiny structures ranging from 1 to 100 nm, held immense promise across diverse fields, from medicine to food processing (Aslani et al., 2014). ZnONPs (Zinc Oxide Nanoparticles) are typically spherical or hexagonal (10–100 nm) as observed via Transmission Electron Microscope (TEM)/ Scanning Electron Microscope (SEM), with larger hydrodynamic sizes in solution due to aggregation. X-ray Diffraction (XRD) and Selected Area Electron Diffraction (SAED) confirm their wurtzite crystalline phase, while Fourier Transform Infra-Red (FTIR) spectroscopy reveals surface chemistry influencing dispersibility of these nano-particles (Shaikhaldein et al., 2021). Surface modifications enhance stability in various substrates. The intersection of nanotechnology and agriculture opened new doors, offering innovative solutions to enhance nutrient uptake in crops. As research continued, scientists and farmers alike found themselves on the cusp of a transformative era. The fusion of ancient wisdom with cutting-edge science was crafting a future where food wasn't just sustenance moreover it was a powerful tool against malnutrition, a bridge between generations and a testament to human resilience (Chen et al., 2016).

The present work demonstrates the first of its kind of assessment revealing the change in physical attributes of edible mushroom species by virtue of ZnONPs presence and also highlights the significant changes that took place in immunity boosting factors of mushrooms by noticing the attributed variation among biochemical parameters studied here in response to ZnONPs presence and absence. The two *Pleurotus* species chosen for this study are consumed largely in the global market and hence biofortifying these will be beneficial to a large group of people consuming them. The usage of wheat straw in cultivating the mushroom species highlights the relevance of the current finding in utilizing the agricultural wastes under sustainable agro-ecosystems management practices.

2 Materials and methods

The present work aimed to explore two *Pleurotus species* (*P. jamor* and *P. florida*) under ZnONP influence. Different concentrations (Table 1) were investigated at *Pleurotus* species' spawning period. The entire research work was carried out at Department of Biochemistry, College of Basic Science and Humanities and Mushroom Research and Training Centre (MRTC), G.B. Pant University of Agriculture and Technology, Pantnagar, India.

Table 1 reflects the treatments of ZnONPs given to oyster mushrooms. PD is designated for *P. djamor* with PD as control while, T1PD and T2PD as treatments for 20 and 40 ppm, respectively. PF is designated for *P. florida* with PF as control while, T1PF and T2PF as treatments for 20 and 40 ppm, respectively.

2.1 Effect of ZnONPs on the mycelial radial growth of oyster mushroom

Experiments were conducted to study the effect of different concentrations of ZnONPs (20 ppm, 40 ppm, 60 ppm, 80 ppm and Control) on the radial growth of the *Pleurotus* spp. Autoclaved potato dextrose agar growth media was supplemented with different concentrations of filter-sterilized ZnONPs (20 ppm, 40 ppm, 60 ppm,

TABLE 1 Treatment details of ZnONPs.

S. no.	Treatments	*Pleurotus* spp.	Concentration of ZnONPs (ppm)
1.	PD Control	*P. djamor*	0
2.	T1PD	*P. djamor*	20
3.	T2PD	*P. djamor*	40
4.	PF Control	*P. florida*	0
5.	T1PF	*P. florida*	20
6.	T2PF	*P. florida*	40

80 ppm and control). 20 mL media was poured into different petri plates under aseptic conditions. The *Pleurotus* spp. mycelia disc, 5 mm in diameter, was cut with the help of a cork borer and was placed in the center of the Petri plates supplemented with different concentrations of ZnONPs. The Petri plates were kept in an incubator at 24°C. The observations were taken after the beginning of mycelium growth in all the treated plates at five days intervals, till full mycelial growth is achieved in one of the plates (mm).

2.2 Method of cultivation of *Pleurotus* spp.

2.2.1 Preparation of master spawns

The wheat grains obtained from the Pantnagar Crop Research Centre were cleaned and boiled (grain: water; 1:25, w/v) till they softened in order to make the master spawn. To eliminate extra moisture, the grains were dried overnight on a sieve bed. To balance and buffer the pH of the developing medium, the grains were finely mixed with calcium sulfate and calcium carbonate at 12 g and 3 g per kg of boiling wheat grains, respectively, thus providing a proper environment for mushroom development and yield. The calcium carbonate raises the pH and thus promotes alkalinity while, calcium sulfate promotes carbonate precipitation thereby lowering the pH indirectly. The final grain mixture was packed into a glass bottle (300 g/bottle) sealed with non-absorbent cotton and autoclaved for 20 min at 15 psi. After removing from the autoclave, the sterilized bottles were gently shaken to prevent grain clumping prior to cooling. After surface sterilizing of laminar flow, these sterilized glass bottles were infected using discs (5 mm) of 12–15 days old culture (*P. djamor* and *P. florida*). The inoculated bottles spent 10 days incubated at 25°C. The bottles were shaken hard to break the mycelia threads consistently all around the number of wheat grains within them. Following 25 days of inoculation, fine mycelia growth (a complete spawn run) covers all of the grains. Commercial spawns were made from the master spawn as well.

2.2.2 Commercial spawn

Master spawns was transferred to finely chopped (soaked in water for overnight) wheat straw which was used as the substrate for preparing commercial spawns. For chemical treatment of the substrate, 90 L of water was taken in a plastic drum of 200-L capacity. 10 kg of wheat straw was completely steeped in the water. In another plastic bucket, 7.5 g. Bavistin (50% WP) and 125 mL formaldehyde (40%) were dissolved in 10 L of water and slowly poured on already-soaked wheat straw. Following addition and mixing, the mouth of the drum was covered with a polyethene sheet and left it for 16 h. After that, the straw was taken out of the mixture, and the extra water was distributed equally on the sterilized surface till the substrate's final moisture content became roughly 70%. Later on, this substrate was employed for spawning.

The resultant mixture of straw was filled in the polypropylene bag and sterilized in an autoclave at 15 psi for 80 min. These sterilized Polypropylene bags were stored in a surface sterilized laminar flow (Yang et al., 2013).

2.2.3 Spawning and bag filling of *Pleurotus* spp.

Standard technique was used to completely mix commercial spawn with prepared wheat straw (2.0%). At 1.5 kg/bag, spawn substrate–substrate following mixing—was packed in polyethylene bags (60 × 45 cm) and the polyethylene bags were tightly closed. Eight to ten small holes were created at specific distances for free diffusion of gases and heat produced within after filling and closing the polyethylene bags. The bags were moved to a dark incubation chamber with 25–28°C temperature and 70–80% humidity for roughly 15 days until the fungus mycelia could tightly bound to the entire straw and moisture content remained at 70%.

2.2.4 Bag opening

Following the complete spawn run, the polyethene sheet was gently removed using a sharp knife. Water was then routinely sprayed twice a day to maintain appropriate humidity and growth of fruiting bodies from then on. Throughout the cropping, temperature and relative humidity were kept at 24–28°C and 75–85% correspondingly.

2.2.5 Harvesting

Harvesting of mushrooms was done when the mature oyster mushrooms became tops flattened and turned inward. Mature fruit bodies of *P. djamor* and *P. florida* were hand-picked under clockwise or anticlockwise rotation before sprinkling with water so that the fruit bodies came out without leaving any stump. Weighing and noting each individual fruit body was recorded. For the second, third, fourth, fifth, and sixth harvests, the same process was followed. Weighing the fruit bodies, they were shade-dried to cause total dehydration. Further biochemical and biological activities were derived from the dried fruit bodies.

2.3 Effect of ZnONPs on mycelial growth, morphology and yield of oyster mushrooms

2.3.1 Chemicals, glassware and culture collection

The chemicals and solvents used in the study were of HighMedia and analytical grade (99% pure). ZnONPs were purchased from Cisco Research Laboratories, Pvt. Ltd. India (Supplementary Figure S1). The cultures of two *Pleurotus* spp. were procured during the year (Figure 1).

2.3.2 Effect of ZnONPs on biochemical attributes of oyster mushrooms

2.3.2.1 Enzymatic analysis

2.3.2.1.1 Enzyme extraction

In a pre-chilled mortar and pestle, the 0.2 g fresh mushroom tissue was pulverized in 2 mL of 0.1 M sodium phosphate buffer (pH 7.0) after washing in sterile distilled water and dried with filter paper. Under 4°C, the homogenate was centrifuged for 20 min at 15,000*g*. The supernatant after centrifugation was taken as enzyme source.

Superoxide dismutase determination. The cuvettes containing 1.5 mL of 100 mM Tris HCI buffer (pH 8.2), 0.5 mL of 6 mM EDTA, 1 mL of 6 mM pyrogallol solution and 0.1 mL of enzyme extract were put in a spectrophotometer. The spectrophotometer measured variations in absorbance at 420 nm over intervals of 30 s to 3 min. One unit of SOD is defined as the quantity of enzyme producing 50% suppression of the auto-oxidation of pyrogallol noted in blanks. Activity is stated in unit of min^{-1} g^{-1} fresh weight (Marklund and Marklund, 1974).

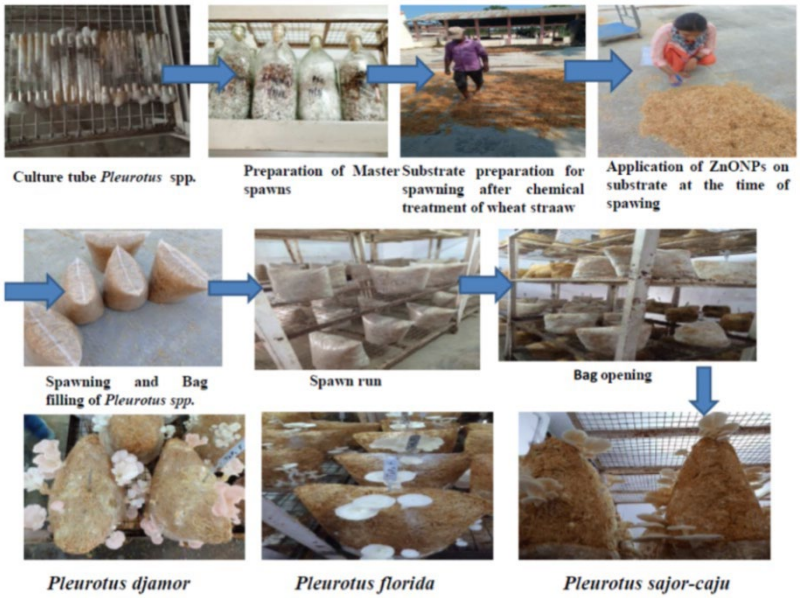

FIGURE 1
Method of cultivation of *Pleurotus* spp. The cultivation of *Pleurotus* mushroom species starting from the preparation of master spawns & substrate proceeded by application of ZnONPs and finally the bag opening was preceded by spawn run and bag opening.

Ascorbate peroxidase determination (APX). Fresh extractions' ascorbate peroxidase activity was assessed right away using Dalton et al. (1987) assaying technique. Potassium phosphate buffer (100 mM, pH 7.0) was employed; it was generated from 39 mL of 0.2 M KH_2PO_4 mixed with 61 mL of 0.2 M K_2HPO_4 and final volume to 200 mL with distilled water. With distilled water, 0.053 g of ascorbic acid (3 mM) was dissolved to get the final volume to 100 mL. Hydrogen peroxide (3 mM): The 3 mL enzyme reaction mixture prepared by diluting 1 mL of 75 mM hydrogen peroxide to 25 mL with distilled water contained 50 mM potassium phosphate buffer (pH 7.0) (1.5 mL of 100 mM), 0.5 mM ascorbic acid (0.5 mL of 3 mM), 0.1 mM EDTA (0.1 mL of 3 mM), 100 µL enzyme extract, 0.6 mL distilled water and 0.1 mM hydrogen peroxide (0.1 mL of 3 mM). Addition of 0.1 mL of 3 mM H_2O_2 set off the process. After hydrogen peroxide dependent oxidation of ascorbic acid, absorbance measured at 290 nm dropped by 3 min at 30 s intervals. The ascorbate oxidized quantity was measured from the molar extinction coefficient (82.8 mM cm^{-1}). Expression of enzyme activity was moles of ascorbate oxidized per milligrams protein per minute.

Guaiacol peroxidase determination (GPX). Using guaiacol as the hydrogen donor, the approach Garg and Bhandari (2016) assessed the rate of breakdown to GPx by the absorbance increase at 424 nm per minute by peroxidase. The reagents applied were potassium phosphate buffer (100 mM, pH 6.1). 1.075 mL analytical grade guaiacol was dissolved in distilled water; the final volume was composed to be 100 mL using distilled water. Dissolving 124 µL of 30% (v/v) hydrogen peroxide in distilled water, the final volume came to be 100 mL. The 3 mL enzyme reaction mixture consisted in 50 mM phosphate buffer (pH 10) (1.5 mL of 100 mM), 16 mM guaiacol (0.5 mL of 96 mM), 100 µL enzyme extract, 0.4 mL distilled water and 2 mM hydrogen peroxide (0.5 mL of 12 mM). Using a spinner, the aforesaid combination was adequately blended for 3–5 s. The process starts with 0.5 mL of 12 mM H_2O_2 added. Measuring at 470 nm for 3 min at 30 s intervals, the rise in absorbance brought about by tetra-guaiacol was recorded. The molar extinction coefficient of its oxidation product, tetra-guaiacol $\varepsilon = 26.6$ mM^{-1}, helped one to estimate the enzyme activity. Expression of enzyme activity was measured as tetra-guaiacol formed mg^{-1} protein min^{-1} in nanomoles.

Catalase determination. The catalase activity was measured promptly in fresh extracts as stated by Halliwell and Gutteridge (2015). The drop in absorbance at 240 mM helped one to estimate hydrogen peroxide-dependent oxidation. The utilized reagent was 75 mM hydrogen peroxide: distilled water helped to dissolve 775 µL of 30% (v/v) hydrogen peroxide, producing a final amount of 100 mL. The 3 mL of potassium phosphate buffer (100 mM, pH 7.0) enzymatic reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.0) (1.5 mL of 100 mM), 200 µL enzyme extract, 800 µL distilled water and 12.5 mM hydrogen peroxide (0.5 mL of 75%). The 0.5 mL of 75 mM H_2O_2 started the process off. The reduction in absorbance at 240 nm was recorded for 3 min at 30 s intervals for the assessment of catalase enzyme activity. The molar extinction coefficient ($\varepsilon = 36$ M^{-1} cm^{-1}) helped to ascertain the concentration of dissociated hydrogen peroxide.

Polyphenol oxidase determination. The method adopted by Okpuzor (1991) was used to assess polyphenol oxidase activity. The reagents used were 0.1 M phosphate buffer (pH 7.0): produced by combining 47.8 mL of 0.2 M NaH_2PO_4 and 76.3 mL of 0.2 M Na_2HPO_4 solution, correcting the pH to 7.0 and producing the final volume 250 mL. The pyragallol reagent (0.01 M) was made freshly by dissolving 0.126 g pyragallol in 100 mL distilled water.

2.3.2.2 Nutrient analysis

2.3.2.2.1 Preparation of extracts

After coarsely ground, shade-dried oyster mushrooms were wrapped in Whatman filter paper and were run sequentially under a

Soxhlet machine. The solvent for the extraction was methanol. To get rid of any last solvent, the extract was concentrated by following evaporation and distillation.

Estimation of total phenol. McDonald et al. (2001) developed a quantitative calculation approach for all oyster mushroom phenol assays. Under alkaline conditions, phenol reacts with the oxidizing agent phosphomolybdate in the Folin–Ciocalteu reagent to produce a blue colored complex detected at 765 nm. The total phenol content was ascertained using 7.5% sodium carbonate, Folin–Ciocalteu reagent, gallic acid (for a standard curve), pipetted into individual test tubes with 0.2 mL of 80% methanolic oyster mushroom extract. Every tube received 1.0 mL of Folin–Ciocalteu reagent (1:10). Next 0.8 mL of a 75% sodium carbonate solution was added. Each test tube held up to 10 mL of double distilled water mixed thoroughly. UV–vis spectrophotometer at 765 nm assessed the color generated in the reaction mixture after 30 min.

Estimation of total flavonoids content. Total flavonoids content was determined by the method of Mandal et al. (2009). 5% $NaNO_3$, 10% $AlCl_3$, 1 M NaOH and catechin were used to determine flavonoids content. 100 µL of methanolic extracts of oyster mushroom was taken in a test tube and to it was added 900 µL of 80%methanol to make up volume of 1 mL. Then 4 mL of distilled water followed by 3 mL of 5.0% $NaNO_3$ was added to it. Incubation for 5 min was done and after that 0.3 mL of 10% $AlCl_3$ was added by following addition of 2 mL of 1 M NaOH and 2.4 mL of distilled water. The solution was mixed well and absorbance of color developed was taken at 510 nm.

Determination of total antioxidants. Prieto et al. (1999) used a technique to measure mushrooms' overall antioxidant content. This test was developed on the reduction of molybdenum (Mo)(VI) to Mo(V) by the extract and consequent synthesis of green phosphate Mo(V) complexes at acidic pH. The reagents needed were ascorbic acid, 4 mM ammonium molybdate (494.36 mg in 100 mL), 28 mM tri-sodium phosphate (397.49 mg in 100 mL), 0.6 M sulfuric acid (3.14 mg in 100 mL). The test tube held 0.1 mL of methanolic extract plus 0.2 mL of 80% methanol. Then 3 mL of the reagent solution (0.6 M sulfuric acid, 28 mM tri-sodium phosphate and 4.0 M ammonium molybdate) was added. Incubation at 95°C in a water bath continued for 90 min. The samples were subsequently brought down to room temperature and at last their absorbance was recorded at 695 nm.

Digestion of dried mushroom sample for N, P, K, S and micronutrients (Zn, Mn, Cu, and Fe) analysis. The nitrogen content in oyster mushroom samples was estimated by using micro kjeldhal method given by Jackson (1967). In this method, dried mushroom samples of 0.2 g were taken and added in digestion tube containing 1 g of catalyst mixture (100 g. potassium sulfate + 10 g cupric sulfate + 1 g metallic selenium powder in ratio of 50:10:1, respectively) + 10 mL concentrated sulfuric acid. The digestion tubes were placed in dark and digestion unit was switched on with an initial temperature of 1,000°C. Later, the temperature was raised up to 410°C. The sample turned colorless or light green color at the end of the digestion. The digested samples were analyzed for nitrogen content. Mushrooms after drying were finely grounded and 0.5 g of dried powdered mushroom samples from different treatments were weighed in 100 mL conical flask. Then, 5 mL of concentrated HNO_3 was added and allowed to stand overnight for prevention of frothing and foaming. The conical flask was placed on hot plate for 10 min. After cooling 10 mL di-acid mixture of concentrated and perchloric acid ($HClO_4$) in the ratio 9:4 was added (Jackson, 1967). Then, the digestion was carried out in hot plate till the white fumes started arising and the mixture became transparent. After cooling, 5 mL of 6 N HCl was added and volume was made up to 50 mL with distilled water. Then, samples were filtered with Whatman no 1 filter paper in to 100 mL volumetric flask and final volume make up was done. These digested samples were analyzed for P, K, and S and micronutrients by using standard methods.

Estimation of nitrogen. The digestion tubes were let to cool and then put into the automated ammonia distillation machine. The digesting unit automatically introduced 40 mL of 40% NaOH at one end of the distillation unit after fitting the tubes. Conversely, 20 mL of 4% boric acid with mixed indicator (methyl red + bromocresol green) was placed into a 250 mL conical flask on the other side of the tube. Steam at an even rate heated the digestion unit; the released ammonia was absorbed into a conical flask filled with boric acid, altering the color from pink to green. About 125 mL of distillate was collected in 5 min in this conical flask. Titrated with 0.1 N sulfuric acid, the distillate collected in the 250 mL conical flask turned from pink to green. The following formula allowed one to determine nitrogen absorption.

$$\text{Nitrogen content}\,(\%) = (\text{sample reading} - \text{blank reading}) \times \text{dilution factor}$$

Estimation of phosphorous (P). Pipette the 10 mL of the digested sample into a 50 mL volumetric flask. After that 10 mL of vanadate-molybdate reagent were added, and distilled water was used to make up the total volume after thorough mixing. After the incubation at room temperature 25°C for 30 min, the absorbance of the sample was measured under a UV–visible spectrophotometer at 420 nm (Jackson, 1967). The standard of P were prepared by taking 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, and 5.0 mL from 50 ppm stock solution of P and made volume 50 mL after adding 10 mL of vanadomolybdate reagent. The standard curve was plotted between P concentration and absorbance at 420 mm and P content in samples was computed.

Estimation of potassium (K). For estimation of total K in oyster mushroom sample, the digested sample was directly measured under Flame Photometer by using red filter (Piper, 1966).

Estimation of sulfur (S). Total sulfur concentration was calculated using a spectrophotometer by employing the barium choride turbidimetric method from oyster mushroom sample. This approach followed transferred 10 mL of the digested sample into a 50 mL volumetric flask. Then 1 mL (6 N HCl) was added, subsequently added 2 mL of 0.25% gum acacia. Then the 0.5 g $BaCl_2$ was added which created turbidity. The solution was left to react for 1 min, and measured the absorbance of the turbidity at 490 nm with the help of spectrophotometer (Chesnin and Yien, 1951).

Estimation of micronutrients (Zn, Mn, Cu, and Fe). For estimation of micronutrients (Zn, Mn, Cu, and Fe) in oyster mushroom sample, the digested sample was directly measured under Atomic Absorption Spectrophotometer (AAS) (Watson and Isaac, 1990). Micronutrients were determined at their respective wavelength using AAS (Perkin-Elmer Model 3100 Atomic Absorption). Each element was calculated based on their respective standards and expressed as mg/g. dry weight (Malavolta et al., 1989). The standard belonging to the element was fed

along with double distilled water (DDW) (0 ppm) to AAS in order to standardize the instrument. Then the sample was fed and the concentration of each element was recorded.

Protein content. The Protein content (%) in mushroom samples was estimated by multiplying the nitrogen content (%) of the *Pleurotus* spp. by the factor 6.25 (A.O.A.C., Association of Official Agricultural Chemists, 1960).

Total soluble sugar content. The method provided by Sadasivam and Manickam (1992) employed to estimate the overall solubility of the sugars. 2.5 N HCI and an anthrone reagent, 200 mg of which was dissolved in 100 mL of ice cold 95% H_2SO_4 were employed. It was prepared fresh before use. Glucose was the benchmark chosen. It dissolved as 100 mg in 100 mL of water for stock. To create the working standard, 100 mL of stock was diluted with 100 mL of distilled water. After adding several drops of toluene, the mixture was refrigerated. It was neutralized by solid sodium carbonate till the bubbling stopped. Centrifugation was done at 3,000 rpm for 5 min following raising the volume to 100 mL. After collecting the supernatant, it was aliquoted in 0.5 and 1 mL quantities for study. The standards were made from 0, 0.2, 0.4, 0.6, 0.8, and 1 mL of the working standard. The blank value was "0." Every tube, including the sample tubes had distilled water added to bring the volume to 1 mL. The 4 mL of anthrone reagent was included in it and was heated for 8 min in a bath set in boiling water. Under ice, the mixture cooled rapidly and the absorbance was recorded at 490 nm.

3 Results

3.1 Enhancement in mycelial growth by virtue of ZnONPs

The radial growth of the fungal mycelium of oyster mushrooms (*P. djamor and P. florida*) was investigated to assess the effect of ZnONPs. The supplementation of ZnONPs significantly ($p < 0.05$) affected the radial mycelium growth of oyster mushrooms on potato dextrose agar (PDA) media. Hyphal extension was measured in treated and untreated plates at five-day intervals, and the data are presented in Figure 2. The fastest radial growth of *P. djamor* (Figure 2) and *P. florida* (Figure 3) was observed on media supplemented with 20 ppm ZnONPs, followed by 40 ppm, at the 15th day compared to the control. Therefore, the minimum concentration of ZnONPs (20 ppm) enhanced the radial growth of mycelium on PDA medium. However, as the nanoparticle concentration increased, the mycelial growth of the oyster mushrooms decreased, potentially due to the toxic effects of metal ions at higher ZnONP concentrations. Concentrations of 60 ppm and 80 ppm of ZnONPs had a negative impact on the radial growth of the mycelium in both species of oyster mushrooms (Tables 2, 3).

3.2 Variation observed in morphology of oyster mushrooms in response to ZnONPs supplementation

The pilus length of all three types of oyster mushrooms was observed to be highest at 8.17 ± 0.58 cm, 7.77 ± 1.39 cm, and 8.06 ± 0.47 cm for *P. djamor* and *P. florida*, respectively, at a concentration of 20 ppm ZnONPs (Figure 3a). The pilus diameter of oyster mushrooms also increased with the use of ZnONPs, with diameters of 10.82 ± 1.53 cm in *P. djamor* and 10.25 ± 1.29 cm in *P. florida* at the 20 ppm concentration (Figure 3b).

The effect of ZnONPs on stipe width varied depending on the species of oyster mushroom. For *P. djamor*, the highest stipe width was recorded at 20 ppm (1.46 ± 0.03 cm), followed by 40 ppm (1.26 ± 0.21 cm), with the lowest stipe width observed in the control group (1.16 ± 0.08 cm). Similarly, for *P. florida*, the highest stipe width was recorded at 20 ppm (1.13 ± 0.08 cm), followed by 40 ppm (1.10 ± 0.05 cm), with the lowest value in the control group (1.06 ± 0.12 cm) (Figure 3c). The findings suggest that the 20 ppm

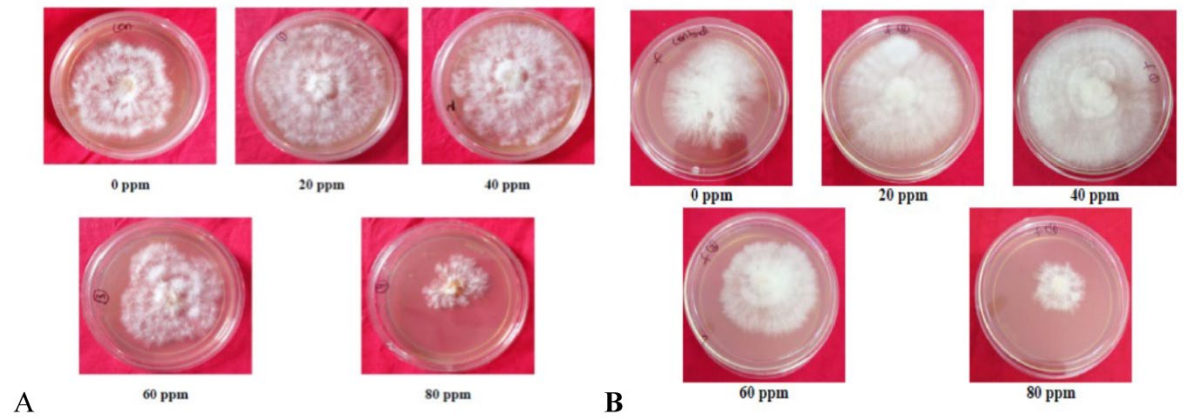

FIGURE 2
Mycelial radial growth of *P. djamor* **(A)** & *P. florida* **(B)** grown on media supplemented with different concentrations of ZnONPs. The decrease in growth area of *P. djamor* **(A)** and *P. florida* **(B)** in media supplemented with ZnOPs of 60 and 80 ppm. But an increase was noticed at 20 and 40 ppm. This sets the criteria of selection of concentration range of ZnOPs with 20 and 40 ppm for further investigation in this study.

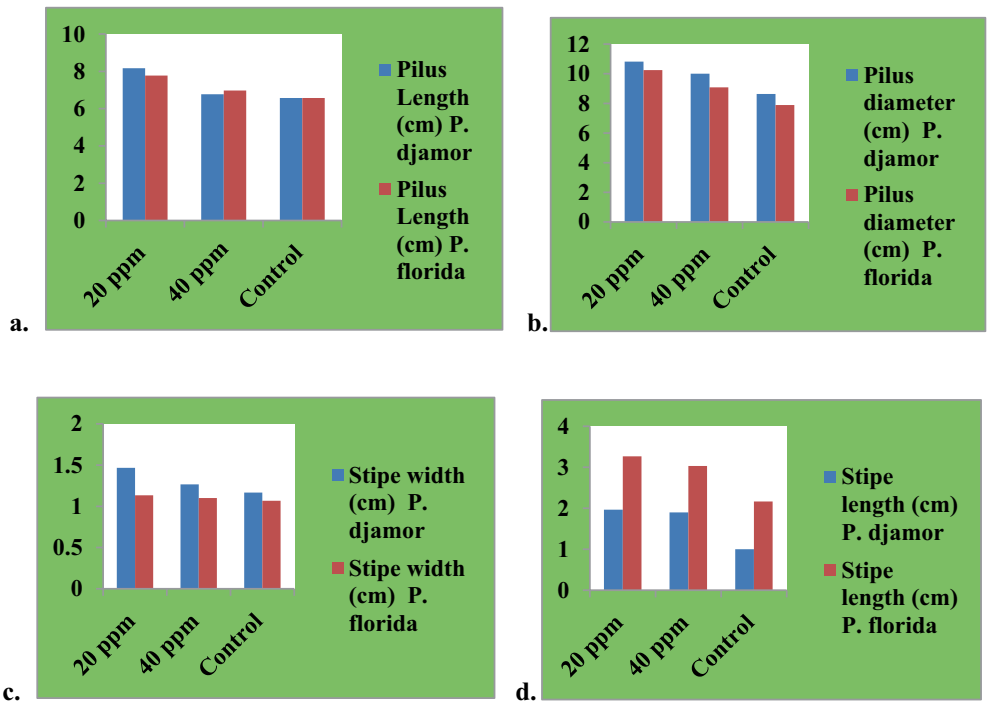

FIGURE 3
Effect of ZnONPs on morphological parameters of oyster mushroom. **(a)** Pilus length; **(b)** Pilus diameter; **(c)** Stipe width; **(d)** Stipe length with respect to 20 ppm and 40 ppm ZnOPs concentration treatments given to oyster mushrooms.

TABLE 2 Effect of ZnONPs on mycelium radial growth (mm) of *P. djamor.*

Treatment	Radial growth (cm) *P. djamor*		
	5th	10th	15th
20 ppm	2.73 ± 0.12	3.56 ± 0.17	8.73 ± 0.12
40 ppm	2.71 ± 0.25	2.93 ± 0.03	8.56 ± 0.08
60 ppm	2.46 ± 0.26	2.94 ± 0.05	6.50 ± 0.11
80 ppm	1.92 ± 0.05	2.00 ± 0.05	3.96 ± 0.03
Control	2.23 ± 0.08	2.71 ± 0.15	6.43 ± 0.26
C.D.	0.56	0.35	0.461
C.V.	12.739	6.852	3.66

This table reflects the radial growth observed at 5th, 10th and 15th day in *P. djamor* on application different concentrations of ZnOPs (ppm: parts per million, cm: centimeters, CD: Coefficient of Deviation; CV: Coefficient of Variation).

TABLE 3 Effect of ZnONPs on mycelium radial growth (mm) of *P. florida.*

Treatment	Radial growth (cm) *P. florida*		
	5th	10th	15th
20 ppm	1.50 ± 0.05	8.367 ± 0.08	8.96 ± 0.03
40 ppm	1.40 ± 0.05	6.367 ± 0.59	8.36 ± 0.18
60 ppm	1.13 ± 0.08	2.967 ± 0.43	5.53 ± 0.29
80 ppm	0.93 ± 0.08	1.633 ± 0.18	3.60 ± 0.30
Control	1.33 ± 0.06	2.767 ± 0.14	5.03 ± 0.14
C.D.	0.233	1.111	0.69
C.V.	10.039	13.637	5.95

This table reflects the radial growth observed at 5th, 10th and 15th day in *P. florida* on application different concentrations of ZnONPs (ppm: parts per million, cm: centimeters, CD: Coefficient of Deviation; CV: Coefficient of Variation).

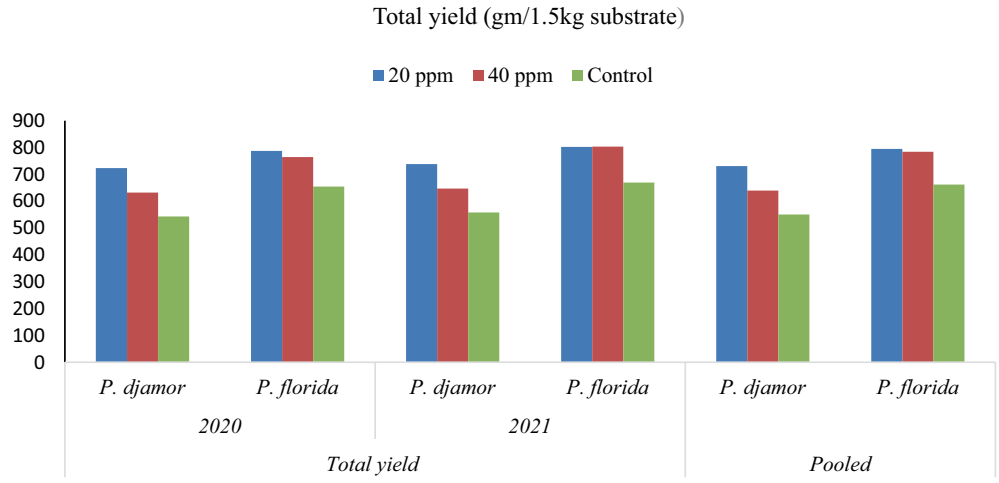

FIGURE 4
Effect of ZnONPs on the total yield of *P. djamor & P. florida*. This figure presents the comparative analysis of two oyster mushrooms with respect to control in 20 ppm and 40 ppm concentrations of ZnONPs (ppm: parts per million, cm: centimeters, CD: coefficient of deviation; CV: coefficient of variation, gm; gram, Kg: kilogram).

ZnONPs treatment positively influenced stipe width in both *P. djamor* and *P. florida*.

ZnONPs concentration also affected stipe length. Both *P. djamor* and *P. florida* exhibited increased stipe length with the application of 20 ppm and 40 ppm ZnONPs compared to the control group (Figure 3d).

3.3 Enhancement in total yield of oyster mushrooms in response to ZnONPs supplementation

The data indicated that varying concentrations of ZnONPs significantly influenced mushroom yield. At a concentration of 20 ppm, the ZnONPs produced the highest yield for *P. djamor*, with 723.27 ± 2.02 g in 2020 and 738.28 ± 3.44 g in 2021, followed by the 40 ppm concentration, which resulted in 631.99 ± 2.45 g in 2020 and 646.99 ± 2.43 g in 2021. The control group yielded 542.87 ± 1.4 g in 2020 and 557.88 ± 2.19 g in 2021.

Similarly, for *P. florida*, the application of 20 ppm ZnONPs significantly enhanced yield to 787.45 ± 2.21 g in 2020 and 802.45 ± 2.50 g in 2021, followed by the 40 ppm concentration, which resulted in yields of 764.45 ± 2.21 g and 803.38 ± 12.54 g in 2020 and 2021, respectively. In comparison, the control group yielded 654.34 ± 1.87 g in 2020 and 669.35 ± 8.70 g in 2021 (Figure 4).

3.4 Biochemical variations observed in oyster mushrooms in response to ZnOPs

The total flavonoid content increased from 30.06% at 20 ppm to 80.16% at 40 ppm in *P. djamor*, and from 43.35% at 20 ppm to 114.88% at 40 ppm in *P. florida*. Similarly, the total phenol content rose from 6.56% at 20 ppm to 53.25% at 40 ppm in *P. djamor*, and from 47.67% at 20 ppm to 139.00% at 40 ppm in *P. florida*. The total antioxidant activity also showed an increase, rising from 6.07% at 20 ppm to 13.85% at 40 ppm in *P. djamor*, and from 3.65% at 20 ppm to 4.16% at 40 ppm in *P. florida* (Table 4).

Protein content, measured as a percentage, and total soluble sugar in the fruiting bodies of oyster mushrooms were also enhanced with the application of ZnONPs (Table 5).

The enzymatic assay of five oxidative enzymes was conducted, and their activities varied with 20 ppm and 40 ppm ZnONPs concentrations, as shown in Figure 5. The specific activities of Superoxide Dismutase (SOD), Ascorbate Peroxidase (APX), Catalase (CAT), Guaiacol Peroxidase (GPX), and Polyphenol Oxidase (PPO) were measured.

In both *P. djamor* and *P. florida*, the specific activities of SOD, CAT, GPX, and PPO decreased with the addition of ZnONPs. However, the specific activity of APX showed differing trends: in *P. djamor*, it decreased with the addition of ZnONPs, while in *P. florida*, it increased.

The nutritional parameters included macronutrients *viz.*, nitrogen, phosphorus, sulfur, and potassium (Table 6) and micronutrient parameters *viz.*, Mn, Cu, Fe, and Zn (Table 7).

3.5 Statistical analysis

Experimental results were the means of three replicates measurements. The results are presented in the form of graphs and tables were subjected to ANOVA at 5% level of significance. The mean values and standard deviation for post-hoc analysis were calculated by Duncan test ($p < 0.05$) using SPSS16.00 and CRD using OPSTAT. Principal component analysis (PCA) of treatments at different concentrations and parameters were done using R software (Figure 6).

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TABLE 4 Effect of ZnOPs in total flavanoid, total phenol and total antioxidant activity of oyster mushroom.

Treatment	Total flavanoid (mg/gm catechin)				Total phenol (mg/gm GAE)				Total antioxidant activity (mg/gm AAE)			
	P. djamor	% Increase	*P. florida*	% Increase	*P. djamor*	% Increase	*P. florida*	% Increase	*P. djamor*	% Increase	*P. florida*	% Increase
20 ppm	18.43 ± 1.32	30.06	25.33 ± 1.31	43.35	22.25 ± 1.64	6.56	19.08 ± 1.26	47.67	24.500 ± 0.505	6.07	28.11 ± 0.669	3.65
40 ppm	25.53 ± 1.55	80.16	37.97 ± 49.39	114.88	32.00 ± 2.00	53.25	30.88 ± 0.75	139.00	27.37 ± 0.474	13.85	28.25 ± 1.091	4.16
Control	14.17 ± 0.40		17.67 ± 0.32		20.88 ± 1.23		12.92 ± 0.56		24.04 ± 0.946		27.12 ± 0.881	

This table represents the % increase in total flavanoid, total phenol and total antioxidant activity of Oyster Mushroom at 20 and 40 ppm concentration treatments of ZnONPs (ppm: parts per million, GAE: gallic acid equivalent, AAE: ascorbic acid equivalent, mg: milligram, gm: gram).

4 Discussion

Zn, being a cofactor for several other enzymes, including superoxide dismutase, is a strong antioxidant. It is essential for the stabilization of subcellular organelles and their membranes' molecular integrity. Furthermore vital for the metabolism of nucleic acids, proteins, carbohydrates, lipids, and secondary metabolites, all of which influence cell division, growth, and tissue healing (Soares et al., 2020). Among the key metals necessary for sustaining fundamental biological functions, zinc ranks second in terms of its association with structurally characterized enzymes (Andreini et al., 2008) and overall cellular distribution, with only iron being more prevalent (Coleman, 1992). Extensive research has been conducted to decipher the mechanisms of zinc transporters in pathogenic fungi, revealing two primary categories: the Zrt- and Irt-like Protein (ZIP) family and the Cation Diffusion Facilitator (CDF) family (Lehtovirta-Morley et al., 2017; Choi et al., 2018). The ZIP transporters, which are membrane proteins, facilitate zinc uptake into the cytosol either from the extracellular medium (Zhao and Eide, 1996) or from intracellular compartments like the vacuole and endoplasmic reticulum (MacDiarmid et al., 2000). Meanwhile, the CDF transporters mediate zinc movement from the cytosol to organelles, ensuring the continuation of zinc-dependent metabolic activities (Eide, 2006) while also rapidly lowering cytosolic zinc concentrations in response to "zinc shock" (Choi et al., 2018). Both transporter families are strictly regulated by a single transcription factor that can detect even slight fluctuations in cytosolic zinc levels (Zhao et al., 1998; Böttcher et al., 2015; Vicentefranqueira et al., 2018). A study conducted by Hoa et al. (2015) examined seven different substrate formulations, including sawdust (SD), corncob (CC), and sugarcane bagasse (SB), both individually and in 80:20 and 50:50 ratios of SD with CC or SB. The findings demonstrated that variations in substrate composition significantly influenced total colonization time, fruiting body characteristics, yield, biological efficiency (BE), as well as the nutritional and mineral content of two oyster mushroom species, *P. ostreatus* (PO) and *Pleurotus cystidiosus* (PC).

High concentrations of Zn are toxic for mushroom growth because they can negatively affect the growth of plants and decrease the contents of soluble protein in mycelia (Yap and Peng, 2019; Shuman and Li, 1997). The toxic effects of Zn on mushrooms may be attributed to high accumulation in fruiting bodies and basidiospores, leading to translocation and accumulation of Zinc in these parts causing disruption in physiological flow of nutrients (Busuioc et al., 2011). Furthermore, the heavy metal contents in mushrooms, including Zn, tend to increase until a certain dose and then decrease with higher doses (Ronda et al., 2022). This suggests that high concentration of Zn may have a detrimental effect on mushroom growth and development processes. Therefore, an optimum 20 and 40 ppm concentration of ZnONPs were utilized in this study so to observe their physical and biochemical effect on edible mushroom species worldwide.

Several studies suggested that nanoparticles can improve the growth and productivity of crop plants. Zn biofortification positively affects the morphological parameters of crops (García-Gómez et al., 2018; Barman et al., 2023; Cakmak and Kutman, 2018). Agronomic practices such as the application of fertilizers in soil, nutri-priming, and foliar spray enhance the availability and uptake of Zn in crops,

TABLE 5 Effect of ZnONPs on total soluble sugar and protein content in fruiting body of oyster mushroom.

Treatment	Protein %				Total soluble sugar (gm/100 gm of dry wt.)			
	P. djamor	% Increase	*P. florida*	% Increase	*P. djamor*	% Increase	*P. florida*	% Increase
20 ppm	29.43 ± 0.042	3.51	20.95 ± 0.196	1.25	3.25 ± 0.004	14.43	3.25 ± 0.073	32.65
40 ppm	29.25 ± 0.28	2.88	20.81 ± 0.175	0.57	2.88 ± 0.039	1.40	2.66 ± 0.042	8.57
Control	28.43 ± 0.429		20.69 ± 0.041		2.84 ± 0.01		2.45 ± 0.005	

This table represents percent increase in the protein and total soluble sugar content in fruit body extracts of *P. djamor* and *P. florida* (%: percent, ppm: parts per million, gm: gram, wt: weight).

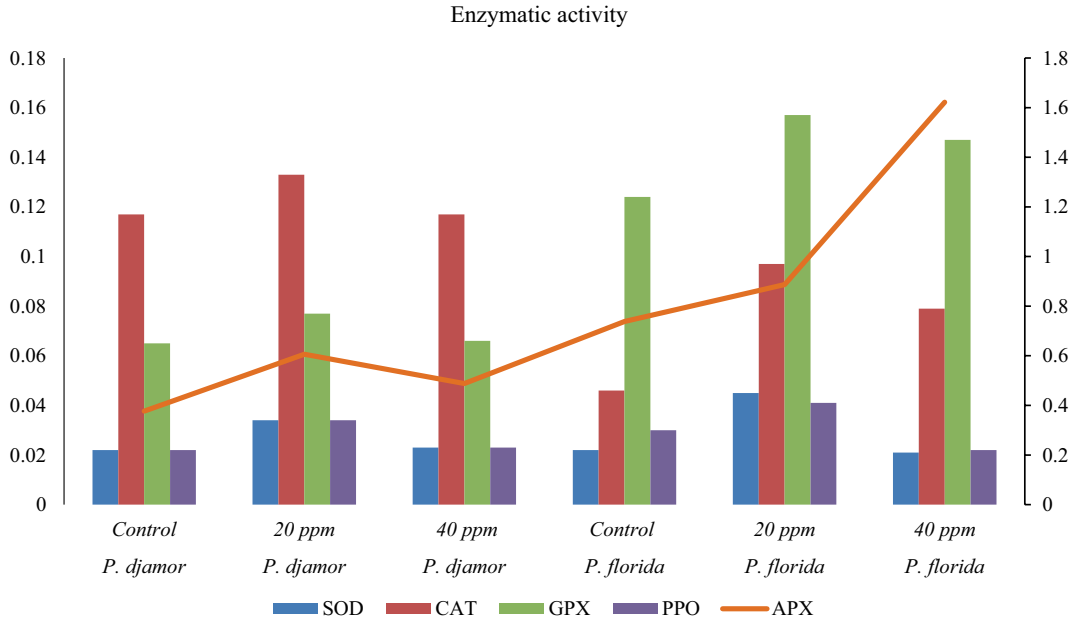

FIGURE 5
Enzymatic activities of different enzymes at 20 and 40 ppm of extracts from *P. djamor* and *P. florida*. This figure represents the variation in enzymatic activities of different enzymes in fruit body extracts of oyster mushrooms at 20 and 40 ppm concentration treatments with ZnONPs (ppm: parts per million).

leading to significant improvements in growth, development, and yield attributes. Additionally, genetic strategies, including the modulation of genes involved in Zn homeostasis, have shown promising results in increasing Zn accumulation (Khush et al., 2012). Furthermore, foliar application and agronomic biofortification have been effective in enhancing the micronutrient content of forage crops, such as Zn and copper (Cu), thereby improving forage productivity and quality. Overall, Zn biofortification through various agronomic and genetic approaches has proven to be a valuable strategy for enhancing the morphological parameters of crops and addressing nutritional deficiencies.

Earlier *Agaricus bisporus* growth, measured by Singh, 2021 in terms of average body weight, body size, pileus width and stipe thickness, observed to be maximal on 9 ppm of iron oxide and 12 ppm of iron sulfide nanoparticles supplemented spawns whereas stipe length were maximum at 15 ppm in both the cases. Similarly, Shivani (2021) measured growth characteristics of *Pleurotus* spp., namely pileus breadth, stipe length, and stipe width, were recorded at 10 ppm concentration of Zn sulfate nanoparticles was greater than the control, although the growth parameters in the measurement decreased as the nanoparticles concentration increased. Despite this, the treated substrate's growth parameter measurement was higher than the control. According to the study conducted by Sahu (2018) showed better results in the 7 ppm (minimum) concentration of Iron sulfide nanoparticles for *Pleurotus florida*, as determined by stipe length, stipe breadth, pileus length and pileus width. Interestingly, there is no particular literature available on other *Pleurotus* species. It has been observed that the growth media augmented with 7 ppm iron sulfide nanoparticles helped to enhance the mycelial growth rate of *P. florida*. This approach also reduced required times for the spawn run's and mycelial colonization's. The iron concentration at 7 ppm as well as the economic yield was noted to be greater than the control.

A study by Poursaeid et al. (2015) revealed that adding zinc to the culture media considerably boosted mycelium development and fruiting body production of *P. florida*, with the best yields obtained at zinc concentrations of 100 mg/liter and 150 mg/liter respectively, Reduced mycelium development and fruiting body production yield in the culture media when zinc (200 mg/L and 300 mg/L) concentrations were higher than in the control medium. Zn positively affects the yield of mushrooms by increasing their content and uptake of the element. The addition of Zn to the growing medium of *Agaricus bisporus* mushrooms resulted in a significant increase in mushroom yield (Spiżewski et al., 2022). The high-yield mushroom culture medium included Zn sulfate as one of the components, providing necessary nutritional substances for the growth of edible mushrooms (Rácz et al., 1998). The use of a

TABLE 6 Effect of ZnONPs on macronutrients in fruiting body of oyster mushroom.

Treatment	Nitrogen content (%)				Phosphorus content (%)				Sulfur content (%)				Potassium content (%)			
	P. djamor	% Increase	*P. florida*	% Increase	*P. djamor*	% Increase	*P. florida*	% Increase	*P. djamor*	% Increase	*P. florida*	% Increase	*P. djamor*	% Increase	*P. florida*	% Increase
20 ppm	4.71 ± 0.044	3.51	4.58 ± 0.083	18.34	0.73 ± 0.018	14.43	0.65 ± 0.008	9.24	3.27 ± 0.102	2.18	3.21 ± 0.026	2.65	1.29 ± 0.009	67.29	1.16 ± 0.015	33.33
40 ppm	4.68 ± 0.021	2.69	4.51 ± 0.074	16.54	0.48 ± 0.007	1.40	0.63 ± 0.015	5.88	3.21 ± 0.038	0.53	3.17 ± 0.054	1.59	1.24 ± 0.03	61.43	0.97 ± 0.01	11.83
Control	4.55 ± 0.095		3.87 ± 0.007		0.47 ± 0.004		0.59 ± 0.002				3.12 ± 0.059		0.77 ± 0.006		0.87 ± 0.01	

This table represents the percent increase in macronutrients of oyster mushrooms (%: percent, ppm: parts per million).

TABLE 7 Effect of ZnONPs on micronutrient content in fruiting body of oyster mushroom.

Treatment	Zinc content (mg/100 gm of dry wt.)				Copper content (mg/100 gm of dry wt.)				Iron content (mg/100 gm of dry wt.)				Manganese content (mg/100 g of dry wt.)			
	P. djamor	% Increase	*P. florida*	% Increase	*P. djamor*	% Increase	*P. florida*	% Increase	*P. djamor*	% Increase	*P. florida*	% Increase	*P. djamor*	% Increase	*P. florida*	% Increase
20 ppm	164 ± 1.857	126.83	77.59 ± 0.171	4.44	16.7 ± 0.156	9.1	45.4 ± 0.751	14.9	113.00 ± 0.92	1.43	152.00 ± 0.55	26.77	32.50 ± 0.54	0.10	36.90 ± 0.93	13.50
40 ppm	116.1 ± 2.055	60.58	146.00 ± 0.278	96.52	41.8 ± 1.044	17.32	46.9 ± 0.562	18.7	141.40 ± 1.24	26.93	193.00 ± 4.01	60.96	34.70 ± 0.56	6,76	45.60 ± 1.13	40.30
Control	72.3 ± 1.692		3.87 ± 0.007		15.3 ± 0.096		39.5 ± 0.329		111.29 ± 2.43		119.90 ± 2.6		32.50 ± 0.64		32.50 ± 0.70	

This table represents the percent increase in micronutrients of oyster mushrooms (%: percent, ppm: parts per million).

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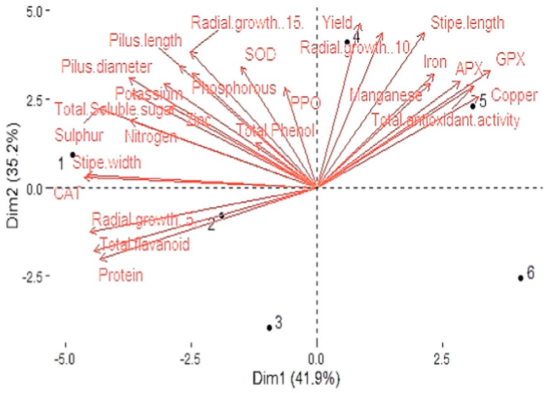

FIGURE 6
Principal component analysis (PCA) of treatments, mushroom varieties and different treatments.

nutrient containing Zn in the production method of mushroom crops led to a yield increase of more than 90% (Paswan and Verma, 2014). The mushroom stick prepared with a nutrition additive that included Zn showed an increase in mushroom yield by 15–20% (Carrasco et al., 2018). Overall, the addition of Zn to the growing medium or nutrient formula positively influenced the yield of mushrooms, leading to higher production.

In a study performed by Shivani (2021) it was observed that in *P. sajorcaju*, the maximum phenol content was obtained with the maximum concentration 40 ppm (2.42 mg GAE/g) of Zn sulfate nanoparticles followed by 30 ppm (2.11 mg GAE/g), 20 ppm (1.89 mg GAE/g) and 10 ppm (1.63 mg GAE/g) as compared to control (1.26 mg GAE/g). Whereas, in *P. florida*, the maximum phenol content was obtained with the maximum concentration 40 ppm (2.46 mg GAE/g) followed by 30 ppm (2.25 mg GAE/g), 20 ppm (1.88 mg GAE/g) and 10 ppm (1.62 mg GAE/g) as compared to control (1.42 mg GAE/g). Similarly, *P. flabellatus*, the maximum phenol content was obtained with the maximum concentration 40 ppm (2.47 mg GAE/g) followed by 30 ppm (2.29 mg GAE/g), 20 ppm (2.06 mg GAE/g) and 10 ppm (1.95 mg GAE/g) as compared to control (1.58 mg GAE/g). Similar study done by Singh (2021) measured total flavonoids content of *Agaricus bisporus* improved on nanoparticles treatment, as there was a 52.77% increase in flavonoid content in case of iron oxide at before casing stage and 45.49% in case of iron sulfide nanoparticles treatment. It has been previously reported that Zn application significantly increased the content of flavonoids, which protected plants from salinity-induced ROS (Shao et al., 2023).

No literature reports are available on the effect of ZnONPs on anti-oxidative activity of oyster mushroom. But as per the study by Mohammadhasani et al. (2017) found that Zn toxicity increased lipid peroxidation and oxidative enzyme activities in pistachio trees colonized by the ecto-mycorrhizal fungus *A. bisporus*. Earlier (Jiang et al., 2009) showed that high Zn concentrations decreased superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities in *Lentinus giganteus* fruit bodies, leading to membrane lipid peroxidation. Kim et al. (2011) demonstrated that ZnONPs affected soil enzyme activity, with a significant decrease in dehydrogenase activity. These findings indicate that Zn can have both positive and negative effects on enzymatic activity in mushrooms, depending on the concentrations of Zn. According to Ali et al. (2022) ZnONPs have a beneficial effect on barley plants by enhancing the enzyme activations and stimulating the physiological and vegetative characteristics. Because Zn is a coenzyme, the nano-scale composition of ZnONPs has a significant impact on general vegetative and physiological features, particularly in enzyme activity (Liang et al., 2023; Shan et al., 2025; Chen et al., 2024; Huang et al., 2025).

The current investigation is first of its kind in assessing the changes in physical and biochemical attributes of largely edible mushroom species worldwide. The non-employment of synthetic chemicals and pesticides in the present research scenario for growing and cultivating the mushrooms using the waste pools of cereal crops (the spawning substrate) makes it impactful. This critically ensures the sustainable management of agro-ecosystems waste. The specific 20 and 40 ppm ZnONPs concentration scale varying effects on edible mushrooms targeted for biofortification values are elucidated and reported here in accordance to the aims and goals of sustainable agricultural management practices.

5 Conclusion

Biofortification was successfully achieved, as the mycelial radial growth of *Pleurotus* spp. was maximized at a concentration of 20 ppm ZnONPs, with growth declining at higher concentrations. The application of ZnONPs significantly enhanced growth parameters, including pilus length, pilus diameter, stipe length and stipe width, particularly at the 20 ppm concentration. Furthermore, the yield of oyster mushrooms increased notably with the 20 ppm ZnONPs treatment. Both macronutrient and micronutrient content improved with ZnONPs application, indicating its potential to enhance the biochemical and nutritional value of oyster mushrooms, although the effects may vary by species. The results also suggest that ZnONPs could serve as a potential antioxidant agent in oyster mushrooms. In an overall enhancement in physical growth parameters and immunity boosting parameters the another advantage of this research is that it has been done while obeying the terms and conditions of sustainable agricultural practices as no synthetic chemical and pesticide has been utilized. In all, the consumption of edible mushrooms when reciprocated with added nutritional benefits the chances of alleviating the malnutrition becomes minimal. To attain such a nutritional benefit, more emphasis should be laid on incorporating the essential metal ions and minerals in edible mushrooms like *Pleurotus* spp. so as to increase their reach in the global market for general public. However, worldwide labs are doing the task of nutritional enhancement in edible mushrooms a more detailed analysis of mineral or, metal ion assimilation in natural biosynthetic pathway of edible mushrooms is in the need of hour so as to eliminate any toxicity. Future research should focus on elucidating the mechanisms underlying multiple nutrient interactions to better understand and mitigate the harmful effects of nutrient deficiencies while suggesting crop-wide incorporation of ZnONPs in the agriculture sector.

Data availability statement

The data that support the findings of the study are available from the corresponding author upon reasonable request.

Ethics statement

Written informed consent was obtained from the individual for the publication of any identifiable images or data included in this article.

Author contributions

Leema: Writing – original draft, Writing – review & editing. ShG: Formal analysis, Writing – original draft, Writing – review & editing. DG: Formal analysis, Methodology, Writing – review & editing. HP: Conceptualization, Writing – review & editing. FA: Supervision, Validation, Writing – review & editing. SaG: Conceptualization, Writing – review & editing. GB: Supervision, Validation, Writing – review & editing. AM: Validation, Writing – review & editing. SS: Supervision, Validation, Writing – review & editing.

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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.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2025.1504781/full#supplementary-material

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