

Effect of Absorption Selenium (Se) on Mycelium Growth of *Pleurotus ostreatus* Mushrooms

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Abstract Selenium (Se) is a critical micronutrient for a variety of human health issues, included cardiovascular health, neurodegeneration and cancer prevention, and appropriate immunological responses. Selenium in the form of sodium selenite (Na₂SeO₃) was measured in the culture medium of *Pleurotus ostreatus* at concentrations of 2.0 mg/L, 5.0 mg/L, 10.0 mg/L, 20.0 mg/L, 40.0 mg/L, and 60.0 mg/L. It is great worthy to mention that as the concentration of Se increased, the amount of biomass produced. At a Se concentration of 20.0 mg/L, biomass reduced from 5.56 g/L in the control to 3.20 g/L, whereas production was entirely suppressed at concentrations of (40.0 and 60.0) mg/L. Even though colony diameters were nearly comparable in the control and media enriched with the lowest three Se concentrations, biomass output was lower at all three Se concentrations compared to the control.

Keywords Selenium (Se), *Pleurotus ostreatus*, Mushrooms

1. Introduction

Mushrooms are fungi, which means they lack chlorophyll. They are typically saprophytic in nature. That is, they obtain sustenance through decomposing non-living organic material [1]. Mushrooms are becoming increasingly popular as a source of nutraceuticals, antioxidants, prebiotics, immunological regulating, anti-inflammatory, cardiovascular, anti-microbial, and anti-diabetic characteristics. [2,3]. *Pleurotus ostreatus* is the binomial name. *Pleurotus* is a genus of roughly 40 species of mushrooms that are popularly referred to as "oyster mushrooms".

Several species of *Pleurotus* have a considerable commercial value in the world market for edible farmed mushrooms at the moment [4]. *Pleurotus* species have important amino acids including arginine, glutamine, and glutamic acid, as well as vitamins and minerals. [5].

Due to its involvement in a variety of key enzymes and proteins, selenium is a necessary microelement for proper human and animal growth and development in minimal amounts. Oyster mushroom mycelium has bioremediation capabilities, meaning it can treat soil polluted with oil

derivatives, polycyclic aromatic hydrocarbons, or heavy metals. *Pleurotus* cultivation can therefore tackle one of the most pressing issues in soil waste management, generate economic benefits, and safeguard the environment. [6].

High Se concentrations can prevent *Pleurotus ostreatus* mushrooms from growing and producing mycelium. [5,7]. As a result, it would be worthwhile to investigate the effects of various Se chemical forms on the development and morphology of *P. ostreatus* mycelium to determine the best concentration to use for enrichment.

P. ostreatus is also one of the world's most frequently cultivated and consumed mushrooms. [8], demonstrating its economic and nutritional significance.

Humans are beginning to recognize selenium as a necessary nutrient. Selenium and its compounds are found in foods in the form of selenoamino acids, selenoproteins, selenide, and selenite, and have been shown to have biological effects through iodothyronine deiodinase, glutathione peroxidases, phospholipid hydroperoxide, sperm capsule, selenoprotein, and thioredoxin. Selenium compounds inhibit tumor-promoting signaling enzymes including protein kinase C (PKC) and act as antioxidants via selenoproteins and thioredoxin reductases. [9].

The International Food and Nutrition Board recommends a daily dose of 40–70 g selenium for males and 45–55 g selenium for women, and 25 g selenium for children [10]. Se can protect against chronic illnesses such as cancer, cardiovascular disease, oxidative stress, and inflammatory

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disorders. Food, on the other hand, has a low Se content. [11].

2. Materials and Methods

Organism and Cultivation Conditions

The culture of *P. ostreatus* originated and was identified by Dr. Mohamed Fathy Salem, GEBRI, Sadat City, Egypt. *P. ostreatus* maintained in Petri dishes containing Potato-Dextrose Agar (PDA) culture medium, pH 5.8. Petri dishes were containing sterilized medium were inoculated with a one-disc of mycelia (about 1 cm² each) and incubated at 25±2°C for 7 days, then stored in a refrigerator at 4°C.

Submerged culture medium for mycelium

The following was the culture media (g/L distilled water): (glucose, 10 g/L; NH₄NO₃, 2 g/L; K₂HPO₄, 1 g/L; NaH₂PO₄, 0.4 g/L; MgSO₄, 0.5 g/L; yeast extract, 2 g/L; pH 6.5). 1) Selenium was tested in the form of sodium selenite (Na₂SeO₃) at concentrations of 2.0 mg/L, 5.0 mg/L, 10.0 mg/L, 20.0 mg/L, 40.0 mg/L and 60.0 mg/L. In addition, added to a sterilized modified synthetic medium that was optimum for biomass production in 400-mL Erlenmeyer flasks containing 100.0 mL modified synthetic medium; as a control, medium without Se was utilized. For each Se concentration, three replicas were created. 2) Incubation at room temperature (25°C) for 7 days on a rotary shaker (100 rpm). 3) Sterile distilled water washing of acquired biomass (3times). 4) Biomass homogenization in a laboratory blender with 100 mL of sterile dH₂O.

Selenium (Se) determined

The concentration of absorbed Se was measured using an HG-AAS Model SP190 hydride generation atomic absorption spectrophotometer (Pye Unicam, England). 0.1 g of dried biomass was dissolved in 10.0 mL concentrated HNO₃ and 3.0 mL concentrated HCl, then diluted with Milli-Q water to a final volume of 20.0 mL. A standard curve was created using solutions containing Se at concentrations of 0.0 g/L, 10.0 g/L, 25.0 g/L, and 50.0 g/L. Data on absorbed Se from the initial incubation medium is reported as a percentage of total absorbed Se and as g of absorbed Se per g of dried biomass.

Statistical Analysis

The mean standard error of measurements from triplicate experiments is used to express the data. Using STATISTICA software, version 5.0, a one-way analysis of variance (ANOVA) was conducted to evaluate the significance of differences among the absorbed Se concentrations (StatSoft, Inc.). Significant values were defined as those smaller than 0.01.

3. Results and Discussion

Mycelium growth and absorption capability are determined as a function of selenium concentrations.

Mycelium growth was good and growth intensity was comparable to that of the control medium in media enriched with Se at concentrations of 2.0 mg/L, 5.0 mg/L, and 10.0 mg/L. Se concentrations of 20.0 mg/L were less favorable for development, resulting in lower mycelium density; Se concentrations of 40.0 mg/L and 60.0 mg/L significantly hampered growth, whereas higher Se concentrations completely stopped it.

As the concentration of Se increased, the amount of biomass produced decreased. At a Se concentration of 20.0 mg/L, biomass reduced from 5.56 g/L in the control to 3.20 g/L, whereas production was entirely suppressed at concentrations of (40.0 and 60.0) mg/L (Figure 1).

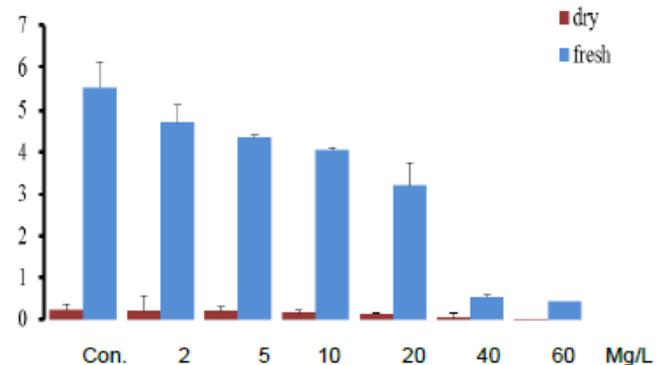


Figure 1. Mycelia growth rate (gram) for Selenium concentration in culture media (mg/L)

Although Mycelia growth rates (gram) were nearly comparable in the control and media enriched with the lowest three Se concentrations, biomass output was lower at all Se concentrations (0.01).

The mycelium of *P. ostreatus* had no Se in the control samples. Se concentrations in the mycelium in the Se-enriched media ranged from (138.87, 260.19, 550.52, 954.33) µg/g (at a Se concentration of (2.0, 5.0, 10.0, 20, 0) mg/L, which was the largest quantity absorbed. Absorption levels increased as Se concentrations in the medium increased, though mycelium concentrations were higher than the observed at Se concentrations of 5.0mg/L and 10.0mg/L ($P \geq 0.01$). The ability of mycelium to absorb selenium, as measured by selenium content in the biomass, was not positively associated with selenium levels in the medium.

The optimum Se content in the medium for the best biomass production and Se absorption is in the range of 5.0, 10.0, and 20.0 mg/L, with biomass production of 4.36, 4.06, and 3.20 g and Se absorption of 260.19, 550.52, and 954.33 µg/g, respectively (Table 1). This should be the Se concentration range where the most Se can be recovered from the medium. However, fungal biomass dropped as Se concentration rose, considerable fungal growth occurred at Se concentrations of up to 20 mg/L, which allowed for significant Se uptake, and the range of the optimum biomass/Se concentration ratio for absorption is illustrated in (Figure 2). When expressed as a percentage of the total Se added to the original media, the amount of Se taken up from the medium and integrated into the mycelium biomass

decreased as the medium Se concentration increased.

Table 1. Show Selenium concentration (Se) absorption $\mu\text{g/g}$ in culture media (mg/L)

Selenium concentration in culture media (Mg/l)	Se absorption $\mu\text{g/g}$			
	R1	R2	R3	average
Control	ND	ND	ND	ND e
2	138.87	138.58	136.83	141.19d
5	260.19	259.77	266.14	254.67c
10	550.52	549.52	587.46	514.57b
20	954.33	955.67	937.18	970.15a

• ND: not detect Se

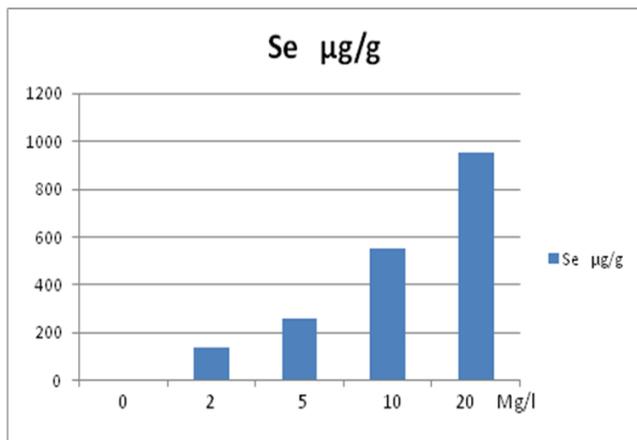


Figure 2. Se absorption $\mu\text{g/g}$ for Selenium concentration in culture media (mg/L)

The greatest percentage of absorbed Se (53.25%) was measured at a median Se concentration of 5.0mg/L. Se concentrations of 10.0 mg/L and 20.0 mg/L in the medium were also acceptable for its absorption (43.58 percent and 41.31 percent, respectively), but the percentages dropped dramatically after culture in media supplemented with 40.0 mg/L and 60.0 mg/L Se (11.17 percent and 3.60 percent, respectively).

When compared to the additional levels, the Se concentrations in the Se-enriched liquid media after sterilization and before inoculation were much lower (about 3- fold). As a result, initial Se concentrations decreased from 300 g/L to 96 g/L, 700 g/L to 210 g/L, 1000 g/L to 296 g/L, and 1300 g/L to 373 g/L, respectively.

Table 2. Correlations between Se absorption $\mu\text{g/g}$ and Fresh and dry Mycelia growth rate

	Fresh	Dry	Se absorption $\mu\text{g/g}$
Fresh	1		
Dry	.958*	1	
Se absorption $\mu\text{g/g}$	-.952*	-.983**	1

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

Regarding the relationship and correlations between Se absorption $\mu\text{g/g}$ and fresh and dry Mycelia growth rate, data illustrated in (Table 2) showed negative correlations

between Se absorption $\mu\text{g/g}$ and fresh and dry Mycelia growth rate with Pearson correlation (-952) and (-983), respectively.

Edible fungi especially *Pleurotus* spp. have been used as dietary supplements when there are deficiencies of certain constituents in natural foods. This is due to their ability to absorb micro and macronutrients from the substrates on which they are grown. [12]. the yield and biological efficiency (BE) of *Pleurotus* spp. revealed that the second flush generated the most mushrooms. However, when Se was added to the substrates, the yield was decreased. This conclusion is in line with the findings of [9,13].

Edible mushrooms such as *Agaricus bisporus*, *P. ostreatus*, and *Lentinula edodes* have been known to contain low Se compounds, interestingly, when it grew on substrates treated with Se, the Se content in the fruiting body was increased up to 347 and 355.7 mg/g dry weight. This suggests that *P. ostreatus* has the potential to become bioaccumulated [14].

Different types and amounts of selenium have different effects on the growth and metabolism of different mushroom species. Thus, Zhao *et al.* [15] showed that *S. cerevisiae* during the exponential phase of development in Na₂SeO₃-enriched medium, which included 30.0 g/ml Se, the bacteria, accumulated Se to concentrations ranging from 1200.0 g/g to 1400.0 g/g dry weight, and greater Se levels caused significant growth inhibition. Agreements our results were obtained by Malinowska *et al.* [16], who observed a substantial reduction in *Hericium erinaceum* mycelium production during culture in a medium containing 100.0 mg/L Se, whereas a concentration of 25.0 mg/L did not influence growth. The results match those of *P. ostreatus* HAI 592. [17].

Our results show that the mushroom under investigation can produce biomass and absorb Se in the form of Na₂SeO₃. Additionally, a previous study has revealed the following benefits: 1) Selenites can be taken up by all species, whereas selenates can only be taken up by terrestrial plants and bacteria. [18]. 2) The absorption of Na₂SeO₃ from aquatic environments is a passive process distinguished from the selenates [19]. 3) The desorption of Se from selenites is 3-fold higher than from selenates [20] and 4) during Se incorporation into selenoproteins, reduction of absorbed selenites to necessary selenide does not need any energy consumption contrary to selenate reduction.

In a study of numerous *Pleurotus* species and strains, Stajić *et al.* [21] Various Se sources had varying effects on mycelial biomass output, In certain species and strains, Se produced an increase or reduction in mycelial biomass production compared to the control, but in others, the yield was the same as the control. The results of this investigation corroborated previous findings of Na₂SeO₃'s growth-inhibitory impact on *P. ostreatus*. Stabnikova *et al.* [22] the similar impact was seen during yeast culture on NaHSeO₃-enriched medium, where biomass decreased according to Se concentration. At Se concentrations more than 100 mg/L, Na₂SeO₃ substantially decreased mycelium

yield in *Hericium erinaceus*, but it had no effect at concentrations less than 25 mg/L. [16]. Chen et al. [23] explained this inhibitory effect by oxidative damage induced by the Se presence when fungal cells react by the production of nonenzymatic components in large amounts.

The shape and concentration of Se have been found to alter fungal biomass production in previous research. Shi et al. [24] found that organic Se is a better form than inorganic Se for human and animal absorption and retention, bioavailability, biotransformation, and accumulation potential in research of numerous *Pleurotus* species and strains. Increased awareness of Se health benefits has led to an increase in daily consumption of Se supplements (multivitamin pills), which could be one explanation for the formation of resistance in some consumers.

However, numerous studies have shown that natural Se sources are much better for health [25]. Mushrooms are a nutritionally high-valued and tasteful food, as well as good sources of Se due to their ability to absorb and incorporate it in proteins, amino acids (selenocysteine, selenomethionine, Se-methylselenocysteine), polysaccharides, nucleic acids, lipids, and several unidentified seleno compounds [15,26,27].

Although mycelium may be produced in a faster and more cost-effective manner than fruiting bodies, only a few studies have attempted to assess mycelium potential for Se accumulation.

According to [21,28], *P. ostreatus* mycelium absorbed the most Se after growing in Na₂SeO₃-enriched synthetic medium, which is consistent with the findings of our investigation [17].

Total Se consumption should not exceed 100 g daily owing to hazardous consequences, according to the European Scientific Committee on Food. Although Falandysz [29] indicated that the maximum safe daily dietary Se dosage is 400 g. Se deficiencies in the diet, on the other hand, have been associated to a variety of diseases [30], which could be alleviated by introducing organic Se-enriched foods and supplements. [24] Found that Se-enriched *G. lucidum* polysaccharides were effective in preventing heart damage by enhancing antioxidant enzyme activity and inhibiting lipid peroxidation.

The study mushroom species might be used as valuable sources of nutrients and cost-effective dietary supplements, according to the findings.

Several studies have shown that different species and strains have different capacities to absorb and accumulate Se based on their developmental stage, substrate, and Se form and concentration. [31,16]. Thus, these researchers found that the Se content of *Lentinula edodes* mycelium and *Saccharomyces cerevisiae* cells grown in a media supplemented with Se at 20.0 mg/L was 748.0 g/g and 1825.0 g/g, respectively, in *Lentinula edodes* mycelium and *Saccharomyces cerevisiae* cells. In contrast to other species that have a high capacity for Se absorption, *Ganoderma lucidum* showed a poor capability for Se incorporation in fruiting bodies, with just 29% of the mean Se concentration

incorporated. [27]. According to [25,32] *P. ostreatus* is a hyperaccumulator since its Se absorption capacity is greater than 100.0 mg per kg of dry matter, which is at least 100 times higher than the values predicted in non-accumulating species on the same substrate.

Selenium bioavailability is also affected by the chemical forms and concentrations of the element in the substrate, as well as the properties of the substrate (composition, type, temperature, pH, water salinity, and oxygen concentration) [33]. A few Se forms can be found in nature: (i) elemental insoluble and less bioavailable Se (Se⁰), (ii) volatile selenide (H₂Se) and metal selenides (metal-Se) which pass into the atmosphere through bioactivity, (iii) less bioavailable selenites (SeO₃²⁻), (iv) mobile selenates (SeO₄²⁻), and (v) organic Se compounds [34]. Besides Na₂SeO₃ is a commercially available form of Se [35], Letavayov'a et al. [36] Because of the production of reactive oxygen species, selenites are significantly more poisonous than organic Se forms.

The methylation of Na₂SeO₃ to seleno compounds, such as methylselenol, dime-thylselenide, and trimethylselenonium, has been proposed as a method for Se absorption by fruiting bodies. This method of ingesting elements might be used to supplement the daily dietary allowances for Se [37].

Although it is known that absorbed Se is incorporated into selenocysteine, selenomethionine, Se-methylseleno cysteine, and several unidentified seleno compounds [38], mechanisms of its uptake, translocation, accumulation, and metabolism depend on the species. According to Ip [34,35,39], water-soluble selenites and selenates could be introduced into two metabolic pathways:

(i) Methylation directly or indirectly (through incorporation into selenomethionine) to methylselenol, dimethyl selenide, and trimethyl selenonium which are vaporized or excreted, and (ii) reduction to insoluble Se⁰, which could be either reoxidized to selenites, rarely selenates, or reduced to H₂Se.

In the reaction with metal cations, H₂Se, a precursor for selenoprotein production, creates metal-Se, which then either precipitates or oxidizes to selenites [17]. More research is needed to establish the right concentration of Se (mg/L) to be added to the bag log so that it can collect on the human body after being absorbed in the fruiting body and eventually meet the body's Se needs.

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