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Growth Rate and Selenium Bioaccumulation in *Pleurotus* species Cultivated on Signal Grass, *Urochloa decumbens* (Stapf) R. D. Webster

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Abstract

Supplementation of edible fungi with essential mineral during cultivation has been an interesting approach to proffer solution to mineral deficiency. Effect of selenium (Se) concentrations (0, 50.0, 100.0 mg mL^{-1}) as sodium selenite on mycelial growth and biomass production of *Pleurotus* spp. was assessed. The biological efficiency and uptake of Se was determined after the cultivation of *Pleurotus* mushrooms on *Urochloa decumbens* (signal grass) with 50.0 g kg^{-1} of Se. The fungal growth rate and biomass production were reduced after addition of Se and their value ranged from 1.0 mm d^{-1} to 3.6 mm d^{-1} and 5.63 g to 30.5 g respectively. Higher biological efficiency (115.23%) was obtained for *P. ostreatus* (P93) and was significantly different ($P<0.05$) from other tested *Pleurotus* spp. Cultivated *P. pulmonarius* aborbed more Se (135.5 µg g^{-1}) followed by *Pleurotus cornucopiae* (120.34 µg g^{-1}) but no Se was detected in the control (mushrooms grown on subatrate without Se). Therefore, signal grass can be used as substrate for cultivation of edible fungi (*Pleurotus* species) enriched with Se. The produced mushrooms can serve as a natural source of mineral supplement for human beings to curb the symptoms of selenium deficiency.

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Introduction

Selenium is a trace element that is fundamental and essential for several metabolic pathways such as thyroid hormone metabolism, antioxidant defence system and immune function[1]. Selenium and its compounds are present in food in form of selenoamino acids, selenoproteins, selenide, selenite, and thus, exhibited significant biological activities through

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iodothyronine deiodinase, glutathione peroxidases, phospholipid hydroperoxide, sperm capsule, selenoprotein and thioredoxin reductase[1,2,3]. Selenium compounds inhibit signaling enzymes such as protein kinase C (PKC) that is responsible for tumor promotion and exert antioxidant effect through selenoproteins and thioredoxin reductases[4].

The quantity of Se in food depends on their content, varies regionally and nationally with Se level in soil relatively low in some countries[5]. Although, soil conditions have been associated with Se toxicity in animals (selenosis) in some areas with high Se content but Se deficiency syndromes occurred in animals and humans in areas of low soil Se[5]**.** Selenium content in foods has been reported as insufficient to facilitate the optimal activity of protective selenoenzymes[6]. The nutritional deficiency of Se in human blood lead to some threatening diseases such as cancer, cardiovascular disease, increasing of viral infections, male infertility, asthma, Kashan disease, thyroid dysfunction and osteoarthritis[7]. Considering the importance of Se in human diet, it is expedient to increase the Se content in foods to rectify the implication of Se deficiency in the body. One of the best methods is to produce foods with adequate amount of Se such as cultivating edible fungi with Se fortification.

Although, wild and edible mushrooms contain some amount of Se but it concentration varied based on the Se content in soil or growth substrate. Hence, supplementation of Se into *Pleurotus* mushrooms will improve the antioxidant status, eliminate dietary aliments and suppress the syndromes associated with deficiency of Selenium. *Pleurotus* spp. have been appreciated as medicinal foods and selected for Se biofortification due to their rapid growth within short time, high volume of production, seasonal independence, safety and wide acceptance by consumers as foods[8]. Therefore, this study is meant to assess the effect of Se on mycelial growth and biological efficiency as well as Se accumulated by edible mushrooms of *Pleurotus* spp. cultivated on signal grass supplemented with Se.

Materials and Methods

Microorganism

The studied fungi namely: *Pleurotus ostreatus* (P93), *Pleurotus ostreatus* (PLO6), *Pleurotus pulmonarius* (Pindo), *Pleurotus cornucopiae* (Plocor), *Pleurotus djamor* (PLO13) and *Pleurotus djamor v. roseus* (Psfarm) were from fungal collection of the Laboratório de Associações Micorrízicas, BIOAGRO, Departamento de Microbiologia, Universidade Federal de Viçosa (UFV), Minas Gerais, Brazil. The fungi were sub cultured on potato dextrose agar (PDA, Merck, Darmstadt, Germany) and incubated at 25±1 °C for seven days.

Effect of Se on mycelial growth rate and biomass of fungi

The fungi were exposed to Se in culture media at different concentrations (0, 50.0 or 100.0 mg L^{-1}). Briefly, one agar disc (8.0 mm) of each actively grown fungi was cut and transferred to the center of culture medium (PDA, Merck, Darmstadt, Germany) containing of Se in form of sodium selenite (Na_2SeO_3) at pH 5.4 ± 0.1. The plates were incubated at 25 °C ± 1°C for seven days. The fungal growth rate was determined after seven days by measuring the colony's diameter in two directions that were perpendicular to each other[9].

The biomass (dry mass) of the fungi was determined by emptying the entire content of Petri dish (mycelium and culture media) into a bottle with distilled water and heated using microwave (5-10 min) to dissolve culture medium. Thereafter, the solution was filtered and the mycelium was dried in an oven at 60 °C until a constant weight was reached[10].

Pleurotus mushroom cultivated on *Urochloa decumbens* with Selenium

The signal grass was obtained from a farmland in Unai, Minas Gerais State, Brazil. It was dried at room temperature and milled into particle of different sizes (4.0-6.0 mm). The milled signal grass was soaked in lime water solution of 2% $Ca(OH)_2$ for 24 h and centrifuged at 1800 rpm for 5 min to remove excess water[11]. Then, experimental units of each fungus were cultivated on signal grass (150 g) supplemented with 50 mg Kg^{-1} of Se. This concentration was chosen based on the fact that there was minimal inhibition of fungal mycelia, which was supported by the findings of Da Silva *et al*.[12] who revealed the Se concentration (50 mg Kg^{-1}) for the optimum production of *P. ostreatus*. The experimental units were incubated at 25 ± 3 ºC for 30 d. After this time, the bags were transferred to a room and incubated for fructification with light, at temperature of 25 ± 3 ºC in 90% air

humidity for about 7 days. The biological efficiency (BE) was calculated as follow:

BE = (fresh weight of mushroom / dry weight of substrate used) x 100

Determination of Se content in cultivated mushrooms

Mushrooms were freeze- dried until they reached a constant weight and were ground in a 2-mm sieve mill. Ground sample of each mushroom (500 mg) was placed into the sealed tube, 6 mL of nitric acid (HNO_3) was added and left in a block of graphite (brand DigiPREP MS) for 24 h at 65 °C. Thereafter, 3 mL of hydrogen peroxide (H_2O_2) was added and the block temperature was raised to 120 °C. The resulting contents of the digested sample was transferred to new tubes and the final volume was made up to 10 mL using ultra-pure water (Milli -Q, Millipore 18.2 MΩ cm^{-1}). The Se in the mushroom samples was quantified using atomic absorption spectrometer Varian (Spectra AA 220FS) and compared to Se standard.

Statistical analysis

Statistical analysis of data obtained from completely randomized experimental design were analysed with the aid of Statistical Package for Social Sciences (SPSS Inc., version 17.0, Chicago, Illinois, USA) using one-way analysis of variance (ANOVA). Mean were compared by Duncan's New Multiple Range Test and considered statistically significant when $P \leq 0.05$.

Results and Discussion

Edible fungi of higher Basidiomycetes, especially *Pleurotus* spp. are used as alternative source of mineral supplement when there are deficient of minerals in natural foods[13]. This is because, they are easily grown within short time and capable of storing essential elements that maintain balanced nutrient in human[14]. The growth rate of *Pleurotus* species on media containing Se, biological efficiency of *Pleurotus* mushroom growing on signal grass added with Se and Se concentration in the produced mushrooms is reported in this study.

The increase in Se concentration from 0 to 100 mg mL^{-1} reduced the fungal growth rate and their biomass production (Table 1). The value of fungal growth rate ranged from 1.5 mm d^{-1} to 3.6 mm d^{-1} and 1.0 to 3.30 mm d^{-1} at 50 and 100 mg mL^{-1} respectively. The growth rate of *P. cornucopiae* and *P. djamor* var. *roseus* were not different ($P<0.05$) at 50 and 100 mg mL^{-1} of Se. Mycelial biomass obtained from the edible fungi at 50 and 100 mg mL^{-1} of Se were within 11.4 g to 30.5 g and 5.63 g to 10.8 g respectively. The biomass produced by *P. cornucopiae, P. djamor* and *P. djamor* var. *roseus* at 50 and 100 mg mL^{-1} were similar ($P<0.05$). The reduction in growth rate and biomass production

Table 1: Mycelial growth rate (mm d^{-1}) and biomass (mg) of edible fungi of genus *Pleurotus* cultivated on Se in culture media at different concentrations (0, 50.0 or 100.0 mg L^{-1})

Se conc.	Mycelial growth rate					
	P93	**PLO 06**	**Pindo**	**Pcor**	**PLO 13**	**Psfarm**
0	8.40 ^{a} ± 0.10	8.42 ^{a} ± 0.14	6.40 ^{a} ± 0.18	5.80 ^{a} ± 0.15	6.11 ^{a} ± 0.10	6.57 ^{a} ± 0.13
50	3.60 ^{b} ± 0.20	3.30 ^{b} ± 0.10	3.40 ^{b} ± 0.20	1.50 ^{b} ± 0.08	1.60 ^{b} ± 0.00	3.60 ^{b} ± 0.02
100	1.40 ^{c} ± 0.14	1.57 ^{c} ± 0.40	2.80 ^{c} ± 0.20	1.20 ^{b} ± 0.02	1.00 ^{c} ± 0.30	3.30 ^{b} ± 0.10
	Biomass					
0	71.90 ^{a}± 1.21	89.96 ^{a} ± 6.90	149.43 ^{a} ± 16.8	34.03 ^{a} ± 1.27	62.20 ^{a} ± 1.90	67.30 ^{a} ± 3.30
50	21.3 ^{b}± 3.60	21.4 ^{b} ±0. 40	30.5 ^{b} ±1.20	12.10 ^{b}± 0.30	16.40 ^{b}±1.60	11.4 ^{b}± 0.70
100	5.63 ^{c} ± 0.80	9.70 ^{c} ± 0.20	10.8 ^{c} ± 0.20	10.6 ^{b}± 0.60	7.30 ^{b} ± 0.20	8.06 ^{b}±0.05

Values are mean±SD of replicates n=3. Mean with different superscript alphabet along column are significantly different at $P<0.05$ when Duncan test was adopted for P93: *P. ostreatus,* PLO6: *P. ostreatus,* Pindo: *P. pulmonarius,* Plocor: *P. cornucopiae,* PLO13: *P. djamor* and Psfarm: *P. djamor* v. *roseus*

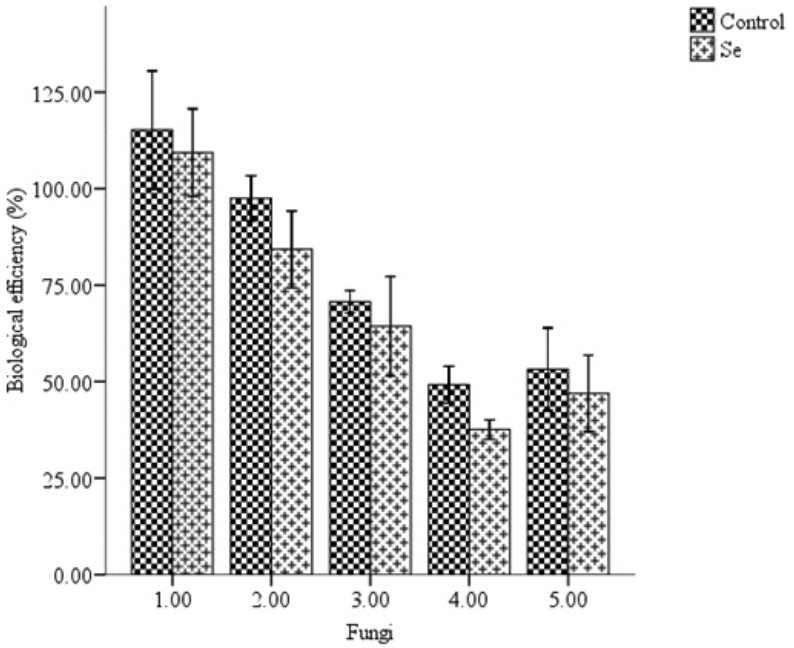

Fig. 1: Biological efficiency of *Pleurotus* mushrooms labelled as 1: *P. ostreatus* (P93), 2: *P. ostreatus* (PLO6), 3: *P. pulmonarius* (Pindo), 4: *P. cornucopiae* (Plocor) and 5: *P. djamor* (PLO13) cultivated on *U. decumbens* without addition of Se and with Se (50 mg kg^{-1})

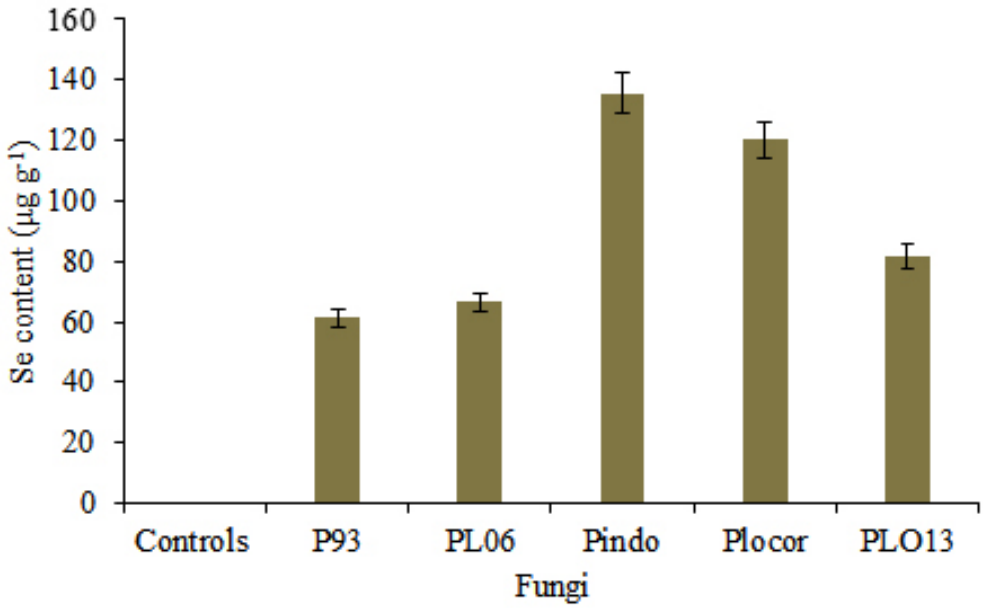

Fig. 2: Se content (µg g^{-1}) absorbed by the *Pleurotus* mushrooms; *P. ostreatus* (P93), *P. ostreatus* (PLO6), *P. pulmonarius* (Pindo), *P. cornucopiae* (Plocor) and *P. djamor* (PLO13) cultivated on *U. decumbens* enriched with Se (50 mg kg^{-1})

indicated that fortification with higher concentration of Se inhibited the growth and production of *Pleurotus* spp. The finding of Da Silva *et al.*[15] revealed decrease in *Pleurotus* spp. growth rate as the concentration of Se increased from 25.4 mg mL^{-1} to 101.8 mg mL^{-1}. Although, Se is required for fungi metabolic activities but found to be toxic when greater than required amount is present in the substrate[16]. Edible mushrooms are known to accumulate Se but the quantity depend on the fungal species, stage of maturity, the amount in soil and substrates used for the cultivation of edible fungi[4]. The concentration of Se required for *Pleurotus* mushroom enrichment may differ based on substrates and fungal species. It is therefore necessary to determine fungal growth rate on media to quantify the amount of Se required in the substrates. Findings of Kalac[4] had earlier revealed that the Se requirement for species or strains of edible mushroom depend on their developmental stage, substrate, Se form and concentration**.**

The biological efficiency of *Pleurotus* mushrooms produced from Se containing substrate was different ($P<0.05$) from control (without Se) as shown in Figure 1. *P. ostreatus* (P93) displayed higher ($P<0.05$) biological efficiency of 115.23% than other cultivated *Pleurotus* spp. The biological efficiency, 62.8% of *P. pulmonarius* (Pindo) from substrate containing Se was similar ($P<0.05$) to what obtained for its control without Se (70.6%). Higher content of Se was obtained for *P. Pulmonarius* (135.50 μg g^{-1}) and was significantly different ($P<0.05$) from other *Pleurotus* spp. (Figure 2). This shows that the fungi may have mechanism for metabolizing Se. No Se detected in mushrooms cultivated on substrate without Se (control), as previously reported by Nunes *et al.* [17] in shiitake. This is in support of Baldrian [18] who had earlier revealed that some heavy metals are essential for the fungal metabolism, whereas others have no known biological role even when present in excess.

The amount of Se (61.45-135.50 μg g^{-1}) absorbed by the cultivated mushrooms are still within the range required for daily dose. The bioavailability of Se in agricultural residue utilized as substrate and supplementation of Se into substrates for the cultivation of *Pleurtotus* species has contributed to the content of amino acids and protein[6]. Mushrooms possess effective mechanism that enables them to readily take up some metals from the ecosystem, which involved bioaccumulation; the active transportation of mineral into cell and intracellular components, biosorption methods like adsorption, ion exchange processes and covalent binding[19, 20].

The ability of *Pleurotus* species to absorb Se and some metals has led to their applications in mycoremediation, often used in the bioconversion of industrial wastes and cultivated on agro-waste residues into fruit bodies of mushroom to control pollution[21]**.** The uptake of organic Se compounds; selenocysteine, selenomethionine, selenite and selenate into various forms of Se metabolites by fungal species is an effective approach to acquire some secondary metabolites, which will promote their therapeutic properties such as antioxidant, antimicrobial, anticancer, cholesterol lowering and immunostimulatory effects[22,23,24]. Hence, the production of edible mushrooms fortified with macro and micro elements can be used as medicinal foods to solve the problem of malnutrition and mineral deficiency in foods.

Conclusion

This research shows that signal grass can be used to produce edible mushrooms enriched with Se, which may serve as nutritional supplement in foods to control the Se deficiency symptoms and syndromes. Hence, biofortification of edible fungi, *Pleurotus* spp. with Se could be a healing option for the Se deficiency symptoms and associated ailments.

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