Acta Alimentaria 50 (2021) 3, 358–368
DOI: 10.1556/066.2021.00005

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Effect of selenium enriched wheat substrate on the nutritional and antioxidant properties of *Pleurotus* spp.

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ORIGINAL RESEARCH PAPER

Received: January 6, 2021 • Accepted: March 5, 2021
Published online: May 13, 2021

ABSTRACT

The study demonstrated that cultivation of *Pleurotus ostreatus* var. *florida*, *Pleurotus eryngii*, *Pleurotus cornucopiae* and *Pleurotus djamor* on selenium enriched substrate did not significantly affect biological efficiency (%), but total soluble protein content, total phenolic content, flavonoid content and free radical scavenging activity (%) were found to be significantly improved in Se enriched fruit bodies as compared to the control. Elemental analysis of the Se biofortified *Pleurotus* mushrooms through SEM-EDS showed signals characteristic for selenium on surface of *P. ostreatus*. var. *florida* and *P. djamor* confirming that selenium was incorporated into the cell wall of these fruiting bodies. The Se content was found to be 22.34 μg g^{-1} dw in Se enriched wheat straw and 0.059 μg g^{-1} dw in respective non-enriched wheat straw. Se contents of Se-enriched fruit bodies were found to be higher compared to non-enriched *Pleurotus* spp. FT-IR spectra of proteins from *Pleurotus* spp. indicated an increase in the flexibility, unfolding, hydrophilicity of the proteins upon Se supplementation.

KEYWORDS

antioxidant activity, biofortification, nutraceutical, SEM-EDS, FT-IR, hydrophilicity

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1. INTRODUCTION

Selenium plays a crucial role in the growth and development of humans and animals and is known for its antioxidant and anticancer properties. It is required in trace amounts for the functioning of a number of enzymes like glutathione peroxidase, thioredoxin reductase or iodine deiodinase (Kaur et al., 2017). It is basically a key component of selenoproteins containing selenocysteine and selenomethionine amino acids. Selenoproteins possess antioxidative activity, which in turn regulates the redox balance (Khurana et al., 2019). Intake of Se biofortified foods are known to protect from carcinogens better than non-Se biofortified foods. An inadequate supply of selenium leads to growth retardation and dysfunctional bone metabolism, which in turn causes abnormalities in thyroid gland function. Some of the diseases associated with insufficient intake of Se in humans and livestock are Keshan disease, Kashin-Beck disease (Yang et al., 2017), atherosclerotic heart disease, or diarrhoeal disease (Zou et al., 2018). As a result, inclusion of selenium in the diet has been recommended by various regulatory agencies. The recommended daily allowance varies with agency to agency, with an average of 70 μg Se day^{-1} per adult person. The World Health Organization (WHO) has recommended the daily intake of Se in the range of 55–200 μg for adults (WHO, 2009).

Mushrooms, the macrofungi, are known to possess excellent abilities to mobilise and accumulate various mineral elements from the environment in which they grow. Growing of *Agaricus bisporus* on substrates enriched with Se at 0.6 mmol L^{-1} concentration resulted in 2.5 times increase in the amount of selenium in the fruiting bodies (Rzymski et al., 2016). Likewise, the ability of the oyster mushroom that is *Pleurotus* spp. could be exploited for the production of Se biofortified mushrooms and is found to be an effective ecofriendly way of recycling organic wastes. It is crucial to precisely optimise the biofortification strategies of mushrooms for enhancing the Se uptake. Therefore, the present study has been planned to produce Se-biofortified *Pleurotus* species using Se rich agricultural residues and to evaluate its antioxidant potential including total selenium content, total soluble protein, total phenol content, flavonoid content, % radical scavenging activity and the chemistry of proteins and polysaccharides on Se biofortification.

2. MATERIALS AND METHODS

The experiments employed *Pleurotus ostreatus* var. *florida Cetto*, *Pleurotus eryngii* (DC. ex Fr.) Quél, *Pleurotus cornucopiae* (Paulet) Rolland and *Pleurotus djamor* (Rumph. ex Fr.) Boedijn cultures procured from germplasm collection bank of Department of Microbiology, PAU, Ludhiana, India. All chemicals used in the present research study were of analytical grade and were procured from HiMedia Laboratories Pvt. Ltd., Mumbai, India.

The Se enriched wheat straw was procured from seleniferous soils of Jainpur village of Nawanshahr-Hoshiarpur region of Punjab, India. Master spawn, generation spawn and the cultivation of *Pleurotus* spp. and biological efficiency were calculated as the ratio of fresh weight (g) of mushrooms to the dry weight (g) of substrate and expressed as percentage according to Garcha and Khanna (2002).

The fruit bodies of four *Pleurotus* species were dried in oven at 55 °C for 24 hours and powdered. The selenium content was estimated from the wheat straw (substrate) and fruit

bodies of *Pleurotus* spp. by using ICP-MS. The dried sample (0.5 g) was weighed and digested in a mixture of HNO_3 and H_2O_2 (3:1). The digestion was carried out in digestion tubes on a hot plate at 70 °C till the solution became transparent and re-dissolved in distilled water (DW), filtered and used for Se estimation.

Selenium biofortified and control fresh fruit bodies of *Pleurotus* spp. were harvested and processed as per the standard protocol for processing of the biological specimen (Bozzola and Russell, 1999) with few modifications. The processed sample was sputter coated and viewed in SEM. Elemental analysis of the sample surfaces was performed by Energy Dispersive Spectroscopy system (EDS) (model Jeol JSM-6100, USA) attached to the SEM (model Jeol JSM-6100, USA). The total soluble protein content of the mushroom fruit bodies were estimated according to the method of Lowry et al. (1951).

Total phenolic content was estimated as per the method of Swain and Hillis (1959) with little modifications. The % free radical scavenging activity for DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was measured for all the four *Pleurotus* spp. as described by Blois (1958). The total flavonoid contents were estimated from the fruit bodies of *Pleurotus* spp. by following the protocol given by Zhishen et al. (1999).

The Se biofortified *Pleurotus* fruit bodies were analysed for their chemical or functional group diversity on Fourier transform infrared spectroscope (FTIR) (model Thermo-Nicolet 6700, ThermoScientific, Rockford, IL, USA) equipped with Attenuated total reflectance assembly (Smart Assembly, ThermoScientific, Rockford, IL, USA). The spectra for the samples were obtained for a range of 500 and 4,000 cm^{-1} wavenumbers.

All experimental results were presented as 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 marked with identical letters in rows or columns exhibit no differences at the significance level $\alpha = 0.05$.

3. RESULTS AND DISCUSSION

3.1. Biological efficiency (%) of *Pleurotus* spp. fruit bodies

No significant differences in the number of fruit bodies and biological efficiency (%) among the Se-enriched and non-enriched treatments were recorded for the four *Pleurotus* spp. (Table 1, Fig. 1). Similar results were obtained by cultivating five mushroom species belonging to different genera, namely *Pleurotus sajor-caju*, *Pleurotus citrinopileatus*, *P. ostreatus*, *A. bisporus* and *Volvariella volvaceae,* for post agricultural residues belonging to both seleniferous and non-seleniferous (control) sites (Solovyeva et al., 2018). No significant differences on the total yield and biological efficiency of mushrooms over non-Se (control) treatment were observed.

3.2. Estimation of selenium content using ICP-MS/AAS (Inductively Coupled Plasma Mass Spectrometry/Atomic Absorption Spectroscopy)

The Se contents of Se enriched and control wheat straw were found to be 22.34 and 0.059 μg g^{-1} dw, respectively. A significant difference in the Se content was recorded between the Se-enriched and non-Se-enriched fruit bodies of all *Pleurotus* spp. studied. The highest Se content was found

Table 1. Phytochemical analysis of selenium biofortified *Pleurotus* spp. fruit bodies

Species	Treatment	Number of fruit bodies/ 100 kg dry substrate	Biological efficiency (%)	Total selenium content (μg Se g^{-1} dw)	Total soluble protein content (mg g^{-1} fresh weight)	Total phenol content (mg GAE/g fresh weight)	Radical scavenging activity (%)	Total flavonoid content (μg QE/g fresh weight)
P. ostreatus var. *florida*	Se straw	6,800 ± 152[a]	66.50 ± 1.11[a]	124.00 ± 7.94[a]	1.98 ± 0.12[a]	2.44 ± 0.16[a]	10.54 ± 0.45[a]	512.5 ± 10.03[a]
	Control	6,712 ± 199[a]	64.57 ± 1.16[a]	2.48 ± 0.39[b]	1.41 ± 0.09[b]	0.42 ± 0.06[b]	8.98 ± 0.24[b]	225.0 ± 10.15[b]
P. eryngii	Se straw	4,200 ± 152[a]	30.41 ± 2.04[a]	57.25 ± 7.33[a]	2.55 ± 0.24[a]	1.28 ± 0.10[a]	4.40 ± 0.20[a]	425.0 ± 16.17[a]
	Control	4,000 ± 208[a]	27.16 ± 0.97[a]	0.49 ± 0.03[b]	1.60 ± 0.24[b]	0.66 ± 0.12[b]	2.01 ± 0.12[b]	250.0 ± 20.60[b]
P. cornucopiae	Se straw	6,631 ± 132[a]	36.74 ± 1.50[a]	46.25 ± 6.59[a]	2.16 ± 0.15[a]	2.00 ± 0.15[a]	10.72 ± 0.26[a]	500.0 ± 19.55[a]
	Control	6,544 ± 208[a]	33.11 ± 1.80[a]	0.161 ± 0.03[b]	1.38 ± 0.11[b]	0.96 ± 0.08[b]	7.56 ± 0.20[b]	275.0 ± 14.74[b]
P. djamor	Se straw	8,612 ± 193[a]	30.14 ± 1.93[a]	156.6 ± 7.66[a]	4.56 ± 0.31[a]	4.08 ± 0.16[a]	9.16 ± 0.28[a]	250.0 ± 13.75[a]
	Control	8,500 ± 208[a]	29.16 ± 1.50[a]	4.33 ± 0.45[b]	3.60 ± 0.24[a]	1.16 ± 0.10[b]	7.53 ± 0.19[b]	50.0 ± 1.53[b]

Mean values ($n = 3$) ± standard deviations; a, b, c, etc.: different letters differ significantly at $P = 0.01$ (Tukey's test).

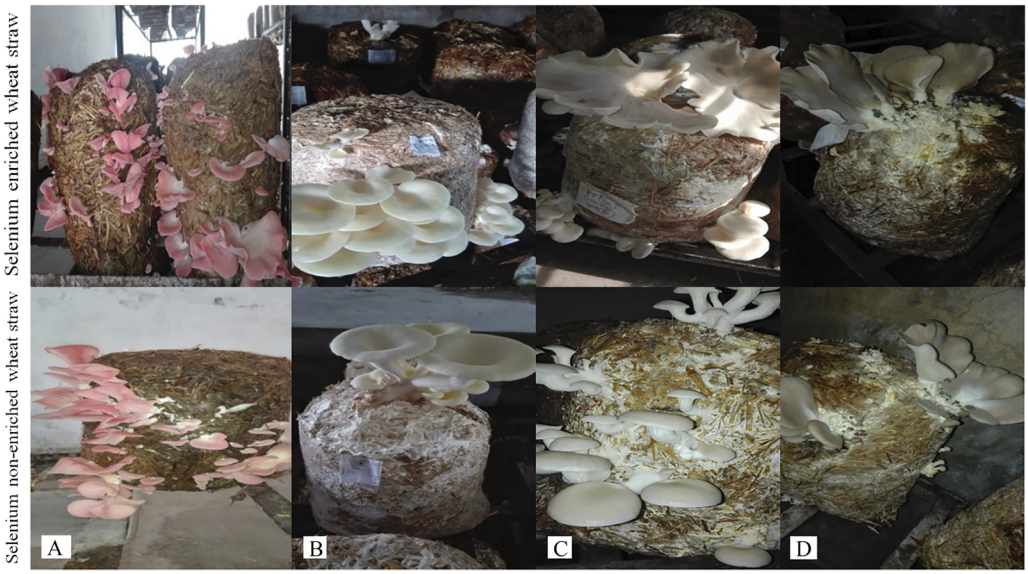

Fig. 1. Fruiting bodies of different *Pleurotus* spp. cultivated on selenium rich and normal wheat straw: (A) *P. djamor*, (B) *P. cornucopiae*, (C) *P. ostreatus* var. *florida* and (D) *P. eryngii*

in *P. djamor*, followed by *P. ostreatus* var. *florida*, *P. eryngii,* and *P. cornucopiae*. Among the control (non Se-enriched) fruit bodies, the order of Se content was *P. djamor* > *P. ostreatus* var. *florida* > *P. eryngii* > *P. cornucopiae* (Table 1). A similar study performed on Se-bioaccessibility and speciation of *P. ostreatus* var. *florida* grown on Se-rich agricultural residues led to increase in Se content (27.9 μg g^{-1} dw) compared to control (0.026 μg g^{-1} dw) (Bhatia et al., 2013).

3.3. Energy Dispersive X-ray Spectroscopy of the fruit bodies

Energy Dispersive X-ray Spectroscopy (EDS) is a useful technique for the estimation of concentration and distribution of different elements on the surface of samples. This technique was utilised to reveal the Se element content present on the surface of the fungal hyphae comprising the mushroom fruit body. Though it is not a direct technique to identify the occurrence of seleno-proteins or organic Se-compounds, but it can possibly indicate the occurrence of Se on the surface of the fruiting bodies of mushrooms. Characteristic peak appeared for Se element on SEM-EDS analysis of the surface of the *P. ostreatus* var. *florida* and *P. djamor* fruit bodies confirming its presence as integral component of the cell wall of mushrooms. On the contrary, the Se peak (in keV) was not observed during the analyses of fruiting bodies of *P. eryngii* and *P. cornucopiae,* indicating that the seleno-proteins and other forms of Se-compounds are concealed as cytosolic moieties (Fig. 2). The Se (% wt) was in order *P. djamor* (2.85% wt) > *P. ostreatus* var. *florida* (0.72% wt) as compared to the respective control fruit bodies (Table 2). Percentage weight and atom percentage of oxygen decreased for Se biofortified *P. ostreatus* var. *florida* and *P. cornucopiae* mushrooms as compared to their control fruit bodies, whereas they increased in Se biofortified fruiting bodies of *P. eryngii* and *P. djamor* mushrooms. The decrease in the oxygen concentration

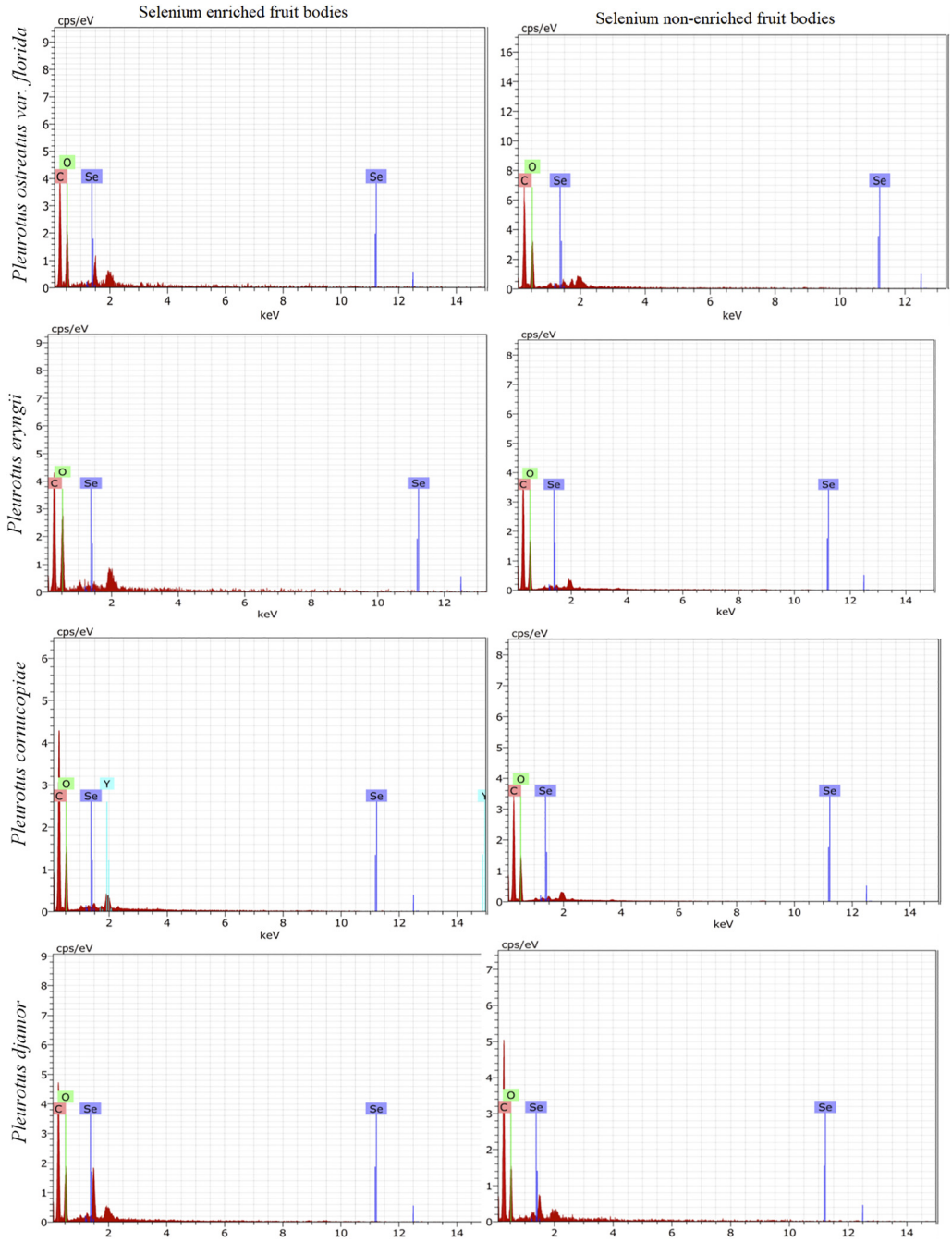

Fig. 2. Energy Dispersive X-ray Spectra for Se-enriched and non-Se-enriched fruiting bodies of four different species of *Pleurotus* spp.

Table 2. SEM-EDS analysis of Se enriched and non-enriched fruiting bodies of *Pleurotus* spp.

Species	Treatment	Weight %			Atom %		
		Carbon	Oxygen	Selenium	Carbon	Oxygen	Selenium
P. florida	Control	52.11	47.66	0.22	59.27	40.69	0.04
	Se straw	53.08	46.20	0.72	60.41	39.47	0.12
P. eryngii	Control	56.36	43.64	0.00	63.24	36.76	0.00
	Se straw	50.35	49.65	0.00	57.46	42.54	0.00
P. cornucopiae	Control	55.72	44.28	0.00	62.64	37.36	0.00
	Se straw	60.06	34.89	0.00	69.08	30.13	0.00
P. djamor	Control	61.24	37.93	0.83	68.17	31.69	0.14
	Se straw	58.33	38.82	2.85	66.36	33.15	0.49

may be due to the replacement of oxygen with Se as both elements belong to the same group in the periodic table. Similar results were obtained by Goyal et al. (2015) in SEM-EDS study of hyphal mass of *Ganoderma lucidum*. It showed difference in the % wt and % atom composition of oxygen.

3.4. Total soluble protein content

The total soluble protein content (TSPC) was estimated from the fruit bodies of *Pleurotus* spp. grown on selenium enriched substrate. The TSPC was found be higher in Se-enriched fruit bodies than control (Table 1). Bhatia et al. (2014) have also observed significantly higher TSPC in Se-enriched mushrooms (307 ± 4.5 mg g^{-1} dw) compared to control fruit bodies (282 ± 2.4 mg g^{-1} dw). The high sulphur amino acid and total protein contents in the fruit bodies of the Se-enriched mushrooms can be responsible for accumulation of large amount of Se, which includes formation of diverse selenoproteins (Zieba et al., 2020).

3.5. Total phenolic content

The total phenolic content (TPC) was higher in Se-enriched than non-enriched fruit bodies of all four *Pleurotus* spp. (Table 1). Rathore et al. (2018) have analysed Se-enriched *Calocybe indica* fruit bodies and observed enhanced TPC (25.29 mg GAE/g) on Se enrichment (5 mg mL^{-1}). However, the TPC decreased significantly with higher concentrations of Se in the substrate.

3.6. Scavenging activity (%)

The DPPH scavenging activity (%) was found to be higher in Se-enriched fruit bodies than respective control for all four *Pleurotus* spp. (Table 1). Rathore et al. (2018) reported that the DPPH radical scavenging activity of the *C. indica* extracts increased with increase in Se concentration, which indicated significantly higher scavenging effects compared to the control.

3.7. Total flavonoid content

The total flavonoid content (TFC) was found to be higher in Se-enriched fruit bodies than non-enriched fruit bodies of all four *Pleurotus* spp. (Table 1). Gąsecka et al. (2016) reported that the TFC for *Hericium erinaceus*, *G. lucidum* and *Agrocybe aegerita* increased after Se

supplementation from 368.6 to 445.6, 469.9 to 627.7 and 318.1 to 393.9 μg g^{-1} of extract, respectively. The results showed that the mushrooms had superior antioxidant properties after Se supplementation.

3.8. Characterisation of selenium biofortified *Pleurotus* spp. fruiting bodies by Fourier Transform Infra-Red Spectroscopy (FT-IR spectroscopy)

Selenium biofortification in *Pleurotus* spp. involves the formation of seleno-proteins and seleno-polysaccharides. The dried powder of the fruiting bodies of different *Pleurotus* species was subjected to the FT-IR spectroscopy analysis to identify the functional group and conformational changes in mushroom polysaccharides and proteins as a result of Se supplementation. The FT-IR spectra of Se enriched fruiting bodies of all four *Pleurotus* species shared the broad peak between 4,000 and 3,000 cm^{-1} representing the stretching vibrations of O–H functional group in sugar residues. Likewise, the peaks in range of 2,300–3,000 cm^{-1} appeared due to the stretching of C–H bonds in carbohydrates. While, the peaks in range of 1,200–900 cm^{-1} are considered characteristic for the stretching vibrations of C–C, C–O–C, and C–O bonds in the glucopyranose structure of carbohydrates. Discriminating peaks representing polysaccharide linked proteins appeared in higher numbers in Se-enriched fruit bodies of *P. ostreatus* var. *florida* [at 1652.8 (Amide-I), 1543.4 and 1533.3 cm^{-1} (Amide-II)] and *P. djamor* [at 1623.6, 1652.8 cm^{-1} (Amide-I) and 1528.3, 1511.2 cm^{-1} (Amide-II)]. However, fewer peaks were observed in the Se-enriched fruit body of *P. eryngii* [at 1639.2 cm^{-1} (Amide-I)], whereas no significant variation in the wavenumbers of Se-enriched fruit body of *P. cornucopiae* (at 1652.6 cm^{-1} (Amide-I) and 1559.7 cm^{-1}(Amide-II)] occurred. The specific peaks representing β-glycosidic bonds were observed at slight variation in wavenumbers for Se-enriched fruit bodies of *P. ostreatus* var. *florida* (at 1311.7, 1369.3 and 1415.0 cm^{-1}), *P. eryngii* (at 1404.3 cm^{-1}), *P. djamor* (at 1409.4, 1338.4, 1368.7 cm^{-1}) and *P. cornucopiae* (at 1380.3 cm^{-1}) as compared to their respective control samples (Fig. 3A). Mohaček-Grošev et al. (2001) analysed the polysaccharide extracts from wild growing mushrooms and toadstools by FT-IR spectroscopy. They have reported that the peaks formed between 750 and 950 cm^{-1}, corresponds to α or β anomer C_1–H deformation. The band at 890 cm^{-1} can be attributed to a β-1,3-glucan, while the bands at 850 and 929 cm^{-1} were assigned to α-1,6-glucan, and the band at 822 cm^{-1} to α-1,3-glucan.

Peaks corresponding to Amide-I region due to C=O stretching were shared for both control and Se-enriched fruit bodies of *P. ostreatus* var. *florida* (at 1652.8 cm^{-1}), *P. eryngii* (at 1639.2 cm^{-1}), *P. djamor* (at 1652.8 and 1623.6 cm^{-1}) and *P. cornucopiae* (at 1652.6 cm^{-1}). Peaks at 1543.4 and 1533.3 cm^{-1} representing the Amide II region arising due to CN stretching and NH bending vibrations were recorded in Se-enriched *P. ostreatus* var. *florida* fruit bodies but were absent in their respective control sample. However, in *P. eryngii* amide-II peak at 1560.0 cm^{-1} was observed only in the control fruit body, whereas, in *P. djamor* and *P. cornucopiae* the peaks appeared with slight change in wavenumbers in Se-enriched (at 1557.4 and 1559.7 cm^{-1} respectively) and control sample (1542.5 and 1571.9 cm^{-1}, respectively). Amide I region showed peaks for α helix at 1652.8 and 1652.6 cm^{-1} in Se enriched and non-enriched fruit bodies of *P. ostreatus* var. *florida*, respectively. In both control and Se enriched fruit bodies of *P. eryngii,* Amide I region showed β sheet conformation with peak at 1639.2 cm^{-1}. In their respective control sample, the peak at 1652.6 cm^{-1} corresponding to Amide I region showed α helix structure of protein. Amide I region showed peaks for α helix conformation of the mushroom

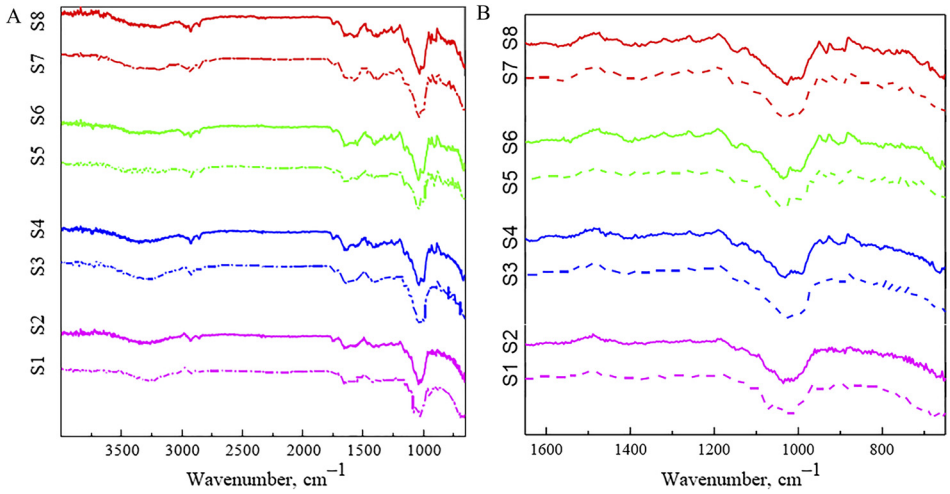

Fig. 3. FT-IR spectra of Se-enriched and non-enriched fruiting bodies of four different *Pleurotus* species for whole mid-IR region i.e. 4,000–650 cm^{-1} (A) and the specific and fingerprint region (1,650–650 cm^{-1}) (B). *P. ostreatus var. florida* (S1 and S2), *P. eryngii* (S3 and S4), *P. djamor* (S5 and S6) and *P. cornucopiae* (S7 and S8). The solid lines indicate the FTIR spectra of the Se-enriched fruit bodies, while the dotted-dashed lines indicate the non-Se-enriched fruit bodies of the respective mushroom

protein with peaks at 1652.8 and 1652.4 cm^{-1} in Se-enriched and non-enriched fruit bodies of *P. djamor*, respectively. Amide I region showed peaks for α helix of protein at 1652.6 and 1652.5 cm^{-1} in Se-enriched and non-enriched fruit bodies of *P. cornucopiae*, respectively (Fig. 3B). FT-IR spectra of proteins from all four *Pleurotus* spp. indicated an increase in flexibility, unfolding and hydrophilicity upon Se supplementation. Bekiaris et al. (2020) studied the *Pleurotus* mushrooms' glucans and ergosterol content by ATR-FTIR Spectroscopy. They have assigned the peaks between 1,800 and 1,500 cm^{-1}, with two major bands around 1,650 and 1,560 cm^{-1} to amide I and amide II region of proteins.

4. CONCLUSIONS

The results revealed that there was no adverse effect on the biological efficiency % of all four *Pleurotus* spp. cultivated on Se-hyperaccumulated wheat straw. There was an improvement in the nutritional values of biofortified mushrooms in terms of total selenium content and total soluble protein content. The antioxidant properties such as total phenol content, total flavonoid content and % DPPH radical scavenging activity were also improved several folds in Se-biofortified *Pleurotus* spp. compared to their respective control samples. FT-IR spectra of proteins from all four *Pleurotus* spp. indicated an increase in flexibility, unfolding and hydrophilicity upon Se supplementation. Thus, this study revealed the variation in Se accumulation potential among the four different species of *Pleurotus* on using Se-rich wheat straw for the production of Se biofortified mushrooms. Consumption of Se-enriched fruit bodies of *Pleurotus* spp. is anticipated to improve the dietary Se-status and can be treated as a functional food.

Conflict of interest: The authors have declared no conflict of interest in this article.

ACKNOWLEDGEMENT

The authors are thankful to the Head, Department of Microbiology and Department of Soil Science for providing the necessary infrastructural facilities to carry out the research work.

REFERENCES

Bekiaris, G., Tagkouli, D., Koutrotsios, G., Kalogeropoulos, N., and Zervakis, G.I. (2020). *Pleurotus* mushrooms content in glucans and ergosterol assessed by ATR-FTIR spectroscopy and multivariate analysis. *Foods*, 9(4): 535. https://doi.org/10.3390/foods9040535.

Bhatia, P., Aureli, F., Amato, M., Prakash, R., Cameotra, S.S., Tejo Prakash, N., and Cubadda, F. (2013). Selenium bioaccessibility and speciation in biofortified *Pleurotus* mushrooms grown on selenium-rich agricultural residues. *Food Chemistry*, 140: 225–230.

Bhatia, P., Bansal, C., Prakash, R., and Tejo Prakash, N. (2014). Selenium uptake and associated antioxidant properties in *Pleurotus fossulatus* cultivated on wheat straw from seleniferous fields. *Acta Alimentaria*, 43: 280–287.

Blois, M.S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, 181: 1199–1200.

Bozzola, J.J. and Russell, L.D. (1999). *Electron microscopy: principles and techniques for biologists*. 2nd ed. Jones and Barlett Publishers, Boston, MA.

Garcha, H.S. and Khanna, P.K. (2002). *Mushroom growing – a manual*. Punjab Agricultural University, Ludhiana, India. pp. 60.

Gąsecka, M., Mleczek, M., Siwulski, M., Niedzielski, P., and Kozak, L. (2016). Phenolic and flavonoid content in *Hericium erinaceus, Ganoderma lucidum* and *Agrocybe aegerita* under selenium addition. *Acta Alimentaria*, 45: 300–308.

Goyal, A., Kalia, A. and Sodhi, H.S. (2015). Selenium stress in *Ganoderma lucidum*: a scanning electron microscopy appraisal. *African Journal of Microbiology Research*, 9(12): 855–862.

Kaur, G., Kalia, A., and Sodhi, H.S. (2017). Selenium biofortification of *Pleurotus* species and its effect on yield, phytochemical profiles and protein chemistry of fruiting bodies. *Journal of Food Biochemistry*, 42(2): 55–67.

Khurana, A., Tekula, S., Saifi, M.A., Venkatesh, P., and Godugu, C. (2019). Therapeutic applications of selenium nanoparticles. *Biomedicine & Pharmacotherapy*, 111: 802–812.

Lowry, O.A., Rosenbrough, N.J., Farr, A., and Randall, R.J. (1951). Protein measurement with the Folin-phenol reagent. *Journal of Biological Chemistry*, 193: 265–275.

Mohaček-Grošev, V., Božac, R., and Puppels, G.J. (2001). Vibrational spectroscopic characterization of wild growing mushrooms and toadstools. *Spectrochimica Acta Part A*, 57: 2815–2829.

Rathore, H., Sharma, A., Prasad, S., and Sharma, S. (2018). Selenium bioaccumulation and associated nutraceutical properties in *Calocybe indica* mushroom cultivated on Se-enriched wheat straw. *Journal of Bioscience and Bioengineering*, 126(4): 482–487.

Rzymski, P., Mleczek, M., Niedzielski, P., Siwulski, M., and Gąsecka, M. (2016). Potential of cultivated *Ganoderma lucidum* mushrooms for the production of supplements enriched with essential elements. *Journal of Food Science*, 81: 587–592.

Solovyeva, N., Prakash, T., Bhatia, P., Prakash, R., Drobyshev, E., and Michalke, B. (2018). Selenium-rich mushrooms cultivation on a wheat straw substrate from seleniferous area in Punjab, India. *Journal of Trace Elements in Medicine and Biology*, 50: 362-366.

Swain, T. and Hillis, W.E. (1959). The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture*, 10: 63–68.

WHO (2009). Global Health Risks: mortality and burden of disease attributable to selected major risks. Available online: http://www.who.int/healthinfo/global_burden_disease/GlobalHealthRisks_reportannex.pdf.

Yang, H.J., Zhang, Y., Wang, Z.L., Xue, S.H., Li, S.Y., Zhou, X.R., Zhang, M., Fang, Q., Wang, W.J., Chen, C., Deng, X.H., and Chen, J.H. (2017). Increased chondrocyte apoptosis in kashin-Beck disease and rats induced by T-2 toxin and selenium deficiency. *Biomedical and Environmental Sciences*, 30(5): 351–362.

Zhishen, J., Mengcheng, T., and Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 64(4): 555–559.

Zieba, P., Kała, K., Włodarczyk, A., Szewczyk, A., Kunicki, E., Ekara, A.S., and Zenamuszýnska, B. (2020). Selenium and zinc biofortification of *Pleurotus eryngii* mycelium and fruiting bodies as a tool for controlling their biological activity. *Molecules*, 25(4), 889.

Zou, Y., Du, F., Zhang, H., and Hu, Q. (2018). Selenium speciation and biological characteristics of selenium-rich Bailing mushroom, *Pleurotus tuoliensis*. *Emirates Journal of Food &Agriculture*, 30(8): 704–708.