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SELENIUM UPTAKE AND ASSOCIATED ANTI-OXIDANT PROPERTIES IN *PLEUROTUS FOSSULATUS* CULTIVATED ON WHEAT STRAW FROM SELENIFEROUS FIELDS

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The present study was carried out to examine the antioxidant activity in oyster mushroom cultivated on selenium (Se) rich substrate. *Pleurotus fossulatus* was cultivated on Se-rich wheat straw collected from the seleniferous belt of Punjab (India) and its potential to accumulate Se from substrate was examined. Using different assay systems the modulations in the anti-oxidant profile of Se enriched mushroom was studied in comparison to the mushrooms cultivated on normal straw. The oyster mushrooms were observed to potentially mobilize Se from Se-rich substrates to fruiting bodies, resulting in significantly high uptake (37.2±0.6 μg g^{-1}) as compared to control (3.57±0.53 μg g^{-1}). The antioxidant activity, as determined by various assays, such as reducing power, 2,2-diphenyl-1-picrylhydrazyl free radical scavenging, and metal chelating activity, was higher in the experimental mushrooms when compared to control. The results obtained demonstrate that Se-fortified mushrooms through cultivation on straw containing organic forms of Se can be considered as natural and effective dietary supplements of organic Se for humans. The present study proposes the use of Se-rich agricultural residues as substrates for mushroom cultivation for human and livestock supplementation.

Keywords: antioxidants, selenium, *Pleurotus* sp., fortification

Selenium (Se) is established as an essential trace mineral of fundamental importance to human health (Papp et al., 2007). Many of its physiologic roles are directly attributed to its presence within selenoproteins, such as cellular GPx (GPx1) and phospholipid hydroperoxide GPx (PHGPx; GPx4), iodothyronine 5'-deiodinases (IDI), and selenoprotein W (Burk & Levander, 1999). However, in several regions of the world the content of Se in diet has been estimated insufficient to facilitate the optimal activity of protective selenoenzymes.

Edible flora that accumulate elements, such as Se, may be used as a natural source of mineral supplement for both animals and human beings, especially in areas that are mineral deficient, through the process of biofortification. In this context, due to their high volume of production, seasonal independence, their safety, and wide acceptance by consumers all over the globe, edible mushrooms have potential to facilitate the bioavailability of such supplements (De Assuncao et al., 2012). Mushrooms have known antioxidant properties provided by different compounds, such as phenolics, ergothioneine, and Se (Beelman & Royse, 2006). The content of Se in mushrooms is species specific (Stijve, 1977).

Mushrooms belonging to genus *Pleurotus* have important medicinal properties, such as anti-tumour and immunostimulatory activity. The products derived from the fruiting bodies of this genus can promote biological responses favouring cancer treatment in humans and have been used as antitumourigenic drugs (Sarangi et al., 2006). These edible mushrooms are known to be Se accumulators. However, the amount of Se is dependent on the species, the

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stage of maturity, the amount in soil, and the substrates used for the growth of cultivated species (Kalac, 2009). Mushroom species grow and yield on a spectrum of agricultural residues and by-products, such as paddy, wheat, sugarcane bagasse, water hyacinth, rubber wood dust, and tree leaves (Khanna, 2003). The agri-wastes generated in seleniferous region of Punjab contain significant levels of Se accumulated in plant parts, as reported in leaves and straw of wheat, rice, and cereals (Dhillon & Dhillon, 2003), and therefore have potential use as substrates for cultivation of Se enriched mushrooms, leading to their appropriate utilization. Extensive research has been carried out on Se uptake by edible mushrooms cultivated on substrates supplemented exogenously with inorganic Se, but to the best of our knowledge no study has been carried out on mushrooms cultivated on substrates hyper-accumulated with Se through natural processes. Keeping this in view, the present study was aimed at exploring the cultivation of *Pleurotus fossulatus* on Se-rich wheat straw and determining its antioxidant profile as induced by Se enrichment.

1. Materials and methods

Standards tert-butylhydroquinone (TBHQ) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma (St. Louis, USA). 2-tert-Butyl-4-methoxyphenol (BHA), gallic acid (GA), quercetin and 2,3-diaminonaphthalene (DAN) were purchased from Hi-Media (India). Methanol and cyclohexane were obtained from SDFine (Mumbai, India). Nitric (HNO_3), hydrochloric (HCl), and perchloric ($HClO_4$) acids were procured from Merck (Bengaluru, India). The rest of the reagents were obtained from Loba Chemie, Mumbai, India.

The strain of *P. fossulatus* was procured from National Research Centre of Mushroom (NRCM), Solan (India). This fungus was cultured on potato dextrose agar (PDA) medium and was stored at 4 °C until use. The Se-rich agricultural residues of wheat crop collected from the village of Jainpur (31°13' N, 76°21' E, Nawanshahr-Hoshiarpur region, Punjab, India) were used as substrates for mushroom cultivation. The mushrooms were cultivated on agricultural residues collected from this site, following the method outlined by Punjab Agricultural University, India (Khanna, 2003) with minor modifications. Similar conditions were followed to grow control mushrooms, with non-Se wheat straw as the substrate. At maturation (22–25 days after inoculation) fruiting bodies were collected and dried at 40 °C for near complete dehydration.

The Se content of powdered samples (from first flush) was analysed using florescence spectrometry (Levesque & Vendette, 1971). The emission spectrum of piazselenol complex formed during the reaction was measured using fluorescence spectrometer (Perkin Elmer LS-45) at excitation and emission wavelength of 360 and 520 nm, respectively. Se quantification in each sample was carried out by the relative method using emission spectrum of NIST certified Se-ICP standard solution (SRM-1349).

Prior to use, the fruiting bodies were milled until a fine powder was obtained. The total protein content in the mushroom was determined by the method of Lowry and co-workers (1951). One gram of the samples in case of estimations of total phenols and total antioxidants and 100 mg in case of other assays were subjected to stirring with 10 ml of 20% and 90% methanol for 2 h, respectively, using ultrasonicator bath, and filtered through Whatman 1 paper. The resultant methanolic extracts (ME) were stored at 4 °C until use. Total phenolic content of both extracts (20% ME and 90% ME) was analysed using the Folin–Ciocalteu reagent according to the method of Singleton and Rossi (1965) using gallic acid as standard.

Thiobarbituric acid reactive substances (TBARS) were assayed fluorometrically (Perkin-Elmer LS45) according to the method of Minotti and Aust (1987). Calibration curves were made using malondialdehyde (MDA; Sigma, St. Louis, USA) in the range of 0.5–5.0 μM. The total antioxidant activity of the methanolic extract (20 and 90% methanol) of *P. fossulatus* was measured using UV-visible spectrometer (Hitachi U-2900) according to the method (phospho-molybdenum assay) outlined by Imran and co-workers (2011), wherein gallic acid (GA) (0.01–0.1 mg ml^{-1}) was used as standard.

The scavenging activity of 20% ME and 90% ME from mushroom on DPPH radicals was measured spectrophotometrically following the method of Chu and co-workers (2000) using quercetin (0.05–0.50 mg ml^{-1}) as standard. The scavenging activity (%SA) of DPPH radicals was calculated using the equation

$$\%SA = \frac{A_{control} - A_{sample}}{A_{control}} \times 100.$$

The chelating activity of the various concentrations of extracts (20% ME and 90% ME) for ferrous ions was measured spectrophotometrically following the ferrozine method (Dinis et al. 1994). The metal chelating activity of the mushroom extracts was calculated as:

$$\%\ \text{chelating activity} = \frac{A_{negative} - A_{sample}}{A_{negative}} \times 100,$$

where A is absorbance. EDTA was used as positive control, while absence of the mushroom extract was the negative control.

All estimations were carried out in triplicates except for total protein content, which was done in duplicates. The comparison between Se and non-Se samples was drawn with Student's *t* test using Graphpad Prism Ver.5.0.

2. Results and discussion

2.1. Protein content in fruiting bodies

Total protein content in methanolic extracts of Se enriched mushrooms (307±4.5 mg g^{-1} d.w.) was found to be significantly higher ($P<0.01$) than of control mushrooms (282±2.4 mg g^{-1} d.w.) (Table 1). The total protein contents (205–246 mg g^{-1} d.w.) of different species of *Pleurotus* grown on non-enriched substrates (Alam et al., 2008) and Se enriched *Lentinus edodes* (213.9±1.09 mg g^{-1} d.w.) (Turlo et al., 2010) were found to be lower than our findings.

The chemical composition of edible mushrooms determines their nutritive value. The Se in mushrooms is dominantly found as selenocysteine, selenomethionine, and methylselenocysteine in addition to inorganic Se (Cremades et al., 2012). Since mushrooms have relatively high protein levels, and can accumulate large amounts of Se, it is reasonable to expect that this element could be significantly incorporated in proteins.

Table 1. Total selenium (n=3) in substrates and fruiting bodies of *P. fossulatus* and total protein content (n=2) in fruiting bodies (n=3)

Sample	Se (μg g^{-1} d.w.)		Total proteins (mg g^{-1} d.w.)
	Wheat straw	Fruiting bodies	Fruiting bodies
Se	24.0±0.2	37.2±0.6	307±4.5
Non-Se	1.90±0.8 ***	3.57±0.53 ***	282±2.4 **

**: P<0.01; *** P<0.001

2.2. Se in substrate and fruiting bodies

The Se contents in straw collected from Se-rich and control (non-Se) sites and in fruiting bodies of Se-rich mushrooms cultivated on the said substrates are presented in Table 1. The fruiting bodies harvested from Se-rich straw containing a total Se concentration of 24.0±0.2 μg g^{-1} were noted to accumulate significantly higher (P<0.001) Se up to 37.2±0.6 μg g^{-1} as compared to control/non-Se mushrooms (3.57±0.53 μg g^{-1}) cultivated on non-Se straw (1.9±0.8 μg g^{-1}). The extent of accumulation was notably higher than the Se concentrations of 4.6 and 9.3 μg g^{-1} reported for *P. eryngii* cultivated on substrates supplemented with 5.0 and 10.0 mg kg^{-1} of sodium selenite, respectively (Estrada et al., 2009), but lower than those reported in *P. ostreatus* (57.6 μg g^{-1}) cultivated on substrate supplemented with 3.2 mg kg^{-1} of sodium selenite (Da Silva et al., 2012).

Many investigations have been carried out with growing Se-enriched mushrooms on substrate supplemented with Se, wherein dominantly inorganic forms of Se compounds were used for the supplementation (Costa-Silva et al., 2011). However, as there is no report on the mobilization of Se by mushrooms, such as *P. fossulatus,* from substrates naturally enriched with Se, use of such substrates can facilitate the availability of bioaccessible forms of Se during growth of mushrooms.

2.3. Se induced transformations in antioxidant properties of mushrooms

The influence of Se hyperaccumulation in *P. fossulatus* was examined on the antioxidant properties of mushrooms using various assay systems. Among the various antioxidant compounds, polyphenols have gained importance due to their large array of biological actions that include free radical scavenging, metal chelation, enzyme modulation activities, and inhibition of LDL oxidation, among others (Rodrigo & Bosco, 2006). In addition, Se is well known for its association with antioxidant mechanisms in humans and animals. The total phenolic content, expressed as mg GA/g d.w. of mushrooms, is shown in Table 2. The amount of phenolic compounds in the methanol (20%) extracts from the Se-enriched mushroom (11.15±0.37 mg GA/g d.w.) was significantly higher (P<0.01) than the control (9.32±0.18 mg GA/g d.w). Similarly, the total phenol content in 90% methanolic extract from Se-enriched mushroom (7.10 ±0.17 mg GA/g d.w.) was also found to be significantly higher (P<0.05) than the control (6.45±0.10 mg GA/g d.w.). However, mushrooms in 20% ME showed higher phenol content than in 90% ME. The results obtained were higher than the total phenol content (4.0±0.32 mg g^{-1}) reported in case of Se-enriched fruiting of *Agaricus bisporus* (Cremades et al., 2012) but comparable to the phenol content (14.3 mg g^{-1} in water extracts; 7.4 mg g^{-1} in methanolic extracts) reported in case of *P. sajorcaju* (Puttaraju et al., 2006)

grown on non enriched substrates. Increase in the phenol content in Se-enriched mushrooms may be due to the influence of Se in promoting the synthesis and activity of antioxidant metabolites, a feature that has been observed in the present study, as well. Various researchers have earlier defined role of Se in inducing antioxidant capacity by facilitating increase in the amounts of tocopherol and phenolic compounds in plants (Xu et al., 2003).

Antioxidant tests could be based on the evaluation of lipid peroxidation or on the measurement of free radical scavenging potency (hydrogen-donating ability). The radical scavengers donate hydrogen to free radicals, leading to non toxic species and therefore to inhibition of the propagation phase of lipid oxidation. Antioxidant activity in terms of free radical scavenging activity of non enriched mushroom extracts is well reported (Puttaraju et al., 2006). Compared to those results, the present study showed that normal (non-Se) fruiting bodies of *P. fossulatus* have higher activity in terms of radical scavenging. On the other hand, Se-rich extracts (20% methanol) showed significantly higher ($P<0.001$) scavenging activity (33.6±1.0%) than non-selenated (21.5±0.4%) extracts (Table 2).

Lipid peroxidation is one of the main manifestations of oxidative damage. Lipid peroxides act on the cellular components, leading to both structural and functional damage of the bimolecular as well as the cellular structure. Mushrooms are considered to generate phenolic antioxidants to inhibit lipid peroxidation (Yen & Chen, 1995). The present observations showed that the MDA content was significantly lower in Se rich extracts (51.5±3.0 nM MDA/g d.w.) ($P<0.001$) compared to control (105.9±2.8 nM MDA/g d.w.) (Table 2), which suggests the inhibition of lipid peroxidation.

Metal ion chelating capacity plays a significant role in antioxidant mechanism since it reduces the concentration of the catalyzing transition metal in the lipid peroxidation process (Dodig & Cepelak, 2004). The chelating effect of Se rich mushrooms from both extractions (20% ME and 90% ME) was found to be significantly higher ($P<0.05$) than non-enriched mushrooms. The 20% ME from Se-enriched and control samples showed the chelating ability of 56.5±2.07% and 44.7±3.5%, respectively, likewise in 90% ME from Se-enriched and control mushrooms showed chelating ability of 53.6±3.3% and 45.8±0.48%, respectively (Table 2).

The results suggested that moderate ferrous-ion chelating ability showed by Se-enriched mushroom extracts could be beneficial to health. Iron can stimulate lipid peroxidation by the Fenton reaction and can also accelerate peroxidation by decomposing lipid hydroperoxide into peroxide and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain of lipid peroxidation (Halliwell, 1999). The high metal chelating activity of Se-enriched mushrooms is assumed to be due to specific metal-Se interactions that greatly affect antioxidant activity based on the type of metal and the specific features of the Se containing biological moieties.

The total anti-oxidant content of the methanol extracts of mushrooms were measured spectrophotometrically with the phosphomolybdenum assay. The total antioxidant content of the methanol extracts of Se-enriched mushrooms was significantly higher than the control (Table 2). Se-enriched mushroom as 90% ME showed higher ($P<0.001$) antioxidant content (41.9±1.15 mg BHT/g d.w.) than non-enriched extracts (34.7±0.65 mg BHT/g d.w.). Similar results ($P<0.05$) were obtained in case of extracts prepared in 20% ME, although lesser amounts were measured than in 90% ME.

The results in the present study revealed that methanolic extracts of Se-enriched mushrooms act as free radical scavengers, indicating the potential use of Se-rich mushrooms for Se supplementation through diet.

Table 2. DPPH scavenging potential, metal chelating efficacy, total antioxidant activity, and lipid peroxidation inhibition capacity of methanolic extracts from Se enriched and control *P. fossulatus* (n=3)

	DPPH scavenging potential (%)		Total phenols (mg GA/g)		Metal chelating activity (%)		Total antioxidant activity (mg GA/g)		Lipid peroxidation (nM MDA/g)
	90% ME	20% ME	90% ME	20% ME	90% ME	20% ME	90% ME	20% ME	
Se-rich	40.60±2.87	33.63±1.01	7.10±0.17	11.1±0.37	53.60±3.33	56.52±2.07	41.93±1.15	32.8±0.3	51.5±2.8
Control	36.03±0.74	21.59±0.40	6.45±0.10	9.32±0.18	45.86±0.48	44.74±3.59	34.76±0.65	31.0±1.0	105.9±3.0
	ns	***	*	**	**	**	***	*	***

*P<0.05; ** P<0.01; *** P<0.001; ns: non-significant

3. Conclusion

The present study demonstrates the use of Se-rich agricultural residues as substrates for cultivation of Se-enriched *P. fossulatus* (oyster mushrooms), which hitherto has been the only one reported with sole exogenous Se supplementation. *Pleurotus fossulatus* indicated notable Se accumulation and corresponding antioxidant activities on cultivation using substrates naturally enriched with selenium. Se-enriched mushrooms with enhanced antioxidant content can therefore serve as effective dietary supplements or neutraceuticals. Thus, the present study proposes the use of Se-rich agricultural residues as substrates for mushroom cultivation for human and livestock Se supplementation.

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