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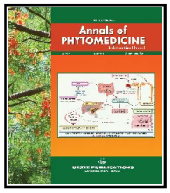

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Influence of selenium biofortification on the biochemical and nutraceutical properties of *Pleurotus* species

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Abstract

A vital mineral, selenium serves a variety of physiological purposes and is crucial to human health. Risks associated with an inadequate intake of selenium are Alzheimer's or Parkinson's disease, male sterility, and abnormal functioning of the thyroid gland. It is well known that mushrooms have exceptional nutraceutical qualities and can effectively mobilize and accumulate different mineral elements from growth media. However, selenium can be toxic to mushrooms based on levels. Hence, it is crucial to screen selenium concentration for the production of selenium biofortified mushrooms. The present research was carried out in the mushroom laboratory at Punjab Agricultural University, Ludhiana, Punjab, India. The present study involved the culturing of *Pleurotus ostreatus* var. florida and *Pleurotus ostreatus* var. eryngii on a medium supplemented with selenium at several concentrations; namely, 0, 5, 10, 15, and 20 mg/l. The results showed maximum radial mycelial growth (9.00 ± 0.25 mm/day) and biomass (88.0 ± 3.20 g/l) in 5 mg/l selenium supplementation for *P. ostreatus* var. florida. The total soluble protein was increased from 1.21 ± 0.10 to 2.28 ± 0.16 mg/g with selenium enrichment. The maximum phenolic (24.22 ± 0.87 mg GAE/g) and flavonoids (462.61 ± 17.84) contents were determined in *P. ostreatus* var. eryngii under 15 mg/l selenium. In addition, scanning electron microscopy and energy dispersive spectroscopy analysis showed maximum weight and atom per cent of oxygen was observed in *P. ostreatus* var. eryngii grown on a selenium-free medium. Furthermore, decreased new hyphae, increased fungal cell wall thickness, and septa number were examined.

1. Introduction

The amount of selenium (Se) in similar kinds of foods varies greatly due to varied origins and growing conditions. Therefore, Se enrichment may be needed to meet its requirement in the human body (Khurana *et al.,* 2019). Selenium which is a constituent of selenoproteins is essential for the body for its physiological activities (Niedzielski *et al.,* 2015). Selenium is an important mineral that plays a vital role in several metabolic pathways like thyroid hormone metabolism, immune system, and antioxidant defense systems (Bhatia *et al.,* 2014). Risks associated with an inadequate intake of selenium were Alzheimer's or Parkinson's disease, male sterility, and abnormal functioning of the thyroid gland (Damato *et al.,* 2020). *In vitro* experiments with rodents reported that the bioactivity and bioavailability of Se varies greatly with the type and concentration. Cellular effects and metabolism vary greatly when selenium is supplemented in organic and inorganic forms (El-Bayoumy, 2001; Kumar *et al*., 2023). Among the various chemical forms of selenium like inorganic and organic, the bioavailability and bioactivity are higher in the organic form. Furthermore, most of the world's population is said to have a sufficient amount of selenium in their diet, several parts of the world like Europe and China where the selenium content of the soil is very low are at high levels of risk due to selenium deficiency (Kuriaa *et al.,* 2020). The recommended dietary allowance (RDA) for children between the age group of 1 to 3 years is 20 µg/day, while the adults and children above 14 years 55 µg/day, and for breast feeding mothers it is 70 µg/day (Mehrotra, 2021; Choudhary *et al*., 2023).

Brazilian nuts, veggies, seafood, and grains were the different sources of selenium. According to Murphy and Cashman (2001), the average selenium concentration in Irish foods, such as gluten-free bread and canned tuna in brine, ranges from 3 µg kg^{-1} dry weight to 701 µg kg^{-1} DW. The study conducted by Klapec *et al*. (2004) involved the examination of a range of foods gathered from rural areas in Eastern Croatia. The results indicated that the concentration of Se in the foods ranged from 7.9 µg kg^{-1} dry weight in tomato to 859.2 µg kg^{-1} DW in canned tuna. Certain wild edible mushrooms, such as *Cantharellus cibarius* or *Xerocomus badius*, have a concentration of Se ranging from less than 0.5 mg kg^{-1} dry weight to more than 20 mg kg^{-1} dry weight, although they are only available during a specific season. Thus, commercially valuable farmed mushrooms such as *Pleurotus, Pholiota*, or *Agaricus* can be grown on selenium enriched substrates to produce them year-round (Bhatia *et al*., 2013; Karuppiah *et al*., 2021). The mushrooms are well known for their exceptional capacity to mobilize and accumulate a variety of mineral elements from their developing environment. However, the degree to which they can mobilize and accumulate varies widely among species when

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grown on selenium enriched substrates, mushrooms such as *Cordyceps militaris* (Dong *et al*., 2013), *P. ostreatus* (Yan and Chang, 2012), and *Lentinula edodes* (Ogra *et al*., 2004) shown a multiplicity of increases in the Se content of fruiting bodies. It is observed that the *Cordyceps indica* supplemented with selenium at 0, 2.5, and 5 µg/ml did not significantly affect the yield as reported by Rathore *et al.* (2018). However, higher concentrations of Se such as 5 mg/ml showed a negative effect on the yield and 40 mg/ml inhibited the growth Therefore, the estimation of the highest concentration of Se in the culture media that does not show adverse effects on the mycelial growth is very crucial to deciding the selenium concentration of substrate used for the cultivation of mushrooms. Therefore, in addition to morphological variations in hyphae when supplemented with different concentrations of selenium, the cultures of *P. ostreatus* var. florida and *P. ostreatus* var. eryngii were chosen for the evaluation of the radial growth, biomass production, selenium content, total soluble protein content, phenolic content, free radical scavenging activity, and total flavonoids.

2. Materials and Methods

2.1 Procurement of *P. ostreatus*

The studies utilized cultures of *P. ostreatus* var. florida *Cetto* and *P. ostreatus* var. eryngii (DC. ex Fr.), which were obtained from the germplasm collection bank of the Department of Microbiology at PAU, Ludhiana, India (Figure 1).

2.2 Mineral estimation methodologies

PDA (potato dextrose agar) and cultures of *P. ostreatus* var. florida and *P. ostreatus* var. eryngii were filled in 90 mm petri plates. Sodium selenium was added at different concentrations: 0 (control), 5, 10, 15, and 20 mg/l. The plates were incubated at 27 ± 1°C for 7 days after being injected with 6 mm sized mycelial pieces. For almost a week, the colony diameter in millimeters was recorded through daily observation of the plates. In 500 ml erlenmeyer flasks with 100 ml of medium each, potato dextrose broth was produced for biomass estimation. Sodium selenate was added to the flasks at several concentrations: 0 mg/l to 5, 10, 15, and 20 mg/l. 6 mm diameter mycelial agar fragments of *P. ostreatus* var. florida and *P. ostreatus* var. eryngii were used to inoculate the flasks. After that, the flasks were incubated for 20 days at 27 ± 1°C. Later, the flasks were removed, the mycelium was filtered using filter paper to remove it from the broth, and the fresh weight was calculated as g/l. ICP-MS was used to assess the selenium concentration of *Pleurotus* spp. mycelium. With minor adjustments, *Pleurotus* spp. mycelium was collected and treated under the typical methodology for handling biological specimens (Bozzola and Russell, 1999). Sputter coating and SEM viewing were performed on the treated sample. The sample surfaces underwent elemental analysis using an energy dispersive spectroscopy system connected to an SEM (Jeol JSM-6100, USA) model. The method developed by Lowry *et al*. (1951) was used to assess the total soluble protein content of the mushroom mycelium. With only minor adjustments, the total phenolic content was calculated using the method of Swain and Hillis (1959). According to Blois (1958), the percentage of free radical scavenging activity for the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was determined for both *Pleurotus* species. Using the method outlined by Zhishen *et al*. (1999), the total flavonoids from the *Pleurotus* spp. mycelium was estimated.

***Pleurotus ostreatus* var. florida**

***Pleurotus ostreatus* var. eryngii**

Figure 1: *Pleurotus* species utilized for selenium supplementation.

2.3 Statistical analysis

All the experimental results were represented as the average mean (standard deviation) of three replicates. Data were evaluated by using Statistica 13.1 (StatSoft, USA) statistical software with one-way analysis of variance, and Tukey's multiple comparison procedure was used. The results were marked with identical letters in rows or columns exhibiting no differences at the significance level $\alpha = 0.01$.

3. Results

The diameter of the mycelium was measured on agar plates at 24 h intervals for 7 days to calculate the growth rate. Both the *Pleurotus*

spp. showed varied responses to selenium supplementation in the growth medium. The addition of sodium selenate @10 mg/l showed a decreasing trend at 15 and 20 mg/l selenium concentration, whereas in *P. ostreatus* var. florida selenium concentration at 5 mg/l was observed in enhancing the growth rate (9.00 ± 0.25 mm/day) as compared to other concentrations (Table 1). In the case of *P. ostreatus* var. eryngii, there was an increasing trend in the growth rate with increasing selenium concentration with 20 mg/l showing the highest growth rate (8.56 ± 0.22 mm/day). For biomass production, *Pleurotus* spp. mycelia were also grown on selenium supplemented and non selenium supplemented potato dextrose broth. In the case of *P. ostreatus* var. florida, the highest biomass production was observed in broth supplemented with 5 mg/l selenium concentration (88.0 ± 3.2 g/l), and then a decreasing trend was observed with increase in selenium concentration from 10 mg/l to 20 mg/l. In the case of *P. ostreatus* var. eryngii, the biomass production increased with an increase in Se concentration from 0 to 20 mg/l and the highest biomass production was observed at 20 mg/l (77.0 ± 2.3 g/l) Se concentration (Table 1). Selenum content was estimated from the Se enriched biomass of *Pleurotus* spp. using ICP-MS. In the control samples, a very low amount of Se was detected, *i.e.,* in *P. ostreatus* var. florida (2.2 ± 0.46 µg g^{-1} dw) and *P. ostreatus* var. eryngii (0.21 ± 0.04 µg g^{-1} dw). As the concentration of supplemented Se increased from 5 to 20 mg/l, the amount of Se accumulated in the biomass of *Pleurotus* spp. also increased, with the highest accumulation detected at 20 mg/l Se supplementation in *P. ostreatus* var. florida (880.61 µg g^{-1} dw) and *P. ostreatus* var. eryngii (850.27 µg g^{-1} dw).

Table 1: Effect of selenium supplementation on the radial growth, biomass production, and total selenium content in *Pleurotus* spp.

Se supplementation (mg/l)	Radial growth (mm/day)		Biomass (g/l)		Total selenium (µg g^{-1} dw)	
	P. ostreatus var. florida	*P. ostreatus* var. eryngii	*P. ostreatus* var. florida	*P. ostreatus* var. eryngii	*P. ostreatus* var. florida	*P. ostreatus* var. eryngii
5	9.00 ± 0.25	6.10 ± 0.09	88.0 ± 3.20	57.5 ± 2.10	240.67 ± 12.98	210.38 ± 21.88
10	7.70 ± 0.29	6.97 ± 0.14	74.7 ± 1.90	60.9 ± 1.60	409.28 ± 15.78	394.22 ± 16.67
15	7.20 ± 0.32	8.32 ± 0.28	58.8 ± 1.30	65.3 ± 2.30	579.81 ± 14.61	562.00 ± 14.74
20	6.40 ± 0.17	8.56 ± 0.22	58.7 ± 1.50	77.0 ± 2.30	880.61 ± 15.78	850.27 ± 20.78
0 (Control)	8.20 ± 0.29	5.56 ± 0.15	63.5 ± 4.40	43.2 ± 1.60	2.20 ± 0.46	0.21 ± 0.04
CD (5 %)	0.85	0.59	8.61	6.40	41.79	52.81

Note: ± values indicated standard error

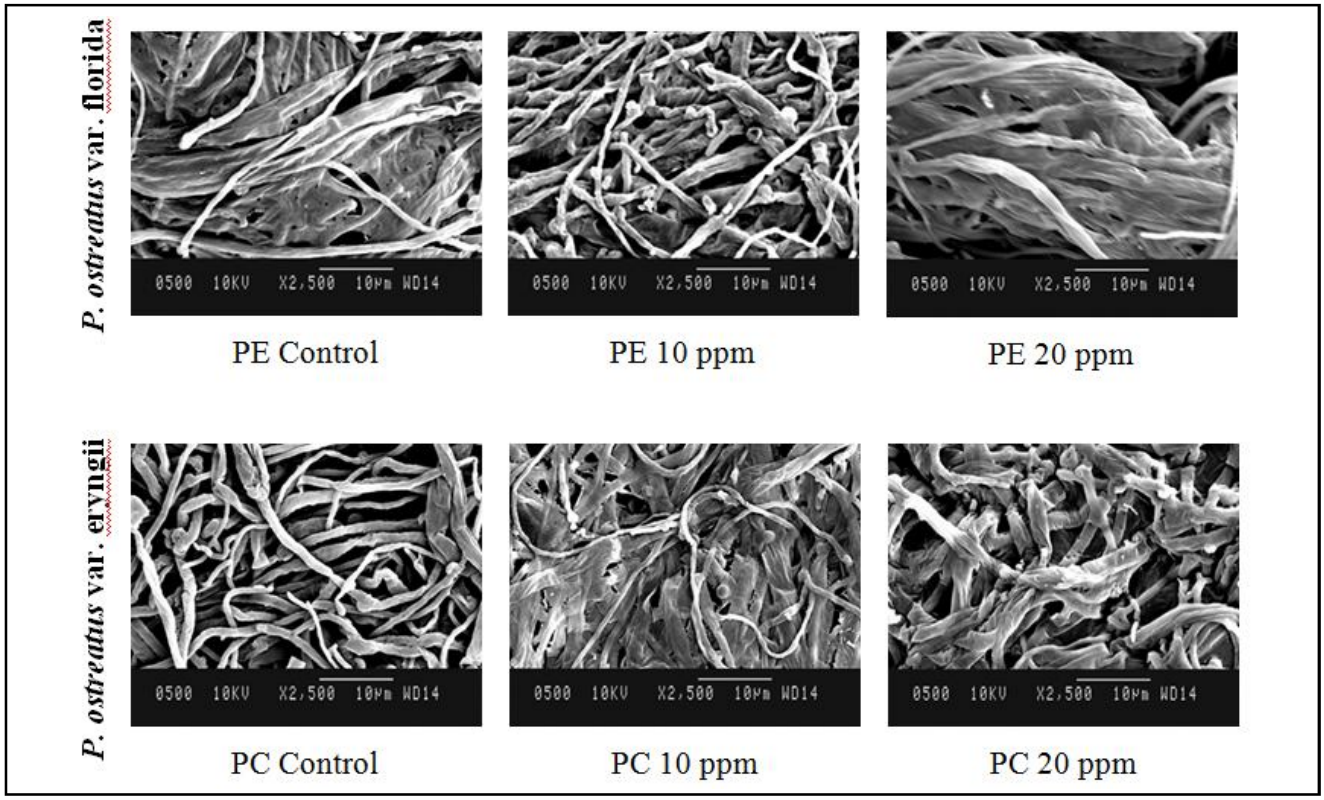

Figure 2: Morphological variations in hyphae of *Pleurotus ostreatus* var. florida and *P. ostreatus* var. eryngii at 0, 10, and 20 mg/l sodium selenate concentration.

The relative occurrence of different elements on the hyphal surface was determined through scanning electron microscopy and energy dispersive spectroscopy analysis. Scanning electron microscopy showed that selenium stress led to a decrease in average hyphal diameter in all four *Pleurotus* species, *i.e.*, *P. ostreatus* var. florida and *P. ostreatus* var. eryngii. In both the species, the hyphal diameter increased @ 10 mg/l Se concentration as compared to control samples. At 20 mg/l Se concentration, the hyphal diameter decreased in both species. This shows that *P. ostreatus* var. florida and *P. ostreatus* var. eryngii have a mechanism to tolerate selenium stress. This also shows that 10 mg/l Se concentration was best for the growth of *P. ostreatus* var. florida and *P. ostreatus* var. eryngii. The mycelial texture of both the *Pleurotus* spp. became coarser and fragile in response to the increased concentration of Se (Figure 2). The numerous thin and long new hyphae appeared in the control treatment as compared to 10 mg/l and 20 mg/l Se concentrations in both the *Pleurotus* spp.: namely, *P. ostreatus* var. florida and *P. ostreatus* var. eryngii. As the concentration of selenium increased, the appearance of new hyphae decreased, besides exhibiting an increase in thickness of the fungal or hyphal cell wall and a reduction in hyphal length. The number of septa and hyphal branching increased, while the clamp connections decreased. The hyphal density increased in 10 mg/l and 20 mg/l Se concentration as compared to the control sample in both species (Figure 2).

Energy dispersive spectroscopy is a valuable method for analyzing the distribution and concentration of various elements on sample surfaces. Using SEM-EDS for elemental analysis of *Pleurotus* spp. mycelia supplemented with different concentrations of Se, signals typical for selenium were observed on the surface of *P. ostreatus* var. florida and *P. ostreatus* var. eryngii mycelia, indicating that the selenium was integrated into the cell wall components of fungal mycelia. The maximum percentage weight and atomic percentage of selenium were observed in the 10 mg/l Se supplementation treatment in both *Pleurotus* spp. The Se content in percentage weight and atom percentage increased at 10 mg/l Se concentration and then decreased at 20 mg/l Se concentration as compared to control samples which exhibited the lowest Se concentration. Percentage weight and atom percentage of oxygen in *P. ostreatus* var. florida increased at 20 mg/l Se concentrations as compared to control and 10 ppm, but it is inversely associated with *P. ostreatus* var. eryngii, showing the decrease in the percentage weight and atomic percentage of oxygen at 10 mg/l and 20 mg/l Se concentration as compared to control (Table 2). Due to their shared grouping in the periodic table, selenium and oxygen may have replaced one another, resulting in a drop in the concentration of oxygen. The percentage weight of carbon slightly decreased and the atomic percentage of carbon slightly increased with an increase in Se concentration in the case of *P. florida* and in the case of *P. eryngii* the percentage weight of carbon slightly increased and the atomic percentage of carbon slightly decreased (Figure 3). From the vegetative mycelium of *Pleurotus* species cultivated on selenium-enriched broth supplemented with 5 mg/l, 10 mg/l, 15 mg/l, and 20 mg/l of selenium, as well as a control group of non-supplemented broth, the total soluble protein content was calculated. In *P. ostreatus* var. florida, the highest total soluble protein content (2.28 ± 0.16 mg/g) was found at 5 mg/g of Se concentration. The total soluble protein content of mycelium increased with an increase in Se concentration in *P. ostreatus* var. *eryngii* and the highest total soluble protein content was observed at 20 mg/l Se concentration (2.55 ± 0.13 mg/g). In the case of *P. ostreatus* var. florida there was an increasing trend in the total soluble protein content of mycelium up to a Se concentration of 10 mg/l and then a decreasing trend was observed (Table 3). The total soluble mycelial protein content of control samples was found to be highest in *P. ostreatus* var. eryngii (1.52 ± 0.09 mg/g), followed by *P. ostreatus* var. florida (1.21 ± 0.10 mg/g). There was a significant difference in the total soluble protein content of both the *Pleurotus* spp. mycelium supplemented with different doses of selenium.

Table 2: Scanning electron microscopy energy dispersive spectroscopy analysis of *P. ostreatus* var. florida and *P. ostreatus* var. eryngii at 0, 10 and 20 mg/l sodium selenate concentration

Species	Sodium selenate concentration in medium (mg/l)	Weight (%)			Atom (%)		
		Carbon	Oxygen	Selenium	Carbon	Oxygen	Selenium
P. ostreatus var. florida	0 (Control)	57.16	42.67	0.17	64.07	35.90	0.03
	10	56.72	42.57	0.71	63.88	36.00	0.12
	20	55.78	44.02	0.20	62.77	37.19	0.03
P. ostreatus var. eryngii	0 (Control)	48.95	50.60	0.45	42.04	57.89	0.08
	10	57.46	41.73	0.81	64.63	35.23	0.14
	20	49.33	50.23	0.44	56.63	43.29	0.08

The total phenolic content, the percent radical scavenging activity, and total flavonoids were determined from the vegetative mycelium of *Pleurotus* spp. grown on selenium enriched broth supplemented with 5 mg/l, 10 mg/l, 15 mg/l and 20 mg/l of selenium along with non-supplemented broth as control. The total phenolic content of mycelium was found to be highest at 15 mg/l Se concentration in *P. ostreatus* var. eryngii (24.22 ± 0.87 mg GAE/g). Whereas, in *P. ostreatus* var. florida (23.23 ± 0.67 mg GAE/g), the highest phenolic content was found at 10 mg/l Se concentration, the response decreased significantly with the higher application of the Se in the broth (Table 3). The highest radical scavenging activity per cent and total flavonoids were found at 15 mg/l Se concentration in *P. ostreatus* var. eryngii (10.86 ± 0.19 % and 462.61 ± 17.84 µg QE/g, respectively). Whereas, in *P. ostreatus* var. florida the highest radical scavenging activity percent and total flavonoids (11.23 ± 0.18 % and 522.7 ± 8.11 µg QE/g, respectively) were found at 10 mg/l Se concentration. There was a significant difference in the total phenolic content, total flavonoids, and radical scavenging activity percentage of both the *Pleurotus* spp. supplemented with different doses of selenium as compared to control (Table 3).

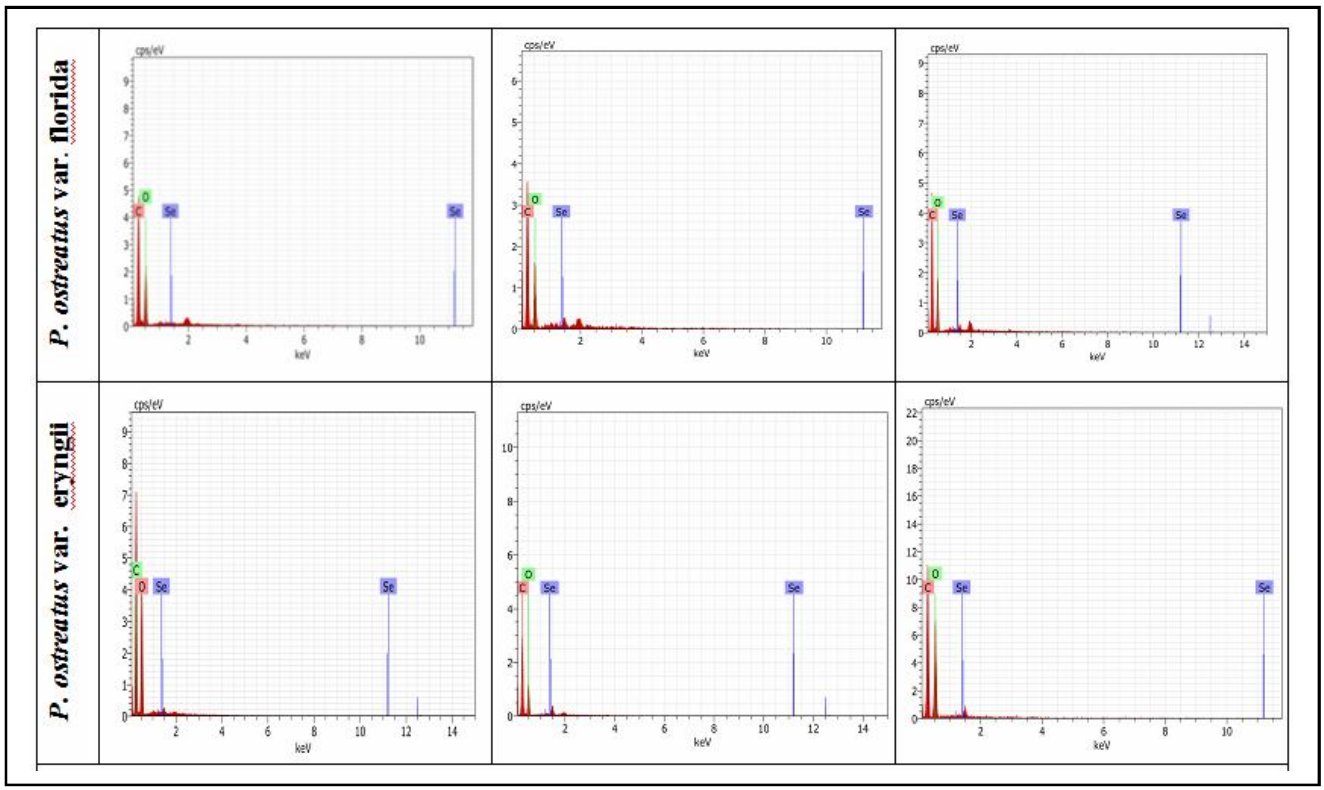

Figure 3: Energy dispersive X-ray spectroscopy signals for *P. ostreatus* var. florida and *P. ostreatus* var. eryngii at 0, 10, and 20 mg/l Se concentration.

Table 3: Effect of Se supplementation on the total soluble protein, radical scavenging activity (%), phenolic and flavonoid content of *Pleurotus* spp. mycelium

Se supplementation (mg/l)	Total soluble protein content (mg g^{-1})		Radical scavenging activity (%)		Total phenol content (mg GAE/g)		Flavonoid content (µg QE/g)	
	P. ostreatus var. florida	*P. ostreatus* var. eryngii	*P. ostreatus* var. florida	*P. ostreatus* var. eryngii	*P. ostreatus* var. florida	*P. ostreatus* var. Eryngii	*P. ostreatus* var. Florida	*P. ostreatus* var. Eryngii
5	2.28 ± 0.16	1.57 ± 0.02	8.76 ± 0.28	5.23 ± 0.14	11.09 ± 0.11	12.67 ± 0.15	189 ± 3.08	210.11 ± 5.71
10	2.08 ± 0.10	2.03 ± 0.20	11.23 ± 0.18	7.98 ± 0.10	23.23 ± 0.67	19.39 ± 0.38	522.7 ± 8.11	320.39 ± 6.97
15	1.83 ± 0.11	2.04 ± 0.23	9.39 ± 0.17	10.86 ± 0.19	20.76 ± 0.70	24.22 ± 0.87	426.32 ± 19.44	462.61 ± 17.84
20	1.29 ± 0.09	2.55 ± 0.13	9.12 ± 0.18	10.13 ± 0.18	18.23 ± 0.27	20.11 ± 0.57	363.24 ± 18.68	401.66 ± 9.79
0 (Control)	1.21 ± 0.10	1.52 ± 0.09	8.98 ± 0.17	8.66 ± 0.23	15.21 ± 0.38	18.23 ± 0.44	310.22 ± 7.97	355.49 ± 12.70
CD (5 %)	0.36	0.48	0.63	0.54	1.53	1.69	41.47	NS

Note: ± values indicated standard error

4. Discussion

Mushrooms grown for human consumption must not pose any health risks due to exposure to harmful substances. As a result, their content has previously been screened in several edible mushrooms by several investigations. Simultaneously, it is crucial to produce fruiting bodies with higher concentrations of essential elements like selenium and zinc and also to know their toxicity levels. The present investigation was carried out to study the mycelial growth characteristics and its nutritional aspects by biofortification of *P. ostreatus* var. florida and *P. ostreatus* var. eryngii with sodium selenate at 0, 5, 10, 15, and 20 mg/l. During biofortification, selenium specifically binds to the chitin present in the *P. ostreatus* cell walls (Muñoz *et al.,* 2006; Riddhi *et al*., 2023). Moreover, they also described that a lower concentration of 2.5 mg/l Se supplementation increased mycelial growth while a higher supplementation of 5 mg/l had an inhibitory effect. Kim *et al.* (2008) reported that concentrations 1, 10, and 100 µM stimulated the mycelial growth while 1000 and 10,000 µM of Se significantly reduced the mycelial growth due to Se toxicity. Our results showed that *P. ostreatus* var. eryngii was more tolerant to Se stress by showing a maximum growth rate at 20 mg/l which showed agreement with the findings of Da Silva *et al*. (2013) which showed that Se addition decreased the septum distance and the diameter of hyphae of all

isolates, except for *P. ostreatus* var. florida and *P. ostreatus* var. eryngii at 25.4 mg/l of selenium which increased the septum distance, and *P. ostreatus* spp. which increased the hyphae diameter. Furthermore, no significant difference was observed in these parameters in Se levels higher than 50.9 mg/l. Thus, this study revealed that biofortification with higher concentrations of Se inhibits the growth and reduction in biomass production of *Pleurotus* spp. Similarly, our results also showed an inhibitory effect on biomass production at higher levels of Se supplementation, *i.e.*, 15 and 20 mg/l in *P. ostreatus* var. florida. This shows that fungi may possess a mechanism for metabolizing metals like selenium from the growing environment (bioaccumulation).

Milovanoviæ *et al.* (2014) studied the potential of *P. ostreatus* mycelium for selenium absorption and reported that no Se content was observed in the mycelial control sample. In the Se enriched media, the concentration of Se in the mycelium ranged from 251.2 5Ø g g^{-1} (5 mg/l Se concentration) to 938.9 5Øßg g^{-1} (20 mg/l Se concentration) which was the highest amount absorbed. At higher concentrations of Se in the medium, there was a decrease in the absorption levels. Even though, the concentrations of Se in the mycelium were higher than those obtained at 5 mg/l and 10 mg/l. Milovanoviæ *et al*. (2014) reported similar outcomes from their transmission electron microscopy study of *P. ostreatus* mycelial development. According to the researchers, mycelial development performed well in Se enriched media at concentrations of 5, 10, and 20 mg/l. The growth was significantly suppressed at a concentration of 500 mg/l, with 1000 mg/l being the minimum inhibitory concentration (Ducros and Favier, 2004**)**. At concentrations of 100 and 500 mg/l Se, the hyphae were significantly shorter, often septated, branched, and had a denser extracellular matrix than the hyaline, thin-walled, branching, and anastomosed hyphae with clamp connections in the control. Goyal *et al*. (2015) found similar outcomes in their SEM-EDS investigation of *Ganoderma lucidum*'s hyphal mass. It revealed variations in the percentages of weight, atom C, and O_2 composition. The findings demonstrated that, in comparison to the control group, which had a maximum percentage weight carbon content of 15 ppm, there was an initial decline in percentage C up to 10 ppm, followed by an increasing trend. On the other hand, the percentage weight of oxygen showed a declining tendency. Comparable outcomes were seen for the percentage of carbon and oxygen atoms. In the case of Se-enriched and non-enriched *Pleurotus* spp. mycelium, our EDS data also revealed variations in the elemental makeup of oxygen, carbon, and selenium in terms of percent weight and atom percentage.

Bhatia (2014) reported that the process of Se biofortification enhanced the total protein and total phenolic content as compared to unfortified mushrooms. Turlo *et al*. (2007) reported that *Lentinula edodes* mycelium can incorporate Se into proteins as selenomethionine. In another study, Turlo *et al.* (2010) revealed that Se toxicity occurred if selenomethionine was present at >300 µg g^{-1} of fungal biomass and a total selenium content of 1100 µg g^{-1}, resulting in substantial reduction of mycelial growth. Mushrooms possess a high amount of protein and are capable of accumulating higher amounts of Se. Therefore, it is reasonable to anticipate that this element might have been incorporated into proteins as selenoproteins. Selenium enriched *Calocybe indica* fruit bodies were analysed for the total phenolic content and found that the Se at the concentration of 5 mg/ml enhanced the total phenolic compounds (25.29 mg GAE/g) whereas, the response decreased significantly with the higher application of the Se in the substrate (Rathore *et al*., 2018). This difference may be due to growing conditions, the type of medium used and the type of extraction solvents used. Similar observations for enhanced DPPH radical scavenging per cent were reported by Rathore *et al*. (2018) for the *Calocybe indica* extracts. This increase indicated significantly higher scavenging effects as compared to the control. Sravani *et al*. (2021) studied the effect of selenium-enriched wheat substrate on the percent radical scavenging activity and total flavonoid content of *Pleurotus* spp. and found a many-fold increase in its content over control samples. The findings demonstrated that after supplementing with selenium, the mushrooms had improved antioxidant capabilities, because the scavenging ability on 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals was improved.

5. Conclusion

Oyster mushrooms possess sufficient physical, legitimate, and antioxidant qualities, and typically may be a powerful vegan source of dietary fiber, protein, and phytochemicals. Selenium can be toxic to mushrooms at higher concentrations. As a mechanism to tolerate its toxic effects fungi change its morphology, especially hyphae diameter, length, number of new hyphae, septa, *etc*. Therefore, the estimation of the highest concentration of Se in the culture media that does not show an adverse effect on the mycelial growth is very crucial to deciding the Se concentration of substrate used for the cultivation of mushrooms. In the food sector, Se-enriched mushrooms are a crucial daily supplement of Se. Selenium contributes to the metabolism of macromolecular substances in addition to influencing the growth and appearance of mushrooms.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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