

Enhanced antioxidant properties as a function of selenium uptake by edible mushrooms cultivated on selenium-accumulated waste post-harvest wheat and paddy residues

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Abstract

Background Majority of the post-harvest agri-residues from agricultural activity in Punjab, India, is burnt in the field resulting in the loss of soil fertility and release of large amounts of air pollutants. In an effort to reutilize the selenium-accumulated waste wheat and paddy straw from seleniferous region of Punjab, two varieties of edible mushrooms, *Pleurotus sajor-kaju* and *Volvariella volvacea*, were cultivated on Se-rich wheat and paddy straw, respectively.

Results Se concentration in Se-enriched *P. sajor-kaju* and *V. volvacea* (43.5 ± 2.1 and 35.0 ± 1.1 $\mu\text{g/g}$) was significantly higher than control (5.2 ± 1.0 and 5.57 ± 0.07 $\mu\text{g/g}$), respectively. The antioxidant activity as depicted by total phenol content, total oxidant activity, DPPH scavenging, metal chelation and lipid peroxidation inhibiting activity of extracts from Se-fortified mushrooms were significantly higher ($p < 0.05$ to $p < 0.001$) than control mushrooms.

Conclusion The increased antioxidant activity is attributed to be induced by the accumulation of selenium by these species of mushrooms, indicating the antioxidant nature of selenium in biological systems. Further, the present study also demonstrates the use of Se accumulated

agricultural residues as substrates for producing Se-rich mushrooms as potential sources for Se supplementation/nutraceutical applications.

Keywords Selenium · Uptake · Straw · *Pleurotus* · *Volvariella* · Antioxidant activity

Introduction

Agricultural production in Punjab, India, results in 35–40 million tonnes of rice and wheat straw, annually in addition to other post-harvest residues. As an environmentally degrading practice, post-harvest agri-residues are being burnt in the field resulting in the loss of soil fertility in addition to release of air pollutants, and predictably contributing to climatic change. The seleniferous region of Punjab bordering the districts of Nawanshahr and Hoshiarpur has approximately 1,000 ha under cultivation. The prominent crop cycle of this region is wheat–rice with marginal interspersed with mustard, sugarcane, potato and millets. In the affected area alone, >8,000 tons of straw is generated with every harvest. In this region, the levels estimated in grains and husk appear to be the highest Se concentrations ever recorded (Sharma et al. 2009; Cubadda et al. 2010).

Selenium is an essential element for antioxidant reactions in humans and animals and an important component of several major metabolic pathways, including synthesis of thyroid hormone metabolism, antioxidant defense systems and immune functions (Kohrle and Gartner 2009; Gandhi et al. 2013). Dietary selenium can also effectively reduce the metal toxicity such as mercury and arsenic poisoning (Ralston and Raymond 2010; Sah et al. 2013). The World Health Organization report advises a Se intake

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of 55–65 µg/day as the average intake level needed to ensure meeting normative requirements of healthy adults. Similarly, the upper tolerable range for adults has been proposed to be 400 µg/day (FAO/WHO 2002). Selenium in diet may behave as two-sided coin, i.e. consumption of food containing <0.1 µg Se/day leads to deficiency disorders, whereas consumption of food containing >1 µg Se/day may result in toxicity.

It is well reported that some mushroom species have the capacity to accumulate selenium (Kalac 2009). Mushrooms constitute a very important and highly appreciated source of food worldwide. These edible foods have many medicinal properties which include cholesterol-lowering, anti-tumor, antiviral, anti-thrombotic and immunomodulating effects (Mau et al. 2002). Mushrooms have known antioxidant properties provided by different compounds such as phenolics, ergothioneine and selenium (Se) (Werner and Beelman 2002; Beelman and Royse 2006; Wong and Chye 2009). Different edible species of mushroom such as *Agaricus*, *Pleurotus*, *Volvariella*, *Ganoderma* and *Lentinus* are cultivated worldwide with *A. bisporus* and *P. sajor-kaju* being the most dominantly produced mushrooms across the globe.

Most of the studies reported till date has been focused on Se uptake by mushrooms through exogenous supplementation of Se dominantly as selenite (Werner and Beelman 2002; Zhao et al. 2004). However, similar studies on mushrooms cultivated on agricultural residues naturally enriched with selenium have hitherto not been reported to the best of our knowledge. Post-harvest agricultural residues such as those generated in seleniferous region of Punjab, India, contain significantly high Se content in plant parts such as grains and straw of wheat and other cereals (Dhillon and Dhillon 2003; Sharma et al. 2009). Our recent report on the bioaccessibility and speciation of Se in mushrooms cultivated on wheat with Se accumulation, showed Se to be mainly present as selenomethionine accounting to 73 % of the sum of detected Se species (Bhatia et al. 2013) indicating the presence of other Se containing moieties.

Keeping it in mind, the present study aimed at studying the selenium uptake of *Pleurotus sajor-kaju* and *Volvariella volvacea* cultivated on Se-rich wheat and paddy straw and determining their comparative antioxidant profile as induced by enrichment of selenium from two different varieties of substrates sourced from selenium-rich agricultural residues.

Materials and methods

The strain of *Pleurotus sajor-kaju* (PSC) and *Volvariella volvacea* (VV) were procured from Punjab Agricultural

University (PAU, Ludhiana, India). Both fungi were cultured on potato dextrose agar (PDA) medium and were stored at 4 °C till use. The Se-rich wheat and paddy straw was collected from Jainpur (31°13'N, 76°21'E, Nawanshahr–Hoshiarpur region, Punjab), India, for cultivation of these mushrooms following method outlined by Punjab Agricultural University India (Khanna 2003). Similarly, straw from non-seleniferous region (30°34'N, 76°38'E Patiala, Punjab, India) was used as control.

In brief, prior to spawning, selenium-rich wheat straw was disinfected by dipping in water containing 1.5 % formalin. The treated straw was then inoculated with spawn (approx. 3.0 %) and was filled in plastic bags (3–4 kg/bag). The spawned bags were then incubated in growth chamber at 25 ± 3 °C for approximately 15 days in suspended position till entire straw got tightly bound by mycelia (spawn run) with moisture content maintained at 70 %. The plastic layers of completely colonized bags were torn off and were left suspended until fruiting. Similar conditions were followed to grow control mushrooms, with non-Se wheat straw as the substrate. The paddy straw mushroom (VV) was cultivated on Se-rich paddy straw collected from seleniferous sites. Se-rich paddy straw was arranged in bundles of 40 cm length and 10 cm width (Fig. 3.2). Bundles were soaked in running water overnight. After wetting, the bundles were stacked in layers of four followed by spawning with the active culture. The spawned stacks were incubated at 35 °C till emergence of fruiting bodies. Similar protocol was followed for non-Se paddy straw.

At maturation (22–25 days after inoculation), fruiting bodies were collected, dried at 40 °C for near complete dehydration and powdered using agate mortar. The Se content of powdered samples was analyzed using fluorescence spectrometry (Perkin-Elmer LS 45) (Levesque and Vendette 1970). Briefly, this method involved digestion with HNO₃ and HClO₄, reduction of Se from Se⁺⁶ to Se⁺⁴, complexing of Se⁺⁴ with 2,3-diaminonaphthalene (DAN) and extraction of the piazselenol in cyclohexane. The emission spectrum of piazselenol complex formed during the reaction was measured using fluorescence spectrometer (Perkin-Elmer LS45) at excitation and emission wavelength of 360 and 520 nm, respectively. Se quantification in each sample was carried out by relative method using emission spectrum of NIST certified Se ICP standard solution (SRM-1349). For determining various antioxidant properties, the samples (1 g of dry powder) were extracted by stirring with 10 ml of 50 % methanol for 3 h, at room temperature using probe ultrasonicator, and filtering through Whatman #1 paper. The filtrates were concentrated with a rotary vacuum evaporator at 40 °C. The resultant extracts were stored at 4 °C until use.

Total phenolic compounds were measured using Lowry reagent (Singleton and Rossi 1965) and expressed as gallic

acid (GA) equivalents. Total antioxidant activity of methanolic extracts of both mushrooms were measured using UV–visible spectrometer (Hitachi U2900) according to the method (phospho-molybdenum assay) (Imran et al. 2011) using gallic acid (GA) (0.01–0.1 mg/ml) as standard. Lipid peroxidation in both Se and NSe samples was monitored fluorimetrically (Perkin-Elmer-LS45) at an excitation wavelength of 532 nm and emission wavelength of 550 nm following method (Minotti and Aust 1987). Calibration curves were made using malondialdehyde (MDA; Sigma) in the range of 0.5–5.0 μ M. The scavenging activity of the methanolic extracts (0.05–1.0 mg/ml) from mushroom on DPPH radicals was measured spectrophotometrically (Chu et al. 2000). The scavenging activity (% SA) of DPPH radicals was calculated using equation [% SA = (1 – Abs in the presence of sample/Abs in the absence of sample) \times 100]. Butylated hydroxyl anisole (BHA) (0.1–1 mg/ml) was used as standard. The chelating activity of the various concentrations of extracts (1–10 mg/ml) 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: % chelating activity = $[(A_{\text{negative}} - A_{\text{sample}})/A_{\text{negative}}] \times 100$, where A is absorbance. EDTA was used as positive control while absence of extract of the mushroom was the negative control.

Results and discussion

The selenium content in paddy and wheat straw collected from Se-rich and control (non-Se) sites, and in fruiting bodies of Se-rich rich mushroom cultivated on the said substrates are presented in Table 1. The fruiting bodies of PSC harvested from Se-rich wheat straw containing a total Se concentration of $27.0 \pm 0.2 \mu\text{g/g}$ were noted to accumulate significantly higher ($p < 0.001$) selenium up to $43.5 \pm 2.1 \mu\text{g/g}$ as compared to control/non-Se mushroom ($5.2 \pm 1.0 \mu\text{g/g}$) cultivated on non-Se straw ($1.9 \pm 0.8 \mu\text{g/g}$). Similar trend was obtained in case of VV wherein the fruiting bodies harvested from Se-rich paddy straw containing a total Se concentration of $29.7 \pm 0.9 \mu\text{g/g}$ were noted to accumulate significantly higher ($p < 0.001$) selenium up to $35.0 \pm 1.1 \mu\text{g/g}$ as compared to control/non-Se mushroom ($5.57 \pm 0.07 \mu\text{g/g}$) cultivated on non-Se straw ($2.0 \pm 0.6 \mu\text{g/g}$). Selenium accumulation from Se-rich substrate to fruiting in PSC and VV varied ($p < 0.01$) significantly (Table 1) with PSC showing higher levels of Se as compared to VV. The amount of selenium found in mushrooms is dependent on the species, the stage of maturity, the amount of selenium in soil, and the substrates used for growth of cultivated species (Werner and Beelman 2002).

Se-rich methanolic extracts belonging to both experimental mushrooms (PSC and VV) showed significantly higher total phenol content as compared to their respective non-Se mushrooms. The amount of phenolic compounds in the methanol extracts of Se-enriched PSC ($8.39 \pm 0.6 \text{ mg GA/g DW}$) was significantly higher ($p < 0.01$) than control ($6.37 \pm 0.2 \text{ mg GA/g DW}$) (Table 1). Similar trend was obtained in case of Se-enriched VV wherein experimental extracts ($17.7 \pm 0.5 \text{ mg GA/g DW}$) contained significantly higher ($p < 0.001$) phenol content as compared to the control ($12.7 \pm 0.7 \text{ mg GA/g DW}$). As individual cases, viz. Se vs NSe, the phenol profile was following similar pattern (increased phenol content with increasing Se) in both experimental mushrooms; however, when compared at organism level (PSC vs VV), it was observed that both Se-enriched mushrooms significantly varied ($p < 0.001$) in terms of phenol content with VV showing higher phenol as compared to PSC. The higher levels of phenol may be due inhibition of enzymatic polyphenol oxidation by strong antioxidant-active selenium compounds.

The total antioxidant activity of the methanol extracts of Se-enriched mushrooms and control increased with increasing concentrations (Table 1). Se-rich methanolic extracts belonging to both experimental mushrooms (PSC and VV) showed significantly higher total antioxidant content as compared to their respective non-Se mushrooms. The total antioxidant content in the methanol extracts of Se-enriched PSC ($4.58 \pm 0.2 \text{ mg BHT/g DW}$) was significantly higher ($p < 0.001$) than control ($1.45 \pm 0.2 \text{ mg BHT/g DW}$) (Table 1). Similar trend was obtained in case of Se-enriched VV wherein experimental extracts ($0.93 \pm 0.01 \text{ mg BHT/g DW}$) contained significantly higher ($p < 0.001$) content as compared to the control ($0.82 \pm 0.07 \text{ mg BHT/g DW}$). The total antioxidant content of Se-PSC was higher than Se-VV and it varied significantly.

Polyunsaturated fatty acid peroxides generate malondialdehyde (MDA) upon decomposition. The measurement of MDA has been used as an indicator of lipid peroxidation (LPO). Table 1 illustrates LPO values of both experimental mushrooms. Se-rich mushrooms in general showed significantly lower values of LPO as compared to control mushrooms. The selenium-rich extracts of PSC ($17.6 \pm 4.4 \text{ nM MDA/g}$) and VV ($475 \pm 4.7 \text{ nM MDA/g}$) showed significant decrease ($p < 0.001$) in MDA levels as compared to control (459.2 ± 4.2 and $640 \pm 5.2 \text{ nM MDA/g}$, respectively) extracts. MDA levels were varying significantly ($p < 0.001$) among Se-rich extracts of both PSC and VV; however, PSC extracts showed very low levels of MDA when compared to VV. The difference in MDA levels between the two organisms might be due to difference in unsaturated fatty acids profiles (which upon decomposition generate large amount of MDA) in

Table 1 Total selenium levels and antioxidant profiles in fruiting bodies Se-enriched *Pleurotus sajor-caju* (PSC), *Volvariella volacea* (VV) and corresponding control mushrooms ($n = 3$)

Sample	Se ($\mu\text{g/g dw}$) [#]		Yield of methanolic extracts (%)	Yield of total phenols (mg GA/g dw)	Total antioxidants (mg BHT/g DW)	Lipid peroxidation (nM MDA/g)		
	Wheat straw	PSC						
Se	27.0 \pm 0.2	43.5 \pm 2.1	28.0 \pm 1.5	8.39 \pm 0.6	4.58 \pm 0.2	17.6 \pm 4.4		
Non-Se	1.9 \pm 0.8	5.2 \pm 1.0	26.0 \pm 1.1	6.37 \pm 0.2	1.45 \pm 0.2	459.2 \pm 4.2		
	***	***	*	**	***	***		
	Paddy straw	VV						
Se	29.7 \pm 0.9	35.0 \pm 1.1	32.7 \pm 1.0	17.7 \pm 0.5	0.93 \pm 0.01	475 \pm 4.7		
Non-Se	2.0 \pm 0.6	5.57 \pm 0.07	29.3 \pm 1.2	12.7 \pm 0.7	0.82 \pm 0.07	640 \pm 5.2		
	***	***	*	***	***	***		
Se-PSC vs Se-VV	*	**	*	***	***	***		
Concentration of methanolic extract (mg/ml)	0.5	1	2	3	4	5	10	
Scavenging effect (%) of mushroom species on DPPH								
PSC								
Se		14.0 \pm 0.55	17.2 \pm 0.70	22.5 \pm 0.50	26.8 \pm 0.52	37.7 \pm 0.6	40.6 \pm 1.0	51.8 \pm 0.3
Non-Se		11.13 \pm 0.9	14.3 \pm 0.75	19.7 \pm 0.64	21.8 \pm 0.32	28.9 \pm 1.0	33.6 \pm 0.72	45.1 \pm 1.8
Se vs NSe		**	**	**	***	***	***	**
VV								
Se		10.9 \pm 0.18	15.4 \pm 0.2	29.6 \pm 0.43	37.9 \pm 1.2	55.2 \pm 0.8	62.0 \pm 0.48	71.1 \pm 0.8
Non-Se		4.50 \pm 0.34	10.3 \pm 0.2	24.1 \pm 0.80	32.1 \pm 1.65	47.4 \pm 0.6	57.1 \pm 0.58	65.1 \pm 0.5
Se vs NSe		***	***	***	**	***	**	**
Se-PSC vs Se-VV		***	*	**	***	***	***	***
BHA [@]		96.7 \pm 0.5						
Methanolic extract (mg/ml)	0.4	1	2	3	4	5	10	
Metal chelating activity (%)								
PSC								
Se	–	19.6 \pm 0.90	45.3 \pm 2.7	67.0 \pm 1.8	73.2 \pm 1.9	90.7 \pm 1.37	95.4 \pm 1.37	
Non-Se	–	11.3 \pm 1.3	31.5 \pm 0.5	45.2 \pm 2.7	59.0 \pm 2.7	75.0 \pm 1.9	83.1 \pm 2.8	
Se vs NSe		***	**	***	**	***	**	
VV								
Se	–	15.2 \pm 1.6	21.8 \pm 1.4	32.0 \pm 1.6	35.0 \pm 1.7	47.0 \pm 0.5	56.1 \pm 1.7	
Non-Se	–	10.4 \pm 1.5	13.8 \pm 1.4	24.2 \pm 0.9	30.0 \pm 0.8	38.0 \pm 0.8	43.0 \pm 1.4	
Se vs NSe		*	**	**	*	***	**	
Se-PSC vs Se-VV	–	*	***	***	***	***	***	
EDTA [@]		85.4 \pm 0.7						

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ [@] positive control[#] Se ($\mu\text{g/g dw}$) in PSC is also published in Bhatia et al. (2013)PSC *Pleurotus sajor-caju*, VV *Volvariella volacea*

mushrooms which in turn get affected with the increasing concentration of Se. The present results can be explained by the important role of Se, in general and that sourced from mushrooms in particular, in preventing lipid peroxidation and in protecting the integrity and functioning of tissues and cells (Fatma and Demerdash 2004; Yan and

Chang 2012). Similarly various researchers have defined selenium role in inducing antioxidant capacity in terms of decrease in lipid peroxidation.

Determination of DPPH scavenging activity represents a rapid method to characterize the antioxidant capacity of extracts against oxidation caused by free radicals. The

scavenging effect of methanolic extract of Se-enriched and control mushrooms increased with increasing concentration of extract (Table 1). The Se-enriched extracts of both experimental mushroom PSC and VV showed significantly higher free radical scavenging as compared to their respective control; however, scavenging ability between the two Se-enriched mushrooms was significantly ($p < 0.001$) different. Se-rich VV extracts showed higher radical scavenging activity which was 71.1 % compared to PSC mushroom which was 51.8 % when tested at the highest concentration (10 mg/ml). In general, the scavenging activity of the VV mushroom was higher than that of PSC mushroom in the concentration range tested. Our results are supported by observations of Turlo et al. (2010), that Se-enriched water and methanol mycelial extracts of *Lentinus edodes* showed excellent scavenging effects as compared to non-enriched extracts.

Metal ions can initiate lipid peroxidation and start a chain reaction that leads to the deterioration of food (Gordon 1990). In the present study, the chelating ability of the mushroom extracts toward ferrous ions (Fe^{2+}) was investigated. The Fe^{2+} -ferrozine complex formation was significantly prevented by methanolic extracts of mushroom species. Table 1 shows the chelating effects of the mushroom species compared with EDTA as standard on ferrous ions. The absorbance of Fe^{2+} -ferrozine complex significantly decreased dose dependently (1–10 mg/ml). The metal chelating capacity at 10 mg/ml concentration of Se-enriched methanolic extract of PSC and VV were found to be 95.4 and 56.1 %, as compared to control with chelation 83.1 and 43.0 %, respectively. Depending on the selenium content, the Se-enriched extracts at concentrations of 1–10 mg/ml had the chelating effect in the following order: Se-enriched PSC extract (19.6–95.4 %) > Se-enriched VV methanolic extract (15.2–56.1 %) with metal chelation ability of Se-PSC and Se-VV varied significantly. Similar observations have also been reported by our group in case of Se-enriched *Pleurotus fossulatus*, wherein Se-enriched extracts showed significantly higher metal chelation than non-Se extracts (Bhatia et al. 2014). Iron-mediated DNA damage inhibition is seen for methyl-selenocysteine, selenocystamine, 3,3-diselenobispropionic acid, and 3,3-selenobispropionic acid but to a lesser extent than with copper (Battin et al. 2006). The enhanced metal chelation ability in the present study might be due to the specific metal-selenium complexes which affects the antioxidant status.

Our earlier observations on mushrooms accumulated with selenium from agri-residues indicated presence of about 23 % of unknown selenium moieties in addition to dominant presence of selenomethioneine (Bhatia et al. 2013). In addition, studies carried out by Beelman and co-workers on mushrooms cultivated on substrates exogenously supplemented with Se as inorganic selenite

indicated the synthesis of selenoergothioneine with Se effectively replacing sulfur in the ergothioneine moiety along with other organoselenium compounds (Beelman et al. 2007). Pioneering work by this group envisaged enhanced potential of selenoergothioneine in mushrooms due to combined benefits of two very strong antioxidant moieties, viz., Se and ergothioneine.

Although there are limited studies on the effect of selenium in mushroom on bioactive properties, some researchers have studied the effect of supplementation of selenium on lipid peroxidation under in vivo conditions. *Pleurotus ostreatus* enriched with selenium and zinc when administered to mice, resulted in significant decrease in MDA levels as compared to those fed on normal diet (Yan and Chang 2012). However, in these studies, the selenium enrichment was carried out through exogenous supplementation using inorganic form of selenium. Therefore, it is clearly evident the selenium uptake and antioxidant activity in mushrooms would complement the nutritional value of these edible species and their use in supranutritional doses required for enhanced health properties such as anti-inflammatory and anti-cancer responses (Clark et al. 1996).

The present study, thus, demonstrates the use of Se-rich agricultural residues as substrates for cultivation of Se-enriched *Pleurotus sajor-kaju* (oyster mushrooms) and *Volvariella volvacea* (paddy straw mushroom) which hitherto has been reported with only exogenous selenium supplementation. Both indicated notable selenium accumulation along with significant antioxidant activities during cultivation on substrates naturally enriched with selenium. These Se-enriched mushrooms, with enhanced antioxidant content, can alternatively be used as effective dietary supplements or nutraceuticals. However, detailed studies need to be carried out to confirm their properties both in vitro and in vivo condition.

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