EVALUATION OF ANTIOXIDANT ACTIVITY IN SELENIUM BIOFORTIFIED OYSTER MUSHROOMS; *Pleurotus* spp.

Thesis

Submitted to the Punjab Agricultural University
in partial fulfilment of the requirements
for the degree of

MASTER OF SCIENCE
in
MICROBIOLOGY
(Minor subject: Biochemistry)

By

Anduri Sravani
(L-2018-BS-266-M)

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2020

CERTIFICATE-I

This is to certify that the thesis entitled, **"EVALUATION OF ANTIOXIDANT ACTIVITY IN SELENIUM BIOFORTIFIED OYSTER MUSHROOMS; *Pleurotus* spp."** submitted for the degree of **M.Sc.**, in the subject of **Microbiology** (Minor subject: **Biochemistry**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Ms. Anduri Sravani (L-2018-BS-266-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

(Dr. Shivani Sharma)
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CERTIFICATE-II

This is to certify that the thesis entitled, **"EVALUATION OF ANTIOXIDANT ACTIVITY IN SELENIUM BIOFORTIFIED OYSTER MUSHROOMS; *Pleurotus* spp."** submitted by **Anduri Sravani** (Admn. No. **L-2018-BS-266-M**) to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **M.Sc.** in the subject of **Microbiology** (Minor subject: **Biochemistry**) has been approved by the Student's Advisory Committee along with External Examiner after an oral examination on the same.

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ABSTRACT

Pleurotus florida, *P. eryngii*, *P. cornucopiae* and *P. djamor* were studied for comparing the effect of selenium biofortification on antioxidant activity. Maximum radial growth, biomass production and total soluble protein content of mycelium were observed @ 5 mg L^{-1} in *P. florida* (9.00 mm/day, 88.0 g L^{-1} and 2.28 mg g^{-1} respectively), @ 10 mg L^{-1} in *P. cornucopiae* (8.02 mm/day, 58.3g L^{-1} and 1.94 mg g^{-1} respectively), *P. djamor* (7.11 mm/day, 88.5g $L^{-1}$and 1.97 mg g^{-1} respectively) and @ 20 mg L^{-1} sodium selenate supplementation in *P. eryngii* (8.56 mm/day, 77.0 g L^{-1} and 2.55 mg g^{-1} respectively). The highest Se content was observed at 20 mg L^{-1} of Se concentration in *P. djamor* (920.32 µg g^{-1}dw), followed by *P. florida* (880.61 µg g^{-1}dw), *P. eryngii* (850.27 µg g^{-1}dw) and *P. cornucopiae* (792.72 µg g^{-1}dw). SEM studies showed that 10 mg L^{-1} Se concentration was best for the growth of *P. florida*, *P. eryngii*, *P. djamor* and *P. cornucopiae* and the mycelial texture became coarser and fragile in response to increased concentration of Se. EDS analysis showed that the % weight and atomic % of Se were highest in 10 mg L^{-1} Se supplementation treatment in all the four *Pleurotus* spp. There was no significant difference in the biological efficiency (%) of *P. florida* (66.50 vs 64.57), *P. cornucopiae* (36.74 vs 33.11), *P. eryngii* (30.41 vs 27.16) and *P. djamor* (30.14 vs 29.16) cultivated on Se-enriched and non-enriched wheat straw respectively. The total soluble protein content, total phenolic content, flavonoid content and free radical scavenging activity (%) were found to be highest in Se enriched fruit bodies when compared to control samples in all the four *Pleurotus* spp. Elemental analysis of Se biofortified *Pleurotus* mushrooms using SEM-EDS showed signals characteristic for selenium on the surface of *P. florida* and *P. djamor* conforming that the selenium was incorporated into the cell wall of these mushroom fruiting bodies and were absent in *P. eryngii* and *P. cornucopiae*. 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 content of Se-enriched fruit bodies were found to be higher as compared to non-enriched fruit bodies of *P. florida* (124.00 vs 2.48 µg g^{-1}dw), *P. eryngii* (57.25 vs 0.49 µg g^{-1}dw), *P. cornucopiae* (46.25 vs 0.161 µg g^{-1}dw) and *P. djamor* (156.6 vs 4.33 µg g^{-1}dw). FT-IR spectra of proteins from all the four *Pleurotus* spp. indicated an increase in flexibility, unfolding and hydrophilicity upon Se supplementation. Therefore, *Pleurotus* spp. can be used as an excellent nutraceutical with numerous health benefits.

Key words: Antioxidant activity, Biofortification, Hydrophilicity, Se supplementation, SEM-EDS.

Signature of Major Advisor **Signature of the Student**

ਖੋਜ ਦਾ ਸਿਰਲੇਖ	:	ਸੇਲੇਨੀਅਮ ਬਾਇਓਫੋਰਟੀਫਾਈਡ, ਔਸਟਰ ਖੁੰਭਾਂ (*ਪਲੀਓਰੋਟਸ* ਸਪੀਸੀਜ਼) ਵਿੱਚ ਐਂਟੀਆਕਸੀਡੈਂਟ ਗਤੀਵਿਧੀ ਦਾ ਮੁਲਾਂਕਣ।
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ਸਾਰ-ਅੰਸ਼

ਪਲੀਓਰੋਟਸ ਫਲੋਰਿਡਾ, *ਪੀ. ਏਰੈਨਜਾਈ, ਪੀ. ਕੋਰਨੋਕੋਪਾਈ* ਅਤੇ *ਪੀ. ਡਿਜਾਮੋਰ* ਦਾ ਅਧਿਐਨ ਐਂਟੀਆਕਸੀਡੈਂਟ ਕਿਰਿਆ ਉੱਪਰ ਸੇਲੇਨੀਅਮ ਬਾਇਓਫੋਰਟੀਫਿਕੇਸ਼ਨ ਦੇ ਪ੍ਰਭਾਵ ਦੀ ਤੁਲਨਾ ਕਰਨ ਲਈ ਕੀਤਾ ਗਿਆ। ਮਾਈਸੀਲਿਅਮ ਦਾ ਅਧਿਕਤਮ ਰੇਡੀਅਲ ਵਾਧਾ, ਬਾਇਓਮਾਸ ਉਤਪਾਦਨ ਅਤੇ ਕੁੱਲ ਘੁਣਨਸ਼ੀਲ ਪ੍ਰੋਟੀਨ ਸਮੱਗਰੀ, *ਪੀ. ਫਲੋਰਿਡਾ*, ਕ੍ਰਮਵਾਰ (9.00 ਮਿਲੀ ਮੀਟਰ/ਦਿਨ, 88.0 ਗ੍ਰਾਮ/ਲੀਟਰ ਅਤੇ 2.28 ਮਿਲੀ ਗ੍ਰਾਮ/ਗ੍ਰਾਮ) ਵਿੱਚ 5 ਮਿਲੀਗ੍ਰਾਮ/ਲੀਟਰ, *ਪੀ. ਕੋਰਨੋਕੋਪਾਈ* ਵਿੱਚ 10 ਮਿਲੀਗ੍ਰਾਮ/ਲੀਟਰ ਕ੍ਰਮਵਾਰ (8.02 ਮਿਲੀ ਮੀਟਰ/ਦਿਨ, 58.3 ਗ੍ਰਾਮ/ਲੀਟਰ ਅਤੇ 1.94 ਗ੍ਰਾਮ/ਲੀਟਰ), *ਪੀ. ਡਿਜਾਮੋਰ* (ਕ੍ਰਮਵਾਰ) 7.11 ਗ੍ਰਾਮ/ਲੀਟਰ, 88.5 ਗ੍ਰਾਮ/ਲੀਟਰ ਅਤੇ 1.97 ਗ੍ਰਾਮ/ਲੀਟਰ) ਅਤੇ *ਪੀ. ਏਰੈਨਜਾਈ* ਵਿੱਚ 20 ਮਿਲੀਗ੍ਰਾਮ ਲੀਟਰ ਸੋਡੀਅਮ ਸੇਲੇਨੇਟ ਪੂਰਕ (ਕ੍ਰਮਵਾਰ 8.56 ਗ੍ਰਾਮ/ਲੀਟਰ, 77.0 ਗ੍ਰਾਮ/ਲੀਟਰ ਅਤੇ 2.55 ਗ੍ਰਾਮ/ਲੀਟਰ) ਦੇਖੇ ਗਏ। ਸੇਲੇਨੀਅਮ ਮਾਤਰਾ ਸਭ ਤੋਂ ਵੱਧ 20 ਮਿਲੀਗ੍ਰਾਮ/ਲੀਟਰ, ਐਸ. ਦੀ ਘਣਤਾ ਤੇ *ਪੀ. ਡਿਜਾਮੋਰ* (920.32 ਮਾਈਕ੍ਰੋ ਗ੍ਰਾਮ / ਗ੍ਰਾਮ ਡੀ ਡਬਲਯੂ) ਵਿੱਚ ਪਾਈ ਗਈ, ਇਸ ਤੋਂ ਬਾਅਦ *ਪੀ. ਫਲੋਰਿਡਾ* (880.61 ਮਈਕ੍ਰੋ ਗ੍ਰਾਮ / ਗ੍ਰਾਮ, *ਪੀ.ਏਰੈਨਜਾਈ* (850.27 ਮਾਈਕ੍ਰੋ ਗ੍ਰਾਮ / ਗ੍ਰਾਮ ਅਤੇ *ਪੀ. ਕੋਰਨੋਕੋਪਾਈ* (792.72 ਮਈਕ੍ਰੋ ਗ੍ਰਾਮ /ਗ੍ਰਾਮ) ਸਨ। ਸਕੈਨਿੰਗ ਇਲੈਕਟ੍ਰਾਨ ਮਾਈਕ੍ਰੋਸਕੋਪੀ (SEM) ਦੇ ਅਧਿਐਨਾਂ ਨੇ ਦਿਖਾਇਆ ਕਿ 10 ਮਿਲੀਗ੍ਰਾਮ/ਲੀਟਰ ਸੇਲੇਨੀਅਮ ਘਣਤਾ *ਪੀ. ਫਲੋਰਿਡਾ*, *ਪੀ. ਏਰੈਨਜਾਈ*, *ਪੀ. ਡਿਜਾਮੋਰ* ਅਤੇ *ਪੀ. ਕੋਰਨੋਕੋਪਾਈ* ਦੇ ਵਾਧੇ ਲਈ ਸਭ ਤੋਂ ਵਧੀਆ ਸੀ ਅਤੇ ਸੇਲੇਨੀਅਮ ਦੀ ਵੱਧ ਰਹੀ ਘਣਤਾ ਦੇ ਜਵਾਬ ਵਿੱਚ ਮਾਈਸੀਅਲ ਮੋਟਾ ਅਤੇ ਕਮਜ਼ੋਰ ਹੋ ਗਿਆ। ਈ ਡੀ ਐਸ ਦੇ ਵਿਸ਼ਲੇਸ਼ਣ ਨੇ ਦਿਖਾਇਆ ਕਿ ਸੇਲੇਨੀਅਮ ਦਾ ਪ੍ਰਤੀਸ਼ਤ ਅਤੇ ਪ੍ਰਮਾਣੂ ਭਾਰ 10 ਮਿਲੀਗ੍ਰਾਮ/ਲੀਟਰ ਸੇਲੇਨੀਅਮ ਪੂਰਕ ਤੇ ਸਾਰੀਆਂ ਚਾਰ ਪਲੀਓਰੋਟਸ ਕਿਸਮਾਂ ਤੇ ਸਭ ਤੋਂ ਵਧ ਸੀ। ਕ੍ਰਮਵਾਰ ਸੇਲੇਨੀਅਮ-ਅਮੀਰ ਅਤੇ ਸੇਲੇਨੀਅਮ ਨਾ-ਅਮੀਰ ਕਣਕ ਦੀ ਤੂੜੀ ਵਿੱਚ *ਪੀ. ਫਲੋਰਿਡਾ* (66.50 ਬਨਾਮ 64.57), *ਪੀ. ਕੋਰਨੋਕੋਪਾਈ* (36.74 ਬਨਾਮ 33.11), *ਪੀ. ਏਰੈਨਜਾਈ* (30.41 ਬਨਾਮ 27.16) ਅਤੇ *ਪੀ. ਡਿਜਾਮੋਰ* (30.14 ਬਨਾਮ 29.16) ਦੀ ਕਾਸ਼ਤ ਦਾ ਜੈਵਿਕ ਕੁਸ਼ਲਤਾ (%) ਵਿੱਚ ਕੋਈ ਮਹੱਤਵਪੂਰਨ ਅੰਤਰ ਨਹੀਂ ਸੀ। ਕੁੱਲ ਘੁਣਨਸ਼ੀਲ ਪ੍ਰੋਟੀਨ, ਸਮੱਗਰੀ, ਕੁੱਲ ਫੇਨੋਲਿਕ ਸਮੱਗਰੀ, ਫਲੇਵੇ ਨੋਇਡ ਸਮੱਗਰੀ ਅਤੇ ਮੁਫਤ ਰੈਡੀਕਲ ਸਕੈਵਿੰਜ਼ਿੰਗ ਗਤੀਵਿਧੀ, (%), ਸਾਰੀਆਂ ਚਾਰ ਪਲੀਓਰੋਟਸ ਕਿਸਮਾਂ ਵਿੱਚ ਨਿਯੰਤਰਣ ਦੇ ਸਮੂਨਿਆਂ ਦੀ ਤੁਲਨਾ ਵਿੱਚ ਸੇਲੇਨੀਅਮ ਅਮੀਰ ਫਰੂਟਿੰਗ ਬਾਡੀਆਂ ਵਿੱਚ ਸਭ ਤੋਂ ਵਧ ਪਾਈ ਗਈ। ਮਾਈਸੀਲੀਆ ਦੇ ਊਰਜਾ-ਵਿਖੰਡਨ ਐਕਸ-ਰੇ (SEM-EDS) ਸੇਲੇਨੀਅਮ ਐਸ ਦੀ ਵਰਤੋਂ ਕਰਦਿਆਂ ਸੇਲੇਨੀਅਮ ਬਾਇਓਫੋਰਟੀਫਾਈਡ ਪਲੀਓਰੋਟਸ ਮਸ਼ਰੂਮਾਂ ਦੇ ਵਿਸ਼ਲੇਸ਼ਣ ਨੇ *ਪੀ. ਫਲੋਰਿਡਾ* ਅਤੇ *ਪੀ. ਡਿਜਾਮੋਰ* ਦੀ ਸਤਹ ਤੇ ਸੇਲੇਨੀਅਮ ਦੀ ਵਿਸ਼ੇਸ਼ਤਾ ਦਰਸਾਈ ਹੈ ਕਿ ਸੇਲੇਨੀਅਮ ਇਨ੍ਹਾਂ ਮਸ਼ਰੂਮਾਂ ਦੀਆਂ ਫਰੂਟਿੰਗ ਬਾਡੀਆਂ ਦੀ ਸੈੱਲ ਦੀਵਾਰ ਵਿੱਚ ਸ਼ਾਮਿਲ ਹੋਇਆ ਪਾਇਆ ਗਿਆ ਅਤੇ *ਪੀ. ਏਰੈਨਜਾਈ* ਅਤੇ *ਪੀ. ਕੋਰਨੋਕੋਪਾਈ* ਵਿੱਚ ਗੈਰ-ਹਾਜ਼ਰ ਸੀ। ਸੇਲੇਨੀਅਮ ਅਮੀਰ ਕਣਕ ਦੀ ਤੂੜੀ ਵਿੱਚ ਸੇਲੇਨੀਅਮ ਦੀ ਮਾਤਰਾ 22.34 µg/g dw ਜਦੋਂ ਕਿ ਨਾ ਅਮੀਰ ਕਣਕ ਦੀ ਤੂੜੀ ਵਿੱਚ 0.059 µg/g dw ਪਾਈ ਗਈ। ਨਾ-ਅਮੀਰ ਫਰੂਟਿੰਗ ਬਾਡੀਆਂ ਦੇ ਮੁਕਾਬਲੇ ਸੇਲੇਨੀਅਮ ਅਮੀਰ ਫਰੂਟਿੰਗ ਬਾਡੀਆਂ ਵਿੱਚ ਸੇਲੇਨੀਅਮ ਦੀ ਵਧੇਰੇ ਮਾਤਰਾ ਪਾਈ ਗਈ- *ਪੀ. ਫਲੋਰਿਡਾ* (124.00 ਬਨਾਮ 2.48 µg/g dw), *ਪੀ. ਏਰੈਨਜਾਈ* (57.25 ਬਨਾਮ 0.49 µg/g dw), *ਪੀ. ਕੋਰਨੋਕੋਪਾਈ* (46.25 ਬਨਾਮ 0.161 µg/g dw), *ਪੀ. ਡਿਜਾਮੋਰ* (156.6 ਬਨਾਮ 4.33 µg / g dw)। ਚਾਰਾਂ ਪਲੀਓਰੋਟਸ ਕਿਸਮਾਂ ਦੇ ਪ੍ਰੋਟੀਨਾਂ ਦੇ ਐਫ-ਟੀ-ਆਈ ਆਰ ਸਪੈਕਟ੍ਰਾ ਨੇ ਸੇਲੇਨੀਅਮ ਯੁਕਤ ਹੋਣ ਤੇ ਲਚਕ, ਫੈਲਣ ਅਤੇ ਹਾਈਡ੍ਰੋਫਲੀਸਿਟੀ ਵਿੱਚ ਵਾਧੇ ਦਾ ਸੰਕੇਤਕ ਦਿੱਤਾ। ਇਸ ਲਈ, ਪਲੀਓਰੋਟਸ ਕਿਸਮਾਂ ਨੂੰ ਬਹੁਤ ਸਾਰੇ ਸਿਹਤ ਲਾਭਾਂ ਕਰਕੇ ਇੱਕ ਪੌਸ਼ਟਿਕ ਆਹਾਰ ਦੇ ਤੌਰ ਤੇ ਵਰਤਿਆ ਜਾ ਸਕਦਾ ਹੈ।

ਮੁੱਖ ਸ਼ਬਦ: ਐਂਟੀਆਕਸੀਡੈਂਟ ਕਿਰਿਆ, ਬਾਇਓਫੋਰਟੀਫਿਕੇਸ਼ਨ, ਹਾਈਡ੍ਰੋਫਲੀਸਿਟੀ, ਸੇਲੇਨੀਅਮ ਪੂਰਕ, SEM-EDS

ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ **ਵਿੱਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ**

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CHAPTER I

INTRODUCTION

Mushroom is a macro fungus with a well defined fruiting body which can be either epigeous or hypogenous and can be seen with the naked eye and to be harvested by hand (Chang and Miles 1992). The size of the fruit body is 5 to 30 cm in diameter. They grow on wet decomposed organic material and played an important role in nutrient cycling (Subramanian 1995). Mushrooms are considered as a culinary wonder across the world since ancient times for their unique taste and subtle flavor. Presently, they are regarded as miniature pharmaceutical factories producing several hundred new ingredients with remarkable biological properties. They have been used in medicinal field since ancient times as they serve as a rich source of nutraceuticals, anti-oxidants, anticancer, prebiotic, immune modulating, anti-inflammatory, cardiovascular, anti-microbial, and anti-diabetics (Patel and Goyal 2012).

Globally mushroom industry has grown very fast in the last 2 decades by the cultivation of new commercial mushrooms. However, mushroom as a vegetable is yet to find a place among the Indian purchasers. Despite of well distributed agro-climate conditions, huge amount of agro wastes, cheap availability of labour and bio diversified fungus, India has a moderately warm response in its growth. In India the mushroom industry from 2010-2017, has reported an average growth rate of 4.3 percent per year. Among the total production, white button mushroom occupies (73%), oyster mushroom (16 %), paddy straw mushroom (7 %) and milky mushroom (3 %) (Sharma *et al* 2017). Per capita consumption of mushroom in India is less than 100 grams per annum as compared to vegetables. White button mushroom generated revenue of Rs. 7282.26 lakhs by exporting 1054 q in frozen and canned form in 2016-2017 (Sharma *et al* 2017). According to National Horticulture Board, in 2017-18 the total mushroom area in India was 1.98 lakh ha and production was 4.87 lakh MT.

Mushrooms play a vital role for the formulation of a healthy diet as they are rich source of high quality proteins, minerals and dietary fibre and poor in calories and in fat (Manzi and Pizzoferrato 2000). The fungal cell wall components like chitin, hemi-celluloses and β-glucans including fibres plays a key role in the enhancement of host resistance to bacterial, viral, fungal and parasitic infections, activation of a nonspecific immune stimulation, reduction of blood cholesterol and blood glucose levels (Cheung 2009). Mushrooms constitute a highly specialized group of fungi, possessing an ability to degrade lignocellulosic agricultural wastes in to useful form of food (Ahmed *et al* 2009). They serve as one of the culinary solution to the present and future generation problems of food world especially to those of vegetarian class as they provide proteins (selenocysteine, selenomethionine) vitamins (B complex, C, D) minerals (potassium, sodium and phosphorous) dietary fiber and various therapeutic compounds (Manzi *et al* 1999).

Mushrooms exist in different forms in terms of colour, shape, texture and edibility. In India, mostly five varieties of mushrooms are cultivated: *Agaricus bisporus*, *Pleurotus* spp., *Lentinus edodes*, *Calocybe indica* and *Volvariella volvaceae* (Singh *et al* 2017).

Pleurotus mushrooms are popularly known as "oyster mushrooms" as their fruiting bodies resembles oyster shells. It is popularly called as "dhingri" in India. It consists of 40 species out of which 12 species are popularly cultivated throughout the country (Ahmed *et al* 2009). It is a lignin degrading mushroom which can be grown throughout the year at varied temperatures both in tropical and subtropical conditions on wide variety of agricultural residues (paddy straw, wheat straw, sugarcane bagasse, maize stalks, corn cobs, and cotton stalks; Wani and Sawant 1999). This mushroom is widely cultivated throughout the world because of its easy production, grows on a wide variety of agricultural substrate, high biological efficiency (Mane *et al* 2007). Now-a-days, the interests of consumers are shifted more towards the oyster mushroom due to its delicious taste, abundant amount of nutrients, medicinal properties (Garcha *et al* 1993). The fruiting bodies of *Pleurotus* has 3 distinct parts; pileus which is shell or spatula shaped with different shades of white, grey, yellow, cream and pink depending on the species; stipe and gills or lamellae underneath the pileus. There are number of oyster species which are important in pharmaceutical industry. *Pleurotus cystidiosus* and *Pleurotus ostreatus* possess antioxidant and antitumor properties (Li *et al* 2007).

Pleurotus florida is cultivated widely in various regions of temperate, subtropical and tropical zones. The pileus colour is light brown at low temperature, but they turn to pale at high temperatures. It could be harvested in moderately high temperature as its fruiting temperature range is higher than other *Pleurotus* mushroom and fruiting induction doesn't require cold shock treatment. Highest yield is recorded in this mushroom among the various *Pleurotus* species.

Pleurotus eryngii is also called as 'king oyster mushroom', its popularity is due to its unique flavour. Since *P. eryngii* is highly prone to diseases and sensitive to growing conditions, grows slowly as compared to *P. ostreatus*. Most of the consumer's prefer bottles or bags filled with sawdust for cultivation. Cold shock (13-18ºC) treatment is required for the initiation of primordia and fruiting bodies. It has cream to grey-brown colour cap with whitish 10-14 cm long stipe. *P. djamor* is famously known as pink oyster or salman pink oyster. Its common name is roseus mushroom. It got this name due to its pink sporophore. It belongs to the family Pleurotaceae and order Agaricales. The optimum conditions required for its growth are temperature between 26°C and 35°C and humidity of 80%. The fruiting bodies will be very large with attractive pink colour and pleasing flavor (Raman *et al* 2018).

Pleurotus cornucopiae is quite similar to *Pleurotus ostreatus*, being distinguished because, the gills are not very decurrent and the cap colour is slate or bluish grey. It is

saprobic on dead wood and can also be a delicate parasite. It has pale yellowish, brownish or greyish coloured cap and grows upto 15 cm. Stem may be up to 11 cm long and 2 cm thick, forked and excentric to fairly central. The gills are decurrent down the stem and criss-cross, at the bottom network of ridges were present. The vigorous smell has an aniseed odour and floury taste when it is cut (Eyssartier and Roux 2013).

Selenium is an important microelement required for 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 number of enzymes like glutathione peroxidase, thioredoxin reductase, iodine deiodinase (Combs 2001; Kaur *et al* 2017). It is basically a key component of selenoproteins. The beneficial effect of Se supplementation has been gaining importance in these years. World Health organization (WHO) has recommended a daily intake of Se in the range of 55-200 µg for adults (USDA 2012). More than 400 µg Se per day might produce toxicity in humans (Dennert *et al* 2011). Different sources of selenium in food are seafood, fish, some vegetables, mushrooms, garlic, brazilian nuts (Rayman *et al* 2008). Cultivation of saprophytic fungi on substrates supplemented with Se might be an effective means for the production of selenium biofortified mushrooms for diet. Bhatia *et al* (2013) examined the ability of *Pleurotus florida*, an edible species of oyster mushrooms by growing it on wheat straw collected from the seleniferous belt of Punjab (India) and found that selenium content in biofortified mushrooms was 800 times higher as compared with those cultivated on normal wheat straw.

Most of the world's population has sub optimal intake of selenium leading to increased risk of many diseases such as cancer, heart and viral diseases. In India, agriculturally rich states (Punjab) generates billions of tons of crop residues in the form of leaves, straw, husks etc annually which is rich in lignocellulosic components (cellulose, hemicellulose and lignin) and are being burnt by farmers in the field resulting in air pollution and loss of soil fertility (Dhillon and Dhillon 1991; Sharma *et al* 2007; Cubadda *et al* 2009). Cultivation of mushrooms on lignocellulosic rich substrate would be one of the best options for subsidiary source of income for farmers. In North eastern parts of Punjab and Haryana, seleniferous soils are found where selenium content of soils ranges from 0.25 to 4.55 mg kg^{-1}. Crops grown in these areas like rice, wheat, maize are found to be hyperaccumulated with selenium (Dhillon and Dhillon 1991). *Pleurotus* spp. possess an ability to mobilise and accumulate selenium in their fruiting bodies when cultivated on these selenium hyperaccumulated straw. This ability of *Pleurotus* spp. could be exploited for the production of Se biofortied mushrooms and is found to be an effective ecofriendly way of recycling organic wastes. The Se biofortified oyster mushrooms contain various chemical compounds like phenols, polysaccharides, selenoproteins like selenomethionine and selenocystine and possess antitumour, immunomodulatory, antigenotoxic, hypocholesterolemic,

antihyperglycaemic, antimicrobial, antioxidant, antiinflammatory and antiviral properties. Se biofortification of mushrooms may represent a safe way to increase Se uptake in humans and animals and could serve as an excellent neutraceutical (Hartikainen 2005). It is very crucial to precisely optimize the biofortification strategies of mushrooms for enhancing the Se uptake. Keeping these knowledge gaps in mind, the present study was planned to meet the following objectives with a view to produce Se-biofortified *Pleurotus* species using Se-rich substrates.

1. To study Se uptake from Se enriched broth culture.
2. To identify Se biofortification in mushroom fruiting body grown on Se enriched substrate.
3. To study the antioxidant potential of Se biofortified mushroom.

CHAPTER II

REVIEW OF LITERATURE

Selenium was discovered in 1817 by Jons Jakob Berzelium (Marian 1984). This trace element is concentrated in the dried regions of alkaline soils of North Eastern parts of Punjab and Haryana (Singh and Kumar 1976; Sharma and Singh 1983; Ruby and Punj 2001). Crops grown in these Se rich areas like rice, wheat and certain vegetables are found to be hyperaccumulated with Se (Dhillon and Dhillon 1991; Sharma *et al* 2007; Cubadda *et al* 2009). Selenium toxicity is observed in human population due to consumption of crops hyperaccumulated with Se and burning of these agricultural residues causes air pollution and even decreases the soil fertility (Ruby and Punj 2001; Hira *et al* 2003; Sharma *et al* 2009; Cubadda *et al* 2010). Selenium is gaining importance in nutrition because it plays an important role for human body as antioxidant, antioncogenic, antiageing, antidiabetic and immunomodulatory (Kaur *et al* 2017). However, its excess concentration (400µg) known as Se toxicity leads to "alkali disease" and " blind staggers" in herbivores, hair loss, nail loss and disorders of nervous system and suboptimal intake leads to "keshans disease" and "white muscle disease" in mammals (Stranges *et al* 2010; Fairweather-Tait *et al* 2011; Tanguy *et al* 2012; Fordyce 2013). Approximately 1 billion people in the world are suffering from Se deficiency also known as "Se-hidden hunger" (Thavarajah *et al* 2008). Rayman *et al* (2008) reported that mushrooms contain higher amount of selenium in comparison to vegetables. Mushrooms or edible fungi not only possess medicinal components in the form of proteins, polysaccharides, bioactive components but also possess an ability to accumulate micronutrients (Manzi *et al* 1999).

Pleurotus spp. is an efficient lignin degrading mushroom which can be grown on a wide variety of suitable substrates like maize straw, rice straw, wheat straw and sugarcane bagasse under varied temperature conditions (Thomas *et al* 1998). Cereals, wheat and other vegetables have been biofortified with Se but their ability to mobilise and accumulate Se is very low in comparison to mushrooms (Hart *et al* 2011). Earlier studies reported that mushrooms possess an ability to transform inorganic Se to organic form in terms of safety, physiological activity and absorption rate (Kaur *et al* 2017). Even though selenium can be gained from various foods like eggs, onions, malts, meat, mushrooms and nuts, but its amount or content is low in most of the natural and processed foods. As a result, there is a need to find a method to increase the Se content in the food or diet to meet the human body requirement.

This can be done in three ways: by supplying nutrients directly, modifying and diversifying the diet and indirectly by biofortification. Biofortification is known as the process of enhancing the amount of essential nutrients and /or it's bioavailability present in

food (Saeid *et al* 2019). Therefore, Se biofortification of *Pleurotus* may prove a safe way to increase Se uptake in humans, animals which could serve as an excellent neutraceutical (Hartikainen 2005). Se biofortification with fungal protein proved that such biofortification in mushrooms improves the nutritional quality of food (Munoz *et al* 2005). The review of literature pertaining to this was discussed under the following headings and subheadings:

2.1 Selenium distribution in soil and environment

2.2 Selenium content in food

2.2.1 Selenium and its importance in human health

2.2.2 Selenium: ill effects due to its shortage in the diet

2.2.3 Selenium bioavailability and speciation in mushrooms

2.3 Nutritional composition of *Pleurotus* spp.

2.3.1 Proteins

2.3.2 Carbohydrates and fibers

2.4 Role of *Pleurotus* spp. in the mobilization of Se from agricultural residues

2.5 Biofortification of *Pleurotus* spp. with Se.

2.6 Therapeutic properties of biofortified mushrooms

2.1 Selenium distribution in soil and environment

The Se content in the Earth's crust is very low i.e. 0.05-0.10 mg kg^{-1} because of its volatilization during crust formation (Ryan and Dittrick 2001). Yet, Se content is highly variable globally i.e. in eruptive, metamorphic, and sedimentary rocks are 0.01-7.0, 0.1-6.5, and 0.01-7.0 mg kg^{-1} respectively (WHO 1987). Sedimentary rocks like lime stones, sandstones and shales are very rich in Se content i.e. <0.1-7.4 mg kg^{-1}, <0.1-1.7 mg kg^{-1} and <0.1- 12.0 mg kg^{-1} respectively (Lopes *et al* 2017). Highest Se concentration has been found in rocks rich in phosphate, marine, black shales, coals and rocks rich in organic carbon. Some elements of sulphide mineral deposits are also considered as Se source. Se concentration in soils is largely determined by the parent rocks. Globally, Se content of soils is very low i.e. 0.4 mg kg^{-1}. Like in earth's crust even in soils, Se content is highly variable globally. Even though igneous volcanic rocks are generally low in Se content, some volcanic soils might be 1 to 2 times higher in Se content when compared to world's average. When volatile-elements come in contact with volatile ashes during volcanic eruptions results in Se rich regions like Keterson reservoir in the USA and the Ziyang Country in China. Various factors influencing geochemical mobility of Se by volcanic ashes are particle size-fractions, temperature within the plume, soil biological reactions, and climate conditions such as wind and humidity. Se mobility and it's bioavailability in the environment depends on adsorption capacity of components of soil. However, adsorption capacity of soil in return depends on various factors such as pH, soil mineralogy, texture, organic matter content, Se chemical form, redox condition, and interactions with other ions. Organic carbon rich soils contain high amount of

Se due to the presence of microorganisms and vegetable waste. The capacity of the soil for adsorption of Se depends on the organo-mineral associations (Tolu *et al* 2014). Intercourse of inorganic Se with organic Se may or may not be bioavailable particularly humic acids. The bioavailability of Se from soils increases when it interacts with fulvic acids as compared to humic acids. In addition to these, inorganic Se immobilization is also affected by plants and microbial activities. Globally, several places like UK, Australia, central Siberia, New Zealand, Thailand, Africa, Finland, Turkey, Nepal, northeast to south central of China, Denmark, and parts of Bangladesh and India contain soils with low Se bioavailability leading to various diseases and disorders (Yasin *et al* 2015). Soils of tropical regions are also found to contain low Se concentrations (Matos *et al* 2017). So, we should find a way to increase its bioavailability.

2.2 Selenium content in food

Some fungi like mushrooms are known to accumulate high concentrations of trace elements like Se, Fe, Zn and Cu which are species specific (Michellot *et al* 1998; Kalac 2009). The wild mushrooms like *Boletus* group which encompass *B. edulis, B. aestivalis, B. aereus* and *B. pinophilus* contains higher concentration of Se than the *Chanterelles* group which includes *C. cibarius* and *C. cornucopioides* and any other wild and cultivated mushrooms. Consumption of the cultivated *Agaricus bisporus* comprising the ‘‘*Portobello*’’ variety in the form of fresh whole or sliced provides 17% and 23% of the Recommended Daily Intake (RDI) of Se respectively. But, consumption in the canned form provides only 9% of the RDI of Se which is higher as compared to *Pleurotus ostreatus* (4.6%) and *Lentinula edodes* (1.7%). Se values of *A. bisporus* fall within the limits of various other food sources like meat, eggs and dairy products and are higher when compared to bread, cereals, fruits and vegetables (Ventura *et al* 2007). Among the various dried wild mushrooms that are available commercially *Marasmius oreades* provides 27% of the RDI of selenium while *L. edodes, Chanterelles cibarius* and *C. cornucopioides* account for only 3% of the RDI and *Auricularia auricular-judae* and *Tuber indicum* could account for approximately 1.5% of the RDI of Se. The Se content provided by various mushroom species are as follows *Boletus aestivalis* 485l g/100 g fresh weight (FW) (882% RDI), *B. pinophilus* 199l g/100 g FW (362% RDI), *B. edulis* 149l g/100 g FW (271% RDI), *B. aereus* 123l g/100 g FW (224% RDI) *B. fragans* and *B. spretus* 21-23l g/100 g FW (40% RDI). Hence, by increasing the consumption of mushrooms, the Se intake would increase which in turn improves the human health.

2.2.1 Selenium and its importance in human health

Selenium (Se) is one among the top most trace elements and it plays a crucial role in human and animal health. It is required for the production of various amino acids like selenocysteine, selenomethionine which in turn required for the synthesis of nearly about 25-35 selenoproteins that are involved in the metabolism of mammals (Rayman 2012; Oliver and

Gregory 2015). Selenoproteins like glutathione peroxidase and thioredoxin reductases (involved in disulfide reduction system) possess antioxidant activity, the one required for synthesis and control of thyroid harmones is iodothyronine deiodinases, a selenoprotein which contains zinc is methionine-R-sulfoxide reductase, selenophosphate synthetase 2 which is required for the synthesis of selenoproteins, a 15-kDa selenoprotein is required for the proper folding of the proteins in the endoplasmic reticulum and H, I, M, T, V, and W (selenoproteins) are some of the other important selenoproteins (Rayman 2012; Labunskyy *et al* 2014). In addition to the selenoproteins, there are some non-protein organic forms of Se that plays a key role in human health. Certain methylated organic compounds of Se are known to possess anticarcinogenic properties. Even though, many researchers reported that some non-methylated organic forms of Se possess chemopreventive properties (Rayman 2012; Riaz and Mehmood 2012), there are even reports confirming the anticancer potential of monomethylated Se forms such as Se-methylselenocysteine and γ-glutamyl-Se-methylselenocysteine (Lee *et al* 2006; Wang *et al* 2009).

2.2.2 Selenium: ill effects due to its shortage in the diet

By understanding the significant role of selenoproteins in the metabolic pathways of human beings and animals, it makes us to realize the ill effects due to selenium deficiency. Selenium enters into the food web via plants, from the soil. As a result, Selenium deficiency has been found in various regions of the World wherever selenium content of the soil is very less like volcanic terrains. Acidic soils and soils with high content of iron or aluminium reduce the plants ability to absorb Se from the soil. Several countries with low Se content in their soil has reports regarding large number of cases of diseases in livestock due to Se deficiency. In animals, there are number of Se deficiency diseases such as defective reproductive system, retarded growth, depression and white-muscle disease, cardiomyopathy and skeletal muscle myopathy especially impacting lambs and calves. In human beings, Se shortage leads to improper functioning of the thyroid gland, irreversible brain damage, peripheral vascular diseases, chronic and degenerative osteoarthropathy (Kashin–Beck disease), impaired immune response to viral infections (such as measles, hepatitis, influenza and HIV/AIDS), male infertility, pre-eclampsia in women, heart diseases and higher risk for different types of cancers (Combs 2000; Pilon-Smits and Quinn 2010; Fairweather-Tait *et al* 2011; Davis *et al* 2012; Rayman 2012; Riaz and Mehmood 2012; Cardoso *et al* 2015). World Health Organization (WHO) has recommended a daily intake of Se in the range of 55-200 µg for adults (USDA 2012). More than 400 µg Se per day might produce toxicity in humans (Dennert *et al* 2011).

2.2.3 Selenium bioavailability and speciation in mushrooms

Selenium (Se) is an important essential element, intake of which is in suboptimal levels in populations of many countries. As edible mushrooms are chiefly included in the

human diet of many countries, biofortified mushrooms can serve as an important food supplement for improving the human health (Przemysław *et al* 2015). The bioavailability is known as the extent to which a nutrient, toxin, or other substances become available to one's body or its deposition after exposure (Reeves and Chaney 2008). When the intake of the substance is oral, then its bioavailability includes body absorption, utilization, and/or deposition (Dernovics *et al* 2009). For assessing the Se-bioavailability, there are two methods-*in-vivo* and *in-vitro*. *In-vitro* method includes simulated gastrointestinal digestion, accompanied by measurement of the dialyzable mineral fraction across a semi permeable membrane (Ca´mara *et al* 2005; Da Silva *et al* 2010) whereas, *in-vivo* estimation is based on the usage of animals or human beings. In case of *in-vivo* system the bioavailability of Se is affected by its interaction with other compounds present in the body.

The bioavailability of Se by *in-vivo* method in enriched *P. ostreatus* mushrooms was estimated by Da Silva *et al* (2010). The experiment was conducted by providing different diets to 8 groups of 64 Wistar male rats: devoid of Se, mushrooms devoid of Se, biofortified mushrooms with 0.15, 0.30 or 0.45 mg of Se kg^{-1} and a normal diet with 0.15 mg of Se kg^{-1} using sodium selenite and their blood plasma was tested for Se concentration and found that blood plasma of rats which had been provided with Se-biofortified mushrooms contained higher Se concentrations in comparison to rats provided with normal diet containing 0.15 mg kg^{-1} sodium selenite. Przemysław *et al* (2015) analyzed three mushroom species namely *P. eryngii*, *P. ostreatus* and *Pholiota nameko* by cultivating them on substrates enriched with different doses of sodium selenite (Na_2SeO_3 (IV) and sodium selenate Na_2SeO_4 (VI) solutions). The most effective dose of Se supplementation was found in the fruiting bodies of *P. ostreatus* cultivated on substrates provided with Se dose of 0.25 and 0.5 mM (53 and 62 mg kg^{-1} DW, respectively). They reported that mushroom enrichment with Se salts triggered biomass growth only in case of *P. eryngii*. However, the bioconcentration factor (BCF) values were found to be highest in the fruiting bodies of *P. nameko*. The BCF values indicated the Se accumulation when the mushrooms were cultivated on substrates supplemented with Se concentration of ≥0.25 mM (*P. nameko*) or ≥0.5 mM (*P. ostreatus and P. eryngii*). These results proved that biofortified mushrooms can serve as an excellent neutraceutical for improving the human health because of their higher bioavailability.

2.3 Nutritional composition of *Pleurotus* spp.

Pleurotus is one among the top most edible mushrooms popularly cultivated in various regions of the world on wide range of substrates and chemicals which in turn effect the concentration of various macronutrients and mycochemicals. Likewise, they are not only a good source of proteins (20–25 %), polysaccharides (37-48 %), fibers (13–24 %), vitamins, minerals (Alam *et al* 2008; Sabaratnam *et al* 2011) but also rich in some of the secondary metabolites like phenolic compounds, polyketides, terpenes and steroids (Cheung *et al* 2003).

However, they are low in calories (Sabaratnam *et al* 2011) and in fat content (4–5 %) (Alam *et al* 2008) providing a balanced nutrition. It is also a good source for crude fiber and β-glucans (10.3% and 25.9% respectively). Especially *P. ostreatus* fulfills the nutritional requirement for adults by providing all the essential amino acids. Similarly, chemical analyses have shown that most of the biologically active compounds separated from mushrooms belongs to hemicelluloses, polysaccharides, lipopolysaccharides, peptides, proteins, glycoproteins, nucleosides, triterpenoids, complex starches, lectins and lipids or other complex compounds (Lindequist *et al* 2005). One way to enhance the nutritive value of *Pleurotus* mushrooms is to use substrates rich in bioactive compounds or by adding minerals or by postharvest treatments. *Pleurotus ostreatus* possess good quality protein and fiber (mainly β-glucans) that may be useful as an ingredient in functional foods. *Pleurotus* mushroom contains special bioactive components with antimicrobial, antiviral, anticancer, antioxidant, hypolipidemic, hypocholesterolemic, antihyperglycemic and immunomodulatory functions (Gonzalez *et al* 2017). Rodrigues *et al* (2015) studied *Pleurotus citrinopileatus var cornucopiae*, *P. salmoneo stramineus* to determine the chemical composition and nutritive value. Protein, sugar and fat contents ranged between 16.2 to 26.6, 52.7 to 64.9 and 2.3 to 3.5 g/100g dry mushroom respectively. Highest total phenolic content was found in *P. citrinopileatus* var. *cornucopiae* i.e. 1140 µg catecol equiv/g dry mushroom. Unsaturated fatty acid content was higher than that of saturated fatty acids with linoleic acid as the predominant one (>30 g FA/100 g fat). Additionally, they are also a good source of potassium (K), magnesium (Mg), phosphorus (P), zinc (Zn), copper (Cu) and iron (Fe) contributing to 15 % of the recommended daily intake.

2.3.1 Proteins

Mushrooms are gaining popularity as a good source of high quality proteins. The protein content depends on various factors in the form of kind of strain, physical and chemical differences in the growing medium (Akyuz and Kirbag 2010), composition of the substrate, pileus size and time of harvest (Mshandete and Cuff 2007). The protein content of *P. ostreatus* fruiting body ranges from 17 to 42 g per 100 g dried weight (Akyuz and Kirbag 2010). A dimeric protein named Lectin was isolated from fresh fruiting bodies of *P. ostreatus* with antihepatoma and antisarcoma functions with a molecular weight of 40 kDa and 41 kDa respectively (Wang *et al* 2000). This mushroom is well known for its nutritional qualities as it contains all the essential amino acids and high quality proteins. In addition to this, it also contains non-essential amino acids especially GABA (a neurotransmitter) and ornithine (precursor for arginine synthesis). Its fruiting body contains 7 mg of amino acids/100 g dry weight (Mattiala *et al* 2001). It was analysed for all the 33 amino acids and meet the adult requirement by providing all the essential amino acids, sometimes it even doubles (Lys, Leu, Val, Ile, Phe + Tyr) or triples (Thr) the body requirement. In children, it meets the

requirement of Thr, Phe + Tyr, Trp and Lys (FAO 2013). It is deficient in leucine which can be supplemented easily by providing mushrooms with cereals (Day 2013). The Protein Digestibility Corrected Amino Acid Score (PDCAAS) of *P. ostreatus* in children was 95.3% and in adults was 103.1% which were the results of amino acid score (AAS) of leucine in children (97.3) and in adults (105.2) multiplied by the digestibility of true protein content of three *Pleurotus* spp. (98%) (Valencia del Toro *et al* 2006). The PDCAAS of *P. ostreatus* in adults proved that its high protein quality is comparable to casein (100), eggs (100), soy and protein isolate (100). In contrast to this, PDCAAS in children proved that its protein quality is higher when compared to beef (92), wheat and pea flour mixture (0.82), pea protein concentrate (0.73), pinto beans (0.63) and other food grains (Hughes *et al* 2011; Day 2013).

2.3.2 Carbohydrates and fibers

Mushrooms contain 50-60% of carbohydrates on dry weight basis like mono, oligo and polysaccharides which helps in maintaining the alimentary tract (Kalac 2012). Among the popularly cultivated edible oyster mushrooms, *P. ostreatus* contains good amount of carbohydrates and dietary fibers. The major polysaccharides present in mushrooms include glycogen, dietary fibers, cellulose, chitin, α and β-glucans and other hemicelluloses like mannans, xylans and galactans (Manzi *et al* 2001; Hossain *et al* 2007). Pleuran (a β-glucan) can serve as an antitumor polysaccharides in *P. ostreatus*. The polysaccharides present in the fruiting bodies are non-starchy in nature which varies widely with strains (37-48g/100 g dry weight) (Synytsya *et al* 2008). Therefore, it is considered as a good source of dietary fibers. Mushroom glucans are also parts of both soluble and insoluble dietary fibers and their solubility is affected by both structure and confirmation of the molecules (Vetter 2007). The insoluble dietary fiber content is higher in stem region of the mushrooms as compared to the cap or pileus portion. Mushrooms contain around 4 to 9% of soluble fibers and 22 to 30% of insoluble fibers (Kalac 2009). Reports said that in addition to chitin, mushrooms also contain various other polysaccharides.

2.4 Role of *Pleurotus* spp. in the mobilization of Se from agricultural residues

More than 1000 ha area in Punjab are considered as seleniferous regions of Punjab (Nawanshahr and Hoshiarpur) where the Se concentration is quite high which is toxic to the plants. Burning of these agricultural residues results in pollution, heavy haze which prevails for long duration and loss of soil fertility (Jaiswal *et al* 2012; Dhanjal *et al* 2016). It is an efficient lignin degrading mushroom which can be grown on a wide variety of suitable substrates like maize straw, rice straw, wheat straw and sugarcane bagasse under varied temperature conditions (Thomas *et al* 1998). Cereals, wheat and other vegetables have been biofortified with Se but their ability to mobilise and accumulate Se is very low while mushrooms possess high accumulation capacity (Hart *et al* 2011). Hence this ability of

mushrooms can be used to biofortify with trace elements which maintains the human health.

The uptake of Se by various mushroom species was evaluated by cultivating *Pleurotus* spp. and *A. bisporus* on Se enriched wheat straw and *Volvariella volvacea* on paddy straw (Solovyev *et al* 2018). They found that *A. bisporus* possessed an ability to uptake high amount of Se i.e. 1,396 µg/g vs 46.8 µg/g in control on dry weight basis as compared to *V. volvacea* which is 231 µg/g vs. 3.77 µg/g dry weight. The results also showed that the biological efficiency and total yield for all mushroom species remains unaffected as compared to control samples. *Pleurotus florida* was able to mobilize and accumulate Se from the Se enriched substrate by growing it on wheat straw collected from seleniferous regions of Punjab. The Se content in biofortified mushrooms was found to be 800 times higher than that of control samples cultivated on non-seleniferous wheat straw (141 vs 0.17l g Se g^{-1} dry weight). By analyzing the gastrointestinal hydrolysate samples by anion exchange HPLC-ICP-MS it was also reported that the bioaccessible selenium was mainly present in the form of selenomethionine which accounts for about 73% in the detected species (Bhatia *et al* 2013). Bhatia *et al* (2014) evaluated 2 kinds of edible mushrooms i.e. *Pleurotus sajor-caju* and *V. volvacea* for their capacity to uptake Se by cultivating them on Se-rich wheat and paddy straw respectively. The results showed that Se concentration was higher in Se enriched *P. sajor-caju* and *V. volvacea* (43.5 ± 2.1 and 35.0 ± 1.1µg/g) as compared to their respective control samples (5.2±1.0 and 5.57±0.07 µg/g). The antioxidant activity represented by the total phenol content, DPPH scavenging activity, metal chelation and inhibition of lipid peroxidation activity from the extracts of Se-biofortified mushrooms were found to be relatively higher ($p<0.05$ to $p<0.001$) than that of control samples. Da Silva *et al* (2012) examined the biological efficiency of *Pleurotus ostreatus* by cultivating it on coffee husks enriched with various doses of sodium selenite. The biological efficiency was found to be affected by the high doses of Se. The maximum level of Se absorption was found at 51 mg kg^{-1} of sodium selenite. The mushrooms harvested from the first flush were found to possess more selenium as compared to other flushes. These results showed that coffee husks can serve as a potential source for the biofortification of mushrooms.

The effect of high selenium (Se) concentrations on morphophysiological and ultrastructural features of *Pleurotus ostreatus* was studied by Milovanovic *et al* (2014). The growth of mycelium was found to be good when grown on media enriched with Se of concentration 5.0, 10.0, and 20.0 mg L^{-1}. However, higher concentration such as 500.0 mg L^{-1} retarted the mycelial growth. They also reported the difference between the hyphal growth. In control (without Se) thin-walled, hyaline, branched, and anastomized hyphae with clamp-connections was observed while at Se concentrations of 100 and 500 mg L^{-1}, the hyphal growth were apparently short, commonly septated and branched, with a more intensive extracellular matrix, and lack clamp connections. At very high Se concentrations, hyphae with

intact membrane, without cellular contents, with a high level of vacuolization and numerous proteinaceous bodies were found. The biomass yield was also reduced at higher concentrations (11.8 g L^{-1}(control), 6.8 g L^{-1} at 100 mg L^{-1} and 0.0 g L^{-1} at 500 mg L^{-1}) of Se. They reported highest Se absorption (53.25%) in media enriched with 5 mg L^{-1} Se while Se absorption reached saturation point (938.9 μg g^{-1}) at 20.0 mg L^{-1} Se.

2.5 Biofortification of *Pleurotus* spp. with Se

Selenium mobilization and their accumulation in mushrooms was observed by Kalac (2009) in terms of conversion of inorganic to organic form as it is more bioavailable but their selenium content greatly varies with respect to soil type, species, stage of maturity and the type of substrate used for cultivation of mushroom species. Beelman *et al* (2007) reported that two strong antioxidant moieties viz Se and ergothioneine increased the potential of selenoergothioneine in mushrooms. They also exogenously supplemented Se as inorganic selenite in mushroom substrate which leads to the formation of selenoergothioneine with Se that replaced sulphur in the ergothioneine along with other organoselenium compounds. The higher Se concentration in the medium negatively affected the mycelium growth rate, hyphae diameter, the colour of the colonies and even toxic to *Pleurotus* spp. as observed by Stajic *et al* (2002); Stajic *et al* (2006); Rodriguez *et al* (2009). Bhatia *et al* (2013) cultivated *P. florida* on Se enriched wheat straw and found 800 times higher Se concentration in biofortified mushroom in comparison with non-enriched wheat straw (141 vs 0.17µg Seg^{-1} dry weight) and observed higher form of selenomethionine in biofortified mushroom. Da Silva (2012) reported that *P. eryngii* was found to be more tolerant to Se in comparison to *P. ostreatus.* They enriched the substrate with high concentration of Se (>25.4 mg kg^{-1}) which resulted in low yield in *P. ostreatus* as compared to *P. eryngii*. The cultivation of *Lentinula edode*s, *Saccharomyces cerevisiae* on Se enriched media accumulated 748 µg g^{-1} of Se, 1825 µg g^{-1} of Se as reported by Zhao *et al* (2004); Sokovi´c and Van Griensven (2006). The liquid medium supplemented with more than 50 mg Se kg^{-1} supported the growth of *P. eryngii* (Hu *et al* 2012). The bioavailability of anticancer and antioxidant selenoprotein has been enhanced in *P. ostreatus* as reported by Marliane *et al* (2010). Sarangi *et al* (2006) reported that the chemical compounds present in the fruiting bodies of *Pleurotus* spp. were responsible for enhancing the biological responses by acting as antitumorogenic, immunostimulatory and antimutagenic chemicals. Activities like DPPH scavenging, chelation of metals and inhibition of lipid peroxidation were higher in biofortified mushrooms in comparison to control samples (Bhatia *et al* 2014). Turlo *et al* (2010) reported that antioxidant or scavenging activity is higher in Se biofortified mushrooms as compared to their control. Supplementation of lithium chloride in the substrate directly increased the concentration of lithium in mushrooms but the biological efficiency remains unaffected with change in concentration. As a result, lithium

biofortified *P. ostreatus* serves as a healthy food with high nutritive values (De Assuncao *et al* 2012). *Pleurotus ostreatus* biofortified with Se and Zn when fed to mice resulted in decrease in the malondialdehyde level as compared to those mice fed with normal food (Bhatia *et al* 2014). Özdal *et al* (2019) prepared extracts from five *Pleurotus* spp. (*P. florida, P. citrinopileatus, P. sajorcaju, P. ostreatus, P. eryngii*) and found antioxidant and antibacterial properties which could be used in the formulation of neutraceutical. Highest Se content between (10 and 30 μg g^{-1} on dry weight basis) was found in mushroom species (*Boletus edulis, B. aestivalis* and *Xerocomus badius*) by Kalac and Svoboda (2000); Bekyarov *et al* (2011). Se biofortification with fungal protein proved that such biofortification in mushrooms improves the nutritional quality of food (Munoz *et al* 2005).

2.6 Therapeutic properties of biofortified mushrooms

Mushrooms which are the macro fungi have been used from several thousands of years in food and folk medicine as they are rich in various nutrients like high protein content (up to 44.93%), vitamins, minerals, fibers, trace elements and even low in calories and devoid of cholesterol. Out of 14,000 well known mushroom species, approximately 850 of them are found to be from India. Around 650 of these are known to contain medicinal properties. They are also known to possess bioactive substances like antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, anticancer, antitumor, anti-HIV and antidiabetic functions. Medicinal mushrooms have been used as a source of neutraceuticals for the improvement of human health in the form of food, medicine and other minerals in India (Thatoi and Singdevsachan 2014**).** Da Silva *et al* (2013) reported that selenium (Se) is an essential element to human. Keshan disease, thyroid dysfunction and osteoarthritis are the diseases caused due to Se deficiency. Recent studies have shown that Se-enriched mushrooms are one of the best source for Se because of its high capacity to absorb and transform the inorganic Se to organic forms, which are more bioavailable. *Pleurotus ostreatus* and *P. eryngii* are some of the mushrooms species which are cultivated and consumed worldwide and are able to bioaccumulate Se from culture media enriched with Se resulted in reduced fungal growth rate, hyphae diameter and septum distance and even alteration in the color of colony. *P. eryngii* mushroom found to be more tolerant to Se as compared to *P. ostreatus*. So, biofortification of mushrooms with selenium was found to be very important to improve the nutritive quality of food. Hu *et al* (2019) conducted an experiment by cultivating a medicinal mushroom *Cordyceps militaris* with five different doses of Se (0, 5, 10, 20, and 40 μg g^{-1}) and three different forms of Se (selenate, selenite, and selenomethionine). It absorbed the inorganic form of Se from the substrate and converted it into the organic form of Se (selenocystine and selenomethionine) in the fruiting bodies. When compared with control sample, there was an increase in Se concentration in the fruiting bodies with increase in Se supplementation. They reported that supplementation of 40 μg g^{-1} selenate and selenite resulted in higher Se

concentration in the fruiting bodies by 130.9 and 128.1 μg g^{-1}, respectively. The antioxidant activity was found to be more in Se-biofortified *C. militaris* as compared to control samples. Hence, these results proved that inclusion of mushrooms biofortified with Se in the diet improved the health of humans and livestock. Selenium biofortified mushrooms such as *A. bisporus, Flammulina velutipes* and *P. ostreatus* has anti-carcinogenic properties which serve as a functional food or neutraceutical (Anonymous 2003). Similar results were obtained by Zaidman *et al* (2005), they reported that Se biofortified mushrooms contains anticancer properties**.** Assuncao *et al* (2014) investigated Se enriched *P. ostreatus* and *L. edodes* mushrooms and found that the protein extract from biomass contain large amounts of selenium methionine which is useful in pharmaceutical industries. The reason behind various diseases like cancer and liver toxicity are free radicals. Mushrooms are rich source of various antioxidants like phenolic compounds, flavonoids, vitamins and selenium which protects from free radicals (Chang and Miles 1989). Rzymski *et al* (2017) reported that an increase in dietary uptake of lithium (Li) results in stabilization of the mood and reduces suicidal rates and violence. Poniedziałek *et al* (2017) investigated the extracts from biofortified mushrooms with Se and Se+Zn (0.6 to 1.2 mM) and found antioxidant activities in human thrombocytes by reducing reactive oxygen species (ROS) and preventing lipid peroxidation, thereby preventing the development of atherosclerosis. A significant hypocholesteremic effect was observed in rats and rabbits along with reduced lipid peroxidation in *P. ostreatus* mushroom. Consumption of 10% of dry wt of fruiting bodies decreased the occurrence and size of atherosclerotic plaques in rabbits (Sano *et al* 2002) Similar results were found in *Auricularia auricularia-judae* and *Tremella fuciformis.* Lindequist *et al* (2005) reported the antiallergic properties in the edible mushrooms such as *Hypsizygus marmoreus, F. velutipes, Pholiota nameko* and *P. eryngii* in mice. They also examined the ethanolic extracts from *P. linteus* and found anti-inflammatory effect for arthritis caused due to collagen, in the croton oil-induced ear edema test and antinociceptive effect in the writhing test in mice. Methanolic extracts from the fruiting bodies of *P. pulmonarius* (500 and 1000 mg/kg) resulted in decreased carrageenan-induced and formalin-induced paw edema in mice due to its antioxidant properties. Its function was comparable to the reference diclofenac (10 mg/kg). In addition to this, it also showed decreased solid tumour formation in mice. The bioactive compounds like polysaccharides are responsible for antioxidant activity in several edible mushrooms like *P. eryngii, P citrinopileatus, P. cystidiosus, P. flabellatus, P. floridanus* and *P. pulmonarius* (Khatua *et al* 2013;Tan *et al* 2015). The polysaccharides of *P. florida* resulted in higher antioxidant activity (Maity *et al* 2011). Similarly in *P. tuberregium* they prevented lysis of erythrocytes, and lipid peroxidation of liver in mice (Wu *et al* 2014). Thus, enhanced antioxidant potential of Se-biofortified *Pleurotus* spp. fruit bodies will help in improving the health of humans and livestock when it was included as a part of diet.

CHAPTER-III

MATERIALS AND METHODS

The present study on evaluating the antioxidant activity in selenium biofortified oyster mushrooms; *Pleurotus* spp. was carried out at Dr. H.S. Garcha Mushroom Laboratories, Department of Microbiology, Electron Microscopy Laboratory, Punjab Agricultural University, Ludhiana. The materials and methods used during this study have been discussed under the following headings and subheadings:

3.1 CULTURE PROCUREMENT AND MAINTENANCE

3.1.1 Source of Cultures

3.1.2 Culture Medium

3.1.3 Maintenance of Cultures

3.2 ESTIMATION OF VARIOUS PARAMETERS OF SELENIUM BIOFORTIFIED MYCELIUM OF *Pleurotus* spp.

3.2.1 Estimation of mycelial radial growth and biomass

3.2.2 Estimation of total soluble protein content

3.2.3 Estimation of Se content in Se biofortified mycelium using Inductively Coupled Plasma Mass Spectrometry/Atomic Absorption Spectroscopy (ICP-MS/AAS)

3.2.4 Analysis of Se biofortified hyphae using Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS)

3.3 CULTIVATION OF *Pleurotus* MUSHROOMS ON SELENIUM ENRICHED AND NON-ENRICHED WHEAT STRAW

3.3.1 Spawn preparation

3.3.2 Substrate collection and preparation

3.3.3 Spawning and bag filling

3.3.4 Fruiting and Harvesting

3.3.5 Mushroom yield and biological efficiency

3.4 ESTIMATION OF VARIOUS PARAMETERS OF SELENIUM BIOFORTIFIED *Pleurotus* MUSHROOMS

3.4.1 Estimation of total phenolic content

3.4.2 Free radical scavenging activity (%)

3.4.3 Estimation of total flavonoids

3.4.4 Characterization of Se biofortified *Pleurotus* mushrooms by Fourier Transform Infra-Red Spectroscopy (FT-IR Spectroscopy)

3.1 CULTURE PROCUREMENT AND MAINTENANCE

3.1.1 Source of cultures

The mushroom cultures of various *Pleurotus* spp. (*P. eryngii*, *P. florida*, *P. djamor* and

P. cornucopiae) were procured from germplasm collection bank of Department of Microbiology, PAU, Ludhiana.

3.1.2 Culture medium

Potato Dextrose Agar (PDA) of the following composition had been used:

Ingredients	Quantity (g L^{-1})
Potatoes	250
D-Glucose/Dextrose	18
Agar	20
Ph	5.5-6.0
Distilled water	1000 mL

Fresh potatoes of about 250 g were washed, peeled, cut into cubes of 1cm and boiled for about 25 minutes in 400-500 mL of distilled water until the potatoes became tender. The extract was collected by filtration using muslin cloth and the volume was made to 1000 mL using distilled water. To this filtered solution, 18g of D-glucose/dextrose was added. The extract was heated and powder of agar-agar (20g) was added without forming lumps till gel consistency was attained and the pH was adjusted to 5.5-6.0. It was transferred into ¼ th capacity of test tubes and plugged with non-absorbent cotton. These test tubes were autoclaved at 15 psi for 20 minutes and allowed it to solidify by placing them in slanting position on a raised platform.

3.1.3 Maintenance of cultures

Cultures of *P. eryngii*, *P. florida*, *P. djamor* and *P. cornucopiae* were maintained on PDA slants at 25±1ºC by sub-culturing them after every 6 weeks. From the culture slants, small agar bits containing mycelium were transferred to freshly autoclaved PDA slants/petriplates in such a way that the mycelium side comes in contact with the agar. The inoculated slants were then incubated at 25±1ºC to allow the mycelium to run on the surface of agar slants and sub-cultured as and when required (Plate 3.1).

3.2 ESTIMATION OF VARIOUS PARAMETERS OF SELENIUM BIOFORTIFIED MYCELIUM OF *Pleurotus* spp.

3.2.1 Estimation of mycelial radial growth and biomass

Cultures of *Pleurotus florida*, *P. eryngii*, *P. djamor* and *P. cornucopiae* were grown on 90 mm Petri plates filled with PDA (Potato Dextrose Agar) and sodium selenate was added @ 0 (control), 5, 10, 15 and 20 mg L^{-1}. The plates were inoculated with 6 mm sized mycelial bits and were incubated at 27 ±1°C for 7 days. The plates were observed daily to record the colony diameter in mm for about a week. For biomass estimation, potato dextrose broth was prepared in 500 mL Erlenmeyer flasks, each containing 100 mL media. Sodium

selenate was added to the flasks at various concentrations such as 0 (control), 5, 10, 15 and 20 mg L^{-1}. The flasks were inoculated with 6 mm diameter mycelial agar bits of *P. florida*, *P. eryngii*, *P. djamor* and *P. cornucopiae*. The flasks were then incubated at 27 ±1°C for 20 days. Later on, the flasks were taken out and the mycelium was separated from the broth by filtration using filter paper and the fresh weight was determined as g L^{-1}.

3.2.2 Estimation of total soluble protein content

The total soluble protein content of the extract was estimated by Lowry *et al* (1951) method where bovine serum albumin was taken for plotting standard curve. Fresh weight of mycelial biomass or mushroom fruit body (0.5 g) was taken and extracted using 5mL of 0.1M phosphate buffer of pH 7, centrifuged at 1500 rpm for 15 min at 4°C. The supernatant was transformed into separate tubes and stored at 4°C for its further use.

Reagents

Solution A: 2 per cent sodium carbonate in 0.1N sodium hydroxide (Dissolve 4.0 g of NaOH in 1000 mL distilled water to get 0.1N NaOH).

Solution B: 0.5 per cent copper sulphate solution in 1 per cent sodium potassium tartarate.

Solution C: Freshly prepared by mixing 100 mL of A and 2 mL of B.

Solution D: Folin phenol reagent diluted with distilled water in 1:1 (v/v) ratio

Assay: 0.5 mL of sample extract was taken in a test tube and the volume was made to 1 mL by using distilled water. To this 2.5 mL of solution C was added, vortexed and incubated for 5 minutes. Blank was also run simultaneously. Then 1 mL of reagent D was added and mixed thoroughly, incubated for 15-20 minutes and the absorbance was measured at 540 nm. The total soluble protein content can be obtained from the standard curve.

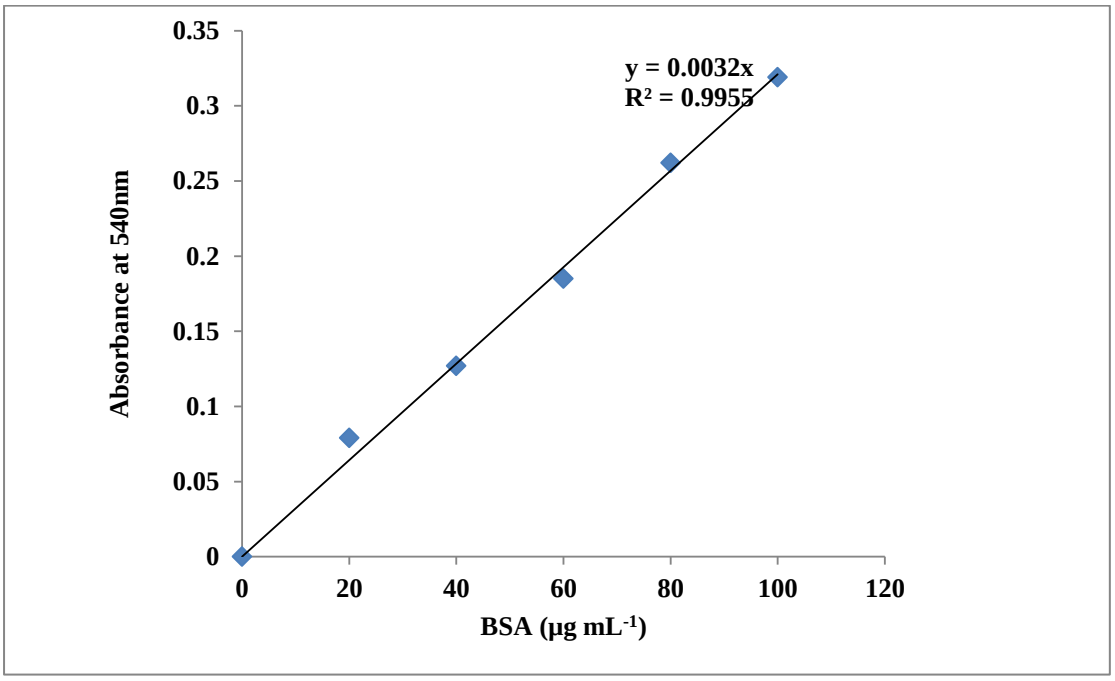
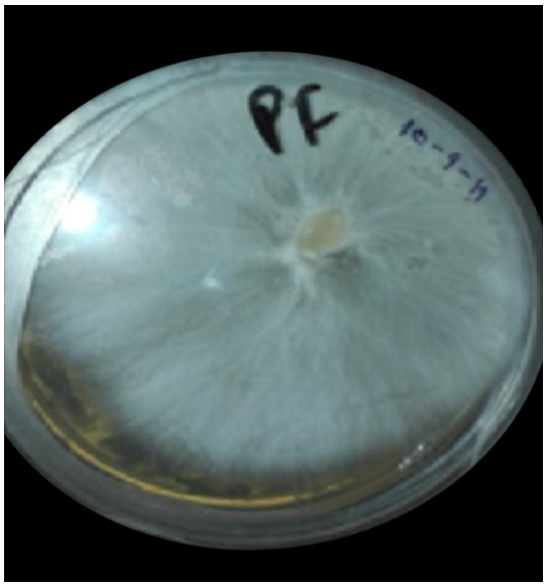
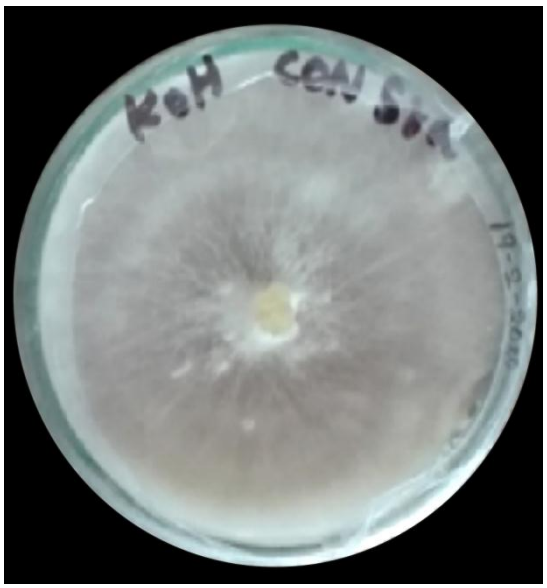


Fig. 3.1 Standard curve for the estimation of total soluble protein content

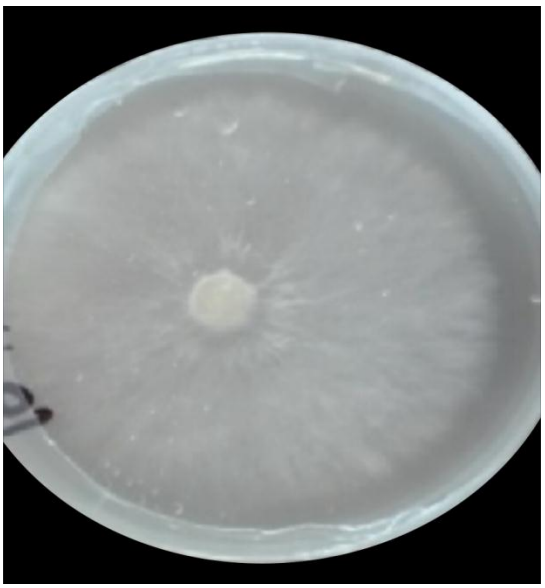


P. florida

P. cornucopiae



P. eryngii



P. djamor

Plate 3.1: Maintenance of various *Pleurotus* spp. cultures

Preparation of standard curve: Standard Bovine Serum Albumin (BSA) (0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL and 1.0 mL) were taken in test tubes and the volume was made to 1 mL with distilled water. To this 2.5 mL of solution C was added, vortexed and incubated for 5 minutes. Then 1mL of reagent D was added, mixed thoroughly, incubated for 15-20 minutes and the absorbance was measured at 540 nm (Fig. 3.1)

3.2.3 Estimation of Se content in Se biofortified mycelium using Inductively Coupled Plasma Mass Spectrometry/Atomic Absorption Spectroscopy (ICP-MS/AAS)

The selenium content was estimated from the wheat straw (substrate), fruit bodies and also from mycelial biomass of *Pleurotus* cultures using ICP-MS/AAS. The mycelial biomass of four *Pleurotus* species namely *P. florida*, *P. eryngii*, *P. djamor* and *P. cornucopiae* were grown on potato dextrose broth enriched with various concentrations of Se such as 0, 5, 10, 15 and 20 mg L^{-1}. The mycelium was separated from the broth by filtration using Whatman No. 1 filter paper. The mycelial biomass was then washed with distilled water twice to remove any traces of the medium present. Then the samples were dried in oven at 55°C for 24 hours. The dried sample (0.5 g) was weighed and digested using di-acid solution (HNO_3 and H_2O_2 in the ratio of 3:1) and re-dissolved in distilled water and filtered. The filtrate was used for estimating Se using Inductively Coupled Plasma Mass Spectrometry/Atomic Absorption Spectroscopy (ICP-MS/AAS).

3.2.4 Analysis of Se biofortified hyphae using Scanning Electron Microscopy and Energy Dispersive Spectroscopy (EDS).

Four cultures of *Pleurotus* namely *P. florida*, *P. eryngii*, *P. djamor* and *P. cornucopiae* were grown on petriplates (size: 90 mm, pre-sterilized polypropylene, Tarsons 460090, India) containing Potato Dextrose Agar (PDA) media supplemented with sodium selenate salt at various concentrations i.e. 0 (control), 10 and 20 mg L^{-1}. The plates were inoculated with uniform sized respective mycelial bits (6 mm diameter) and incubated at 27°C for 7 days. Control and biofortified fresh fruit bodies of *P. florida*, *P. eryngii*, *P. djamor* and *P. cornucopiae* were harvested and processed as per the protocol of Bozzola and Russell (1999) with few modifications. The processed sample was placed on double sided sticky carbon tape on an aluminium stub and sputter coated with 2nm thick gold in an ion sputter coater machine (model Jeol JFC-1100, USA). The sputter coated samples were viewed in Scanning Electron Microscope (model Jeol JSM-6100, USA) operated at an accelerating voltage of 10kV. Elemental analysis of the samples were performed using the Energy Dispersive Spectroscopy (EDS) system (model Jeol JSM-6100, USA) attached to the SEM (Plate 3.2).

3.3 CULTIVATION OF *Pleurotus* MUSHROOMS ON SELENIUM ENRICHED AND NON-ENRICHED WHEAT STRAW

3.3.1 Spawn preparation

Master spawn and the generation spawn of *Pleurotus* spp. were prepared from culture slants by growing them on wheat grains in glucose glass bottles according to the standard methodology given by Garcha and Khanna (2002). Fresh, healthy and disease free wheat grains were selected and washed properly with fresh water, boiled in open pan for about 40 minutes until the seeds become tender without rupturing the seed coat. Then the water was drained out using sieve and the grains were spread on the clean floor until cooled. Then commercial grade lime powder ($CaCO_3$) @ 2 percent w/w and gypsum ($CaSO_4.2H_2O$) @ 4 percent w/w were added to these grains and mixed properly. Then glucose glass bottles of 500 mL volume were filled with approximately 200-300 g of these grains, plugged with non-adsorbent cotton, wrapped with paper and autoclaved at 20 psi pressure for 1 hour. The autoclaved bottles were left overnight to cool at room temperature, next day the bottles were shaken to restore the transparency of glass. The bottles were inoculated with 5-7 days old culture from the culture slants, the mycelial bits of approximately 3x1 cm rectangular shaped were cut from the culture slants and placed on the surface of the grains inside the bottles in such a way that the mycelium comes in contact with the grains. The inoculated bottles were incubated at 25±1°C until the mycelial run was completed. Such culture bottles produced were called as Master Spawn. For preparing generation spawn, the mother spawn was shaken properly and used to inoculate the freshly autoclaved grain bottles in 20:1 ratio and incubated at 25±1°C until the mycelial run was completed.

3.3.2 Substrate collection and preparation

The Se enriched wheat straw was procured from seleniferous soils of Jainpur village of Nawanshahr-Hoshiarpur region of Punjab, India for the production of Se biofortified oyster mushrooms. For the cultivation of control mushrooms the wheat straw was also procured from the non-seleniferous regions of Punjab Agricultural University, Ludhiana, Punjab, India.

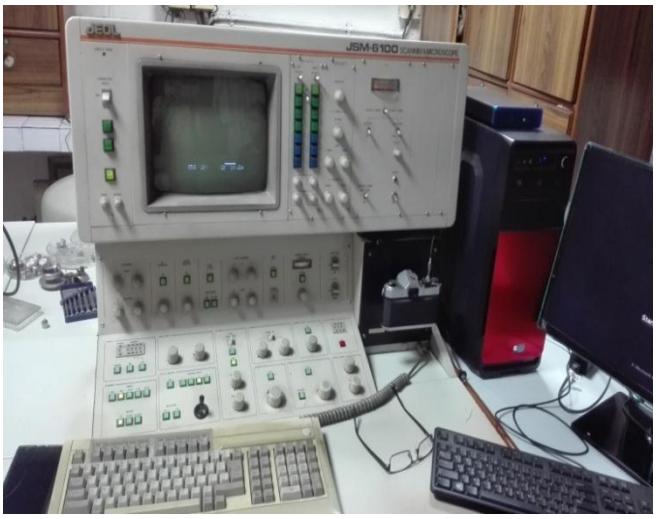
Wheat straw was spread on the clean concrete pucca floor and wetted with fresh water and left for 16-20 hours to achieve 65-70 per cent moisture.

3.3.3 Spawning and bag filling

The wetted straw was spread on the clean floor and spawned @ 10 % (w/w) of dry straw and mixed thoroughly. The straw was filled in polythene bags of size 12” x 16” up to $3/4^{th}$ of its capacity. The edges of the polythene bags were cut at the bottom for aeration and tied with rope at the top. The spawned bags were then incubated at 25±1°C and 70% relative humidity on racks in growing rooms for spawn run.

(a) Ion sputter coater machine (model Jeol JFC-1100, USA)

(b) Energy Dispersive Spectroscopy (EDS) system (model Jeol JSM-6100, USA)



(c) Scanning Electron Microscope (model Jeol JSM-6100, USA)

Plate 3.2 (a) Ion sputter coater machine, (b) Energy Dispersive Spectroscopy (EDS) system and (c) Scanning Electron Microscope

3.3.4 Fruiting and harvesting

Once the pinhead stage was reached, the bags were opened with the help of a blade, tagged and arranged on the racks of the growing room. Later, these bags were watered daily in the form of mist using a nap-sac sprayer fitted with a nozzle. Within a week, the pin heads developed into mature fruiting bodies. After 3-4 days of emergence of fruiting bodies, they were harvested by gentle twisting (Fig. 3.2; Plate 3.3).

3.3.5 Mushroom yield and biological efficiency

The total yield of *Pleurotus* spp. was calculated as the fresh weight of total harvested mushrooms per 0.5 kg of dry substrate. Biological efficiency was calculated as the ratio of fresh weight (g) of mushrooms to the dry weight (g) of substrate and expressed as percentage (Chang *et al* 1981).

Biological Efficiency (%) =Fresh weight of mushrooms (g) / dry weight of substrate (g) * 100

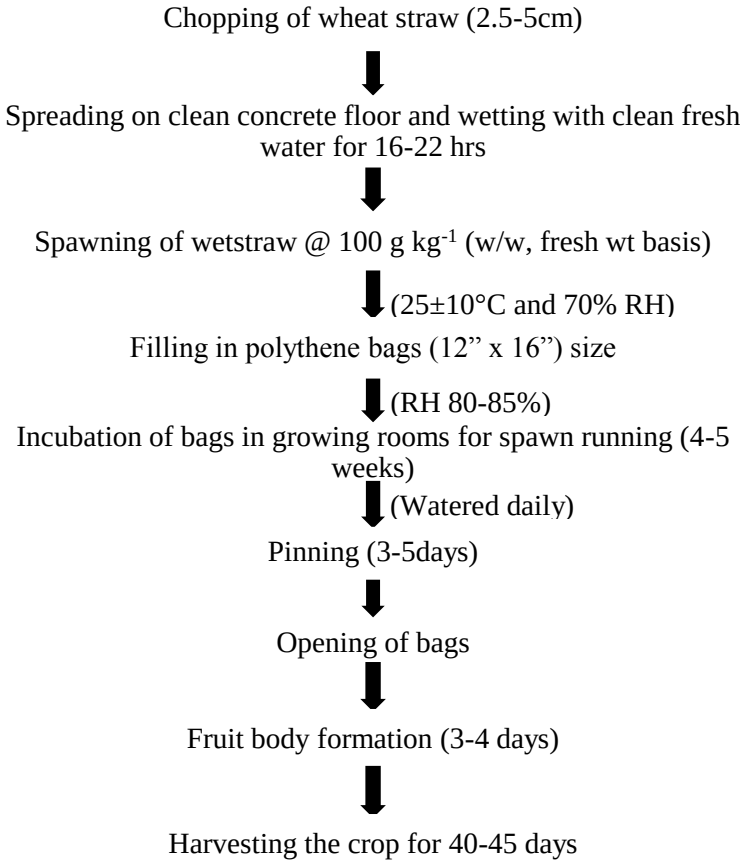


Fig. 3.2 Flow Chart for Cultivation of *Pleurotus* spp.

3.4 ESTIMATION OF VARIOUS PARAMETERS OF SELENIUM BIOFORTIFIED *Pleurotus* MUSHROOMS

3.4.1 Estimation of total phenolic content

Total phenolic content was estimated by following the protocol given by Swain and

Hillis (1959) with little modifications. The fruit bodies of *P. florida*, *P. eryngii*, *P. djamor* and *P. cornucopiae* were dried at 55°C for 24 hours and were grinded into fine powder. Thereafter, powdered sample (0.5 g) was extracted with methanol (5 mL) and centrifuged. The supernatant was collected and the volume was made to 5 mL with methanol and stored at 4°C for further use. 0.5 mL of extract was taken in a test tube and refluxed it until entire methanol was evaporated. To the extract, distilled water (6.5 mL) and then 0.5 mL of Folin-ciocalteu reagent was added and vortexed. Thereafter, 1 mL of Na_2CO_3 (2%) was added. Later, 1 mL of saturated sodium carbonate was added, vortexed and incubated it for 1 hour and the absorbance was measured at 760 nm. Standard curve was plotted by using various concentrations of gallic acid (Fig. 3.3). The total phenolic content of the mushroom sample was expressed as mg of gallic acid equivalents (GAE) g^{-1} of dry sample.

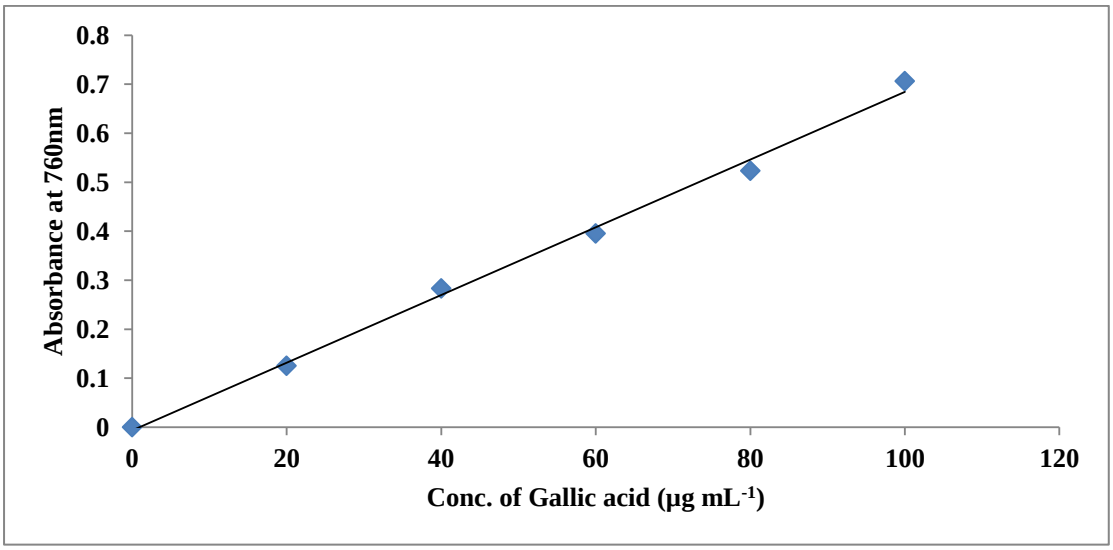


Fig. 3.3 Standard Curve for Estimation of Total Phenolic Content

3.4.2 Free radical scavenging activity (%)

For the determination of % free radical scavenging activity on DPPH (2, 2-diphenyl-1-picrylhydrazyl) of fruit bodies of *Pleurotus* spp., the protocol given by Blois (1958) was followed. The fruiting bodies of *P. florida*, *P. eryngii*, *P. djamor* and *P. cornucopiae* were dried at 55°C for 24 hours. The dried fruit bodies were grinded into fine powder. 0.5 g of powdered sample was then extracted with 5mL of methanol and centrifuged. Supernatant was collected and the volume was made to 5 mL with methanol and stored at 4°C for further use. 0.1 mL of methanolic extract was taken and 0.9 mL of methanol was added to this. Then 3 mL of 0.1mM DPPH solution was added, incubated in dark for 30 minutes and absorbance was measured at 517 nm. For control sample, 1 mL methanol and 3 mL of 0.1mM DPPH was added. The percentage of free radical scavenging activity was calculated from the following formula (where abs sample was the absorbance of sample and abs control was the absorbance of control).

% inhibition of DPPH = Abs control- Abs sample ÷ Abs control × 100

Mother spawn of *P. eryngii*

Generation spawn of *P. florida*

Substrate preparation

Spawning

Bag filling

Plate 3.3: Cultivation of various *Pleurotus* spp. on selenium enriched wheat straw

3.4.3 Estimation of total flavonoids

The total flavonoids were estimated from the fruit bodies of *Pleurotus* spp. by following the protocol given by Zhishen *et al* (1999). The fruiting bodies of *P. florida*, *P. eryngii*, *P. djamor* and *P. cornucopiae* were dried at 55°C for 24 hours, grinded into fine powder. 0.1 g of dried powder sample was added to 10 mL of distilled water, boiled for 1 hour and filtered using Whatman No. 1 filter paper. The filtrate was stored at 4°C for further use. 0.25 mL of filtrate was taken and 1.25 mL of distilled water, 75 µL of 5% sodium nitrite ($NaNO_2$) were added. After 6 minutes, 150 µL of 10% $AlCl_3.6H_2O$ was added and after 5 minutes, 0.5 mL of 1M sodium hydroxide was added and the final volume was made to 2.5 mL with distilled water and the absorbance was measured at 510 nm. Various concentrations of Quercetin were used for preparing the standard curve (Fig. 3.4).

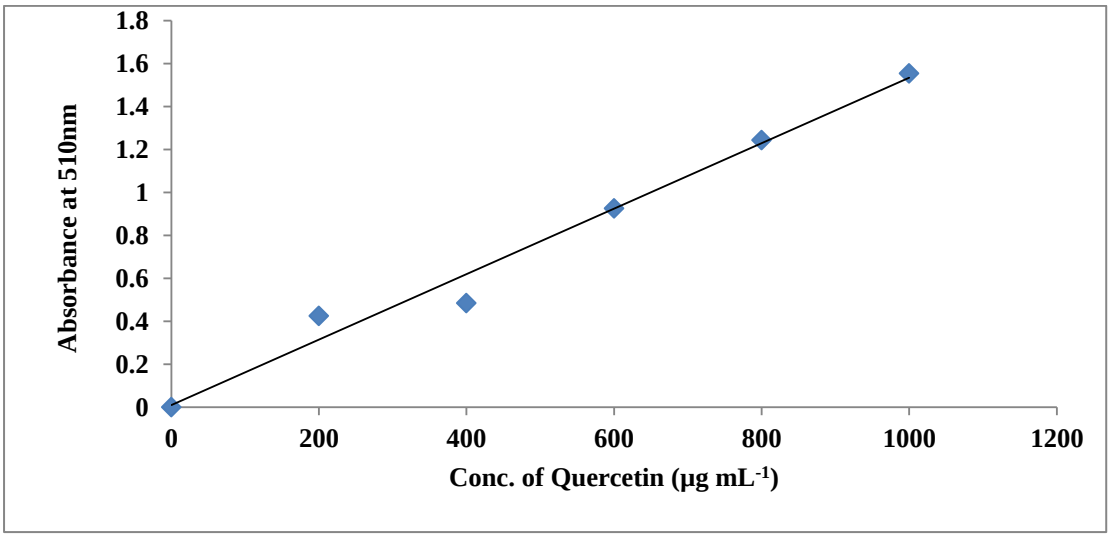


Fig. 3.4 Standard Curve for Estimation of Total Flavonoids

3.4.4 Characterization of Se biofortified *Pleurotus* mushrooms by Fourier Transform Infra-Red Spectroscopy (FT-IR Spectroscopy)

The FTIR is the most powerful tool for identification of the types of the chemical bonds. The wavelength of the IR light absorbed is characteristics of the chemical bond. A beam of infrared light is focused on the sample using all-reflective optics. Depending on the sample composition, different amount of light is absorbed at different wavelengths. This pattern of light absorption is unique for almost every organic compound (except optical isomers) and many inorganics. Selenium biofortified *Pleurotus* spp. fruit bodies were analyzed for their chemical structure using a Fourier Transform Infra-Red (FT-IR) spectrophotometer (model Thermo-Nicolet 6700, ThermoScientific, Rockford, IL, USA) equipped with Potassium bromide (KBr) beam splitter with DTGS (deuterated triglycinesulphate) detector (7800-350cm^{-1}). Spectra obtained were recorded in between the wavelength range of 500 and 4000 cm^{-1}. The completely dried samples were kept on the probe of attenuated total reflectance for the spectroscopic analysis.

CHAPTER-IV

RESULTS AND DISCUSSION

The present study comparing the effect of selenium biofortification on antioxidant activity of four different species of selenium biofortified *Pleurotus* spp. i.e. *P. florida*, *P. eryngii*, *P. cornucopiae* and *P. djamor*. Effect of selenium supplementation on the radial hyphal growth, biomass production and the total soluble protein content of mycelium were observed. The Se content of the Se biofortified mycelium and fruit bodies were estimated using Inductively Coupled Plasma Mass Spectrometry/Atomic Absorption Spectroscopy (ICP-MS/AAS), Scanning Electron Microscopy and Energy Dispersive Spectroscopy (EDS) techniques. The four *Pleurotus* species were cultivated on selenium enriched and non-enriched wheat straw. Fruit bodies for total protein content, total phenol and flavonoid content, and percent radical scavenging activity were analyzed. Se biofortified *Pleurotus* spp. fruit bodies were also characterized by FT-IR Spectroscopy. The results of these experiments have been discussed under the following headings and subheadings:

4.1 EFFECT OF SELENIUM SUPPLEMENTATION ON THE RADIAL GROWTH AND BIOMASS PRODUCTION OF *Pleurotus* spp. MYCELIUM

4.2 EFFECT OF SELENIUM SUPPLEMENTATION ON THE TOTAL SOLUBLE PROTEIN CONTENT OF *Pleurotus* spp. MYCELIUM

4.3 ESTIMATION OF SELENIUM CONTENT IN SELENIUM ENRICHED *Pleurotus* spp. MYCELIUM USING INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY/ATOMIC ABSORPTION SPECTROSCOPY (ICP-MS/AAS)

4.4 ANALYSIS OF SE BIOFORTIFIED *Pleurotus* spp. HYPHAE USING SCANNING ELECTRON MICROSCOPY AND ENERGY DISPERSIVE SPECTROSCOPY (SEM AND EDS)

4.5 CULTIVATION OF *Pleurotus* spp. ON SELENIUM ENRICHED AND NON-ENRICHED WHEAT STRAW

4.6 ANALYSIS OF SELENIUM BIOFORTIFIED *Pleurotus* spp. FRUIT BODIES

- 4.6.1 Total soluble protein content
- 4.6.2 Total phenolic content
- 4.6.3 Free radical scavenging activity (%)
- 4.6.4 Total flavonoids

4.7 ESTIMATION OF SELENIUM CONTENT IN SELENIUM BIOFORTIFIED *Pleurotus* spp. FRUITING BODIES BY ENERGY DISPERSIVE SPECTROSCOPY (EDS)

4.8 ESTIMATION OF SELENIUM CONTENT IN SE BIOFORTIFIED *Pleurotus* spp. FRUITING BODIES USING INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY/ATOMIC ABSORPTION SPECTROSCOPY (ICP-MS/AAS)

4.9 CHARACTERIZATION OF SELENIUM BIOFORTIFIED *Pleurotus* spp. FRUITING BODIES BY FOURIER TRANSFORM INFRA-RED SPECTROSCOPY (FT-IR SPECTROSCOPY)

4.1 EFFECT OF SELENIUM SUPPLEMENTATION ON THE RADIAL GROWTH AND BIOMASS PRODUCTION OF *Pleurotus* spp. MYCELIUM

Pleurotus species namely *P. florida*, *P. eryngii*, *P. cornucopiae* and *P. djamor* were grown both under *in-vitro* and *in-vivo* conditions on normal and selenium supplemented substrates. For *in-vitro* studies, *Pleurotus* spp. mycelium were grown on potato dextrose agar (PDA), on petriplates supplemented with sodium selenate @ 5 mg L^{-1}, 10 mg L^{-1}, 15 mg L^{-1} and 20 mg L^{-1} with non-Se supplemented agar plates as control. The diameter of the radial mycelial growth was measured on agar plates at 24 hours interval for 7 days to calculate the growth rate. All *Pleurotus* spp. showed varied response to Se-supplementation in the growth medium. Addition of sodium selenate @ 10 mg L^{-1} showed a positive effect on the growth rate of *P. djamor* (7.11±0.19 mm/day) and *P. cornucopiae* (8.02±0.16 mm/day) while a decreasing trend was found at 15 and 20 mg L^{-1} Se concentration, whereas in *P. florida*, Se concentration @ 5 mg L^{-1} was observed in enhancing the growth rate (9.00±0.25 mm/day) as compared to other concentrations (Table 4.1). In case of *P. eryngii*, there was an increasing trend in the growth rate with increasing selenium concentration with 20 mg L^{-1} showing the highest growth rate (8.56±0.22 mm/day). Hence, 5 and 10 mg L^{-1} Se concentrations were selected to carry out biofortification studies so that no adverse effect on the radial growth rate could occur. At concentrations higher than 10 mg L^{-1}, the radial growth decreased significantly which may be due to the toxicity caused by Se (Fig. 4.1). During biofortification, Se specifically binds to the chitin present in the *P. ostreatus* cell walls (Munoz *et al* 2006). Moreover, they also described that lower concentration of 2.5 mg L^{-1} Se supplementation increased the mycelial growth while higher supplementation of 5 mg L^{-1} had an inhibitory effect. The growth and enzymatic activity of *P. eryngii* grown on sodium selenite supplemented medium (1, 10, 100, 1000, and 10,000 µM) was studied by Kim *et al* (2008). They also reported that concentration 1, 10, 100 µM stimulated the mycelial growth while 1000 and 10,000 µM of Se significantly reduced the mycelial growth due to Se toxicity. These results are similar to the findings of Goyal *et al* (2015) which studied the effect of selenium supplementation on the radial mycelial growth of *Ganoderma lucidum*. A decreasing trend was observed on the radial growth with increase in sodium selenate concentration from 0 (1.375cm) to 25 ppm (1.05cm) on the mushroom minimal agar post 24h on the basis of stereomicroscopic studies. However, Se supplementation resulted in equivalent radial diameter with respect to control, 5 and 10 ppm at 48h of incubation. There was a slight increase in the radial growth at Se concentration from 0 (3.49 cm) to 10 ppm (3.52 cm) followed by a decreasing trend at higher concentrations i.e. 15 (3.46 cm), 20 (3.25 cm) and 25

ppm (3.05 cm) at 92h of incubation. Our results showed that *P. eryngii* was more tolerant to Se stress by showing maximum growth rate at 20 mg L^{-1} which showed agreement to the findings of Da silva *et al* (2013) which showed that Se addition decreased the septum distance and the diameter of hyphae of all isolates, except for *P. ostreatus* 09 and *P. eryngii* 02 at 25.4 mg L^{-1} of Se which increased the septum distance, and *P. ostreatus* 06 which increased the hyphae diameter. Furthermore, no significant difference was observed in these parameters in Se levels higher than 50.9 mg L^{-1}. Although, at a Se concentration of 101.8 mg L^{-1}, decrease in septa distance and hyphae diameter were observed in most of the strains when compared with non-supplemented medium. They also reported highest reduction in growth rate of *P. ostreatus* as compared to *P. eryngii* which indicated its more tolerance towards Se stress. The effect of various concentrations of selenium (0, 50, 100 mg mL^{-1}) in the form of Na_2SeO_3 (sodium selenite) on the mycelial growth of *Pleurotus* spp. was studied by Ogidi *et al* (2017). They observed decreased fungal growth rate when Se concentration increased from 0-100 mg mL^{-1}. The fungal growth rate ranged between 1.5-3.6 mm/d and 1.0-3.30 mm/d @ 50 and 100 mg mL^{-1} respectively. The growth rate of *Pleurotus spp.* i.e. *P. cornucopiae* and *P. djamor var. roseus* were not significantly different @ 50 and 100 mg mL^{-1} Se concentration. Zięba *et al* (2020) demonstrated that the 1 g of dry *P. eryngii* mycelium enriched with selenium could provide 268% of the RDI (recommended daily intake) while, zinc sulfate supplementation could provide 58% of the recommended daily intake. They also concluded that such biofortifications could be used as a diet supplement that provides these essential nutrients along with bioactive components present in the mushrooms.

Table 4.1 Effect of Se supplementation on the radial growth of *Pleurotus* spp.

Se supplementation (mg L^{-1})	**Radial growth (mm/day)**			
	P. florida	***P. eryngii***	***P. djamor***	***P. cornucopiae***
5	9.00±0.25	6.10±0.09	5.78±0.16	5.37±0.26
10	7.70±0.29	6.97±0.14	7.11±0.19	8.02±0.16
15	7.20±0.32	8.32±0.28	5.04±0.12	6.87±0.21
20	6.40±0.17	8.56±0.22	4.15±0.14	6.63±0.21
0 (Control)	8.20±0.29	5.56±0.15	5.98±0.26	5.80±0.25
CD (5 %)	0.85	0.59	0.56	0.69

No. of replicates-3

Medium used: Potato Dextrose Agar (PDA)

Incubation temperature-25±1°C

Incubation period-7 days

± values indicated standard error

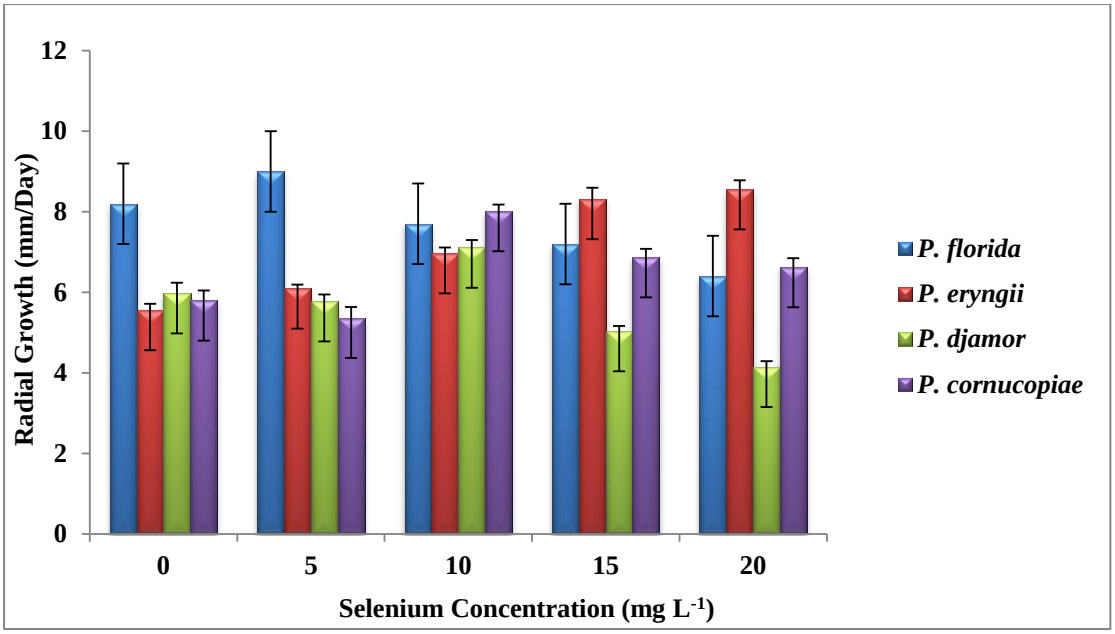


Fig. 4.1 Effect of Se supplementation on the radial growth of *Pleurotus* spp.

For biomass production, *Pleurotus* spp. mycelia were also grown on selenium supplemented and non-Se supplemented potato dextrose broth. In case of *P. florida*, highest biomass production was observed in broth supplemented with 5 mg L^{-1} Se concentration (88.0±3.2 g L^{-1}) and then a decreasing trend was observed with increase in Se concentration from 10 mg L^{-1} to 20 mg L^{-1}. In case of *P. eryngii*, the biomass production increased with increase in Se concentration from 0 to 20 mg L^{-1} and highest biomass production was observed at 20 mg L^{-1} (77.0±2.3 g L^{-1}) Se concentration. In case of *P. cornucopiae* and *P. djamor*, the biomass production increased up to 10 mg L^{-1} Se concentration and then a decreasing trend was observed from 10 mg L^{-1} to 20 mg L^{-1} Se concentration (Fig. 4.2). The highest biomass production was observed in *P. cornucopiae* (58.3±1.7 g L^{-1}) and *P. djamor* (88.5±3.0 g L^{-1}) at 10 mg L^{-1} Se concentration (Table 4.2).

The mycelium grown under submerged conditions facilitated the more accessibility of Se from medium with less chances of contamination in order to achieve high biomass yield and bioactive components of consistent quality as compared to solid state fermentation (Muñoz *et al* 2006). The results obtained were in agreement with the previously published data where there was a reduction in the fungal growth rate and biomass production with increase in Se concentration from 0-100 mg mL^{-1} (Ogidi *et al* 2017). Thus, this study revealed that biofortification with higher concentrations of Se inhibits the growth and reduction in biomass production of *Pleurotus* spp. Similarly, our results also showed an inhibitory effect on biomass production at higher levels of Se supplementation i.e. 15 and 20 mg L^{-1} in *P. florida*, *P. djamor* and *P. cornucopiae.* Da silva *et al* (2013) reported that the dry mass of *Pleurotus* spp. (*P. eryngii* and *P. ostreatus*) decreased (2.5 to 8 folds) as the concentration of Se increased from 25.4 to 101.8 mg mL^{-1}. Moreover, the highest biomass decrease in *P.*

ostreatus indicated that this fungal species was less tolerant to Se than *P. eryngii*. Selenium is required by fungi for its metabolic activities but found to be toxic when present in higher concentration than the required amount in the substrate as reported by Spallholz (1994). A significant inhibitory effect of sodium selenite (100 mg Se L^{-1}) on the biomass yield of *Hericium erinaceum* was also reported by Malinowska *et al* (2009). Turlo *et al* (2010) reported that the *Lentinula edodes* yield (% dry weight of mycelium) from both water and alcohol extracts was much lower for mycelium cultivated on Se-enriched as compared to non-enriched medium. They reported that biomass yield in the methanol extract was 37.6% for non-enriched medium in comparison to Se-enriched medium (22.2%). While the biomass yield in the water extract from Se-enriched (38.6%) and non-enriched medium (25.1%). In *P. ostreatus*, the supplementation of inorganic Se lead to a change in mycelial morphology in terms of less dense hyphae with thick cell wall, abundant septa and lower branch frequency (Milovanović *et al* 2014). They also reported an optimal selenium concentration (15-20 mg L^{-1}) for higher biomass yield and Se accumulation in *P. ostreatus*. Miletić *et al* (2019) compared two selenium compounds (selenourea and Na_2SeO_3) @ 10 mg Se mL^{-1} for submerged cultivation of *Coriolus versicolor*. They found that Se accumulation was more effective with Na_2SeO_3 but with less biomass production (1.89 g DW L^{-1}) in comparison to samples supplemented with selenourea (4.48 g DW L^{-1}). The Se supplementation in liquid medium results in production of stable mycelium with respect to dry weight while supplementation of growing substrate with Se results in reduction in fruiting body yield Zięba *et al* (2020). Thus, it was concluded from our study that supplemented mycelium with essential elements could be used as a dietary supplement.

Table 4.2 Effect of Se supplementation on the biomass production of *Pleurotus* spp.

Se supplementation (mg L^{-1})	**Biomass (g L^{-1})**			
	P. florida	***P. eryngii***	***P. djamor***	***P. cornucopiae***
5	88.0±3.2	57.5±2.1	83.0±3.1	47.2±1.7
10	74.7±1.9	60.9±1.6	88.5±3.0	58.3±1.7
15	58.8±1.3	65.3±2.3	71.0±2.0	33.0±1.7
20	58.7±1.5	77.0±2.3	49.8±2.6	28.9±1.7
0 (Control)	63.5±4.4	43.2±1.6	85.0±3.5	49.5±1.1
CD (5 %)	8.61	6.40	8.98	5.1

No. of replicates-3

Medium used: Potato Dextrose broth (PDB)

Incubation temperature:25±1°C

Incubation period-20days

± values indicated standard error

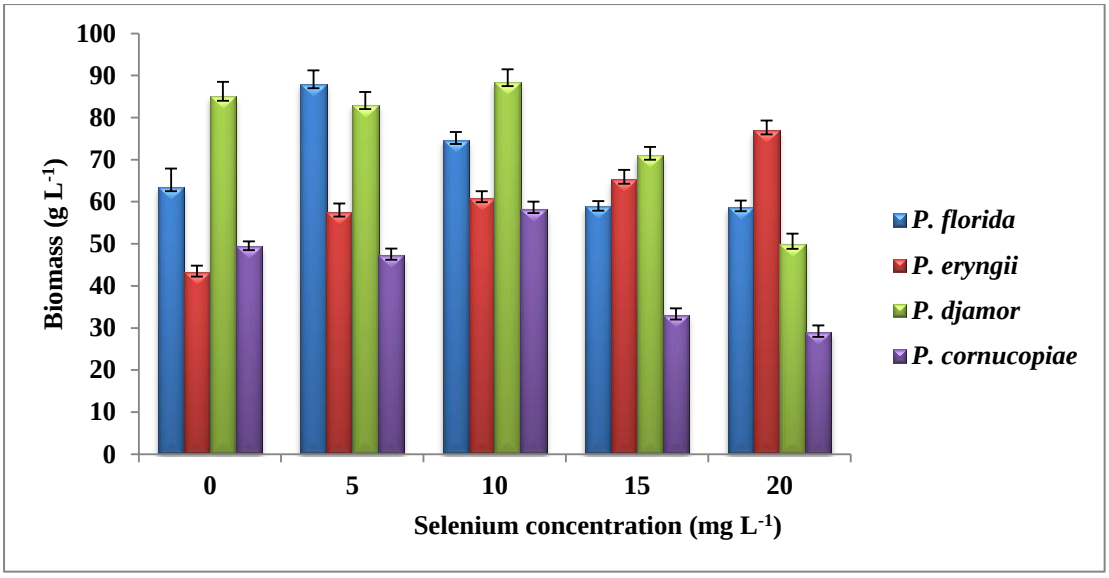


Fig. 4.2 Effect of Se supplementation on the biomass production of *Pleurotus* spp.

4.2 EFFECT OF SELENIUM SUPPLEMENTATION ON THE TOTAL SOLUBLE PROTEIN CONTENT OF *Pleurotus* spp. MYCELIUM

The total soluble protein content was estimated from the vegetative mycelium of *Pleurotus* spp. grown on selenium enriched broth supplemented with 5 mg L^{-1}, 10 mg L^{-1}, 15 mg L^{-1} and 20 mg L^{-1} of selenium along with non-supplemented broth as control. The total soluble protein content of mycelium was found to be highest at 10 mg L^{-1} Se concentration in *P. djamor* (1.97±0.15 mg g^{-1}) and *P. cornucopiae* (1.94±0.10 mg g^{-1}). In *P. florida*, the highest total soluble protein content (2.28±0.16 mg g^{-1}) was found at 5 mg L^{-1} of Se concentration. The total soluble protein content of mycelium increased with increase in Se concentration in *P. eryngii* and highest total soluble protein content was observed at 20 mg L^{-1} Se concentration (2.55±0.13 mg g^{-1}). In case of *P. florida*, *P. djamor* and *P. cornucopiae* there was an increasing trend in the total soluble protein content of mycelium up to a Se concentration of 10 mg L^{-1} and then a decreasing trend was observed (Table 4.3). The total soluble mycelial protein content of control samples were found to be highest in *P. eryngii* (1.52±0.09 mg g^{-1}) followed by *P. florida* (1.21±0.10 mg g^{-1}), *P. djamor* (1.02±0.12 mg g^{-1}) and *P. cornucopiae* (0.99±0.13 mg g^{-1}). There was a significant difference in the total soluble protein content of all the four *Pleurotus* spp. mycelium supplemented with different doses of Se (Fig. 4.3). Mushrooms possess high amount of protein and are capable of accumulating higher amounts of Se. Therefore, it is reasonable to anticipate that this element might have incorporated in to proteins as selenoproteins. Bhatia (2014) reported that the process of Se biofortification enhanced the total protein and total phenolic content as compared to unfortified mushrooms. Turlo *et al* (2007) reported that *Lentinula edodes* mycelium can incorporate Se into proteins as selenomethionine. In another study, Turlo *et al* (2010) revealed that Se toxicity occurred if selenomethionine was present at >300 µg g^{-1} of fungal biomass

and a total selenium content of 1100 µg g^{-1}, resulting in substantial reduction of mycelial growth. The accumulation of Se in mushrooms favors the formation of more bioaccessible selenoproteins (Oliveira and Naozuka 2019). In *P. ostreatus*, biofortification with Se lead to an increase in protein content and antioxidant properties of mushrooms (Poniedziałek *et al* 2017). It was reported by Muszyńska *et al* (2020) that Se accumulated in the *Lentinula edodes* mycelium as selenite triglycerides and therefore, constitutes a good source of Se for livestock feed and protein for human with immunostimulatory, antioxidative and anti-inflammatory effects.

Table 4.3 Effect of Se supplementation on the total soluble protein content of *Pleurotus* spp. mycelium

Se supplementation (mg L^{-1})	Total Soluble Protein Content (mg g^{-1})			
	P. florida	***P. eryngii***	***P. djamor***	***P. cornucopiae***
5	2.28±0.16	1.57±0.02	1.19±0.11	1.68±0.13
10	2.08±0.10	2.03±0.20	1.97±0.15	1.94±0.10
15	1.83±0.11	2.04±0.23	1.92±0.12	1.18±0.07
20	1.29±0.09	2.55±0.13	1.90±0.06	1.11±0.19
0 (Control)	1.21±0.10	1.52±0.09	1.02±0.12	0.99±0.13
CD (5 %)	0.36	0.48	0.36	0.38

No. of replicates-3

Medium used: Potato Dextrose broth (PDB)

Incubation temperature:25±1°C

± values indicated standard error

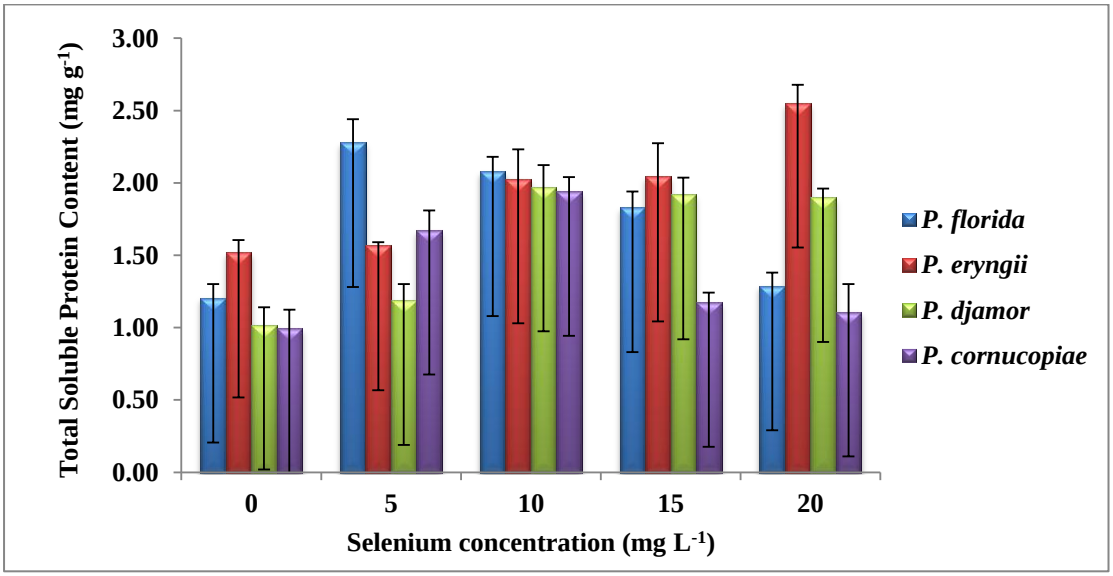


Fig. 4.3 Effect of Se supplementation on the total soluble protein content of *Pleurotus* spp. mycelium

4.3 ESTIMATION OF SELENIUM CONTENT IN SELENIUM ENRICHED *Pleurotus* spp. MYCELIUM USING INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY/ATOMIC ABSORPTION SPECTROSCOPY (ICP-MS/AAS)

Se content was estimated from the Se-enriched biomass of *Pleurotus* spp. using ICP-MS/AAS and expressed as µg Se g^{-1} dw. In the control samples, very low amount of Se was detected i.e. in *P. florida* (2.2±0.46 µg g^{-1} dw), followed by *P. cornucopiae* (1.9±0.26 µg g^{-1} dw), *P. djamor* (0.39±0.06 µg g^{-1} dw) and *P. eryngii* (0.21±0.04 µg g^{-1} dw). As the concentration of supplemented Se increased from 5 to 20 mg L^{-1}, the amount of Se accumulated in the biomass of *Pleurotus* spp. also increased, with highest accumulation detected at 20 mg L^{-1} Se supplementation in *P. djamor* (920.32±17.99 µg g^{-1} dw), followed by *P. florida* (880.61 µg g^{-1} dw), *P. eryngii* (850.27 µg g^{-1} dw) and *P. cornucopiae* (792.72 µg g^{-1} dw; Table 4.4). Se absorbed from the medium and incorporated into the mycelial biomass, represented as total Se (%) supplemented to the original medium showed a decreasing trend with increase in Se concentration of the medium (Fig. 4.4). Highest percentage of absorbed selenium was found at 5 mg L^{-1} Se concentration in the medium in all the four *Pleurotus* spp. namely *P. djamor* 57.27% (920.32±17.99 µg g^{-1} dw), followed by *P. florida* 51.02% (880.61±15.78 µg g^{-1} dw), *P. eryngii* 44.60% (850.27±20.78 µg g^{-1} dw) and *P. cornucopiae* 43.70% (792.72±20.93 µg g^{-1} dw). At 10, 15 and 20 mg L^{-1} Se concentration in the medium, there was a slight decrease in the percentage of absorbed Se in all the four *Pleurotus* species i.e. in *P. djamor* (40.97%, 40.60% and 40.49% at 10, 15 and 20 mg L^{-1} respectively), *P. florida* (39.25%, 38.71% and 38.75% at 10, 15 and 20 mg L^{-1} respectively), *P. eryngii* (37.81%, 37.52% and 37.41% at 10, 15 and 20 mg L^{-1} respectively) and *P. cornucopiae* (35.74%, 35.25% and 34.89% at 10, 15 and 20 mg L^{-1} respectively). This shows that fungi may possess a mechanism for metabolizing metals like selenium from the growing environment (bioaccumulation). Milovanović *et al* (2014) studied the potential of *P. ostreatus* mycelium for selenium absorption and reported that no Se content was observed in the mycelial control sample. Whereas, in the Se-enriched media, the concentration of Se in the mycelium ranged from 251.2 μg g^{-1} (@ 5 mg L^{-1} Se concentration) to 938.9 μg g^{-1} (@ 20 mg L^{-1} Se concentration) which was the highest amount absorbed. At higher concentrations of Se in the medium, there was decrease in the absorption levels. Even though, the concentrations of Se in the mycelium were higher than those obtained @ 5 mg L^{-1} and 10 mg L^{-1}. When the absorbed Se by mycelial biomass was represented as % of total Se supplemented to the original medium there was a decreasing trend with increasing Se concentration of medium. The highest percentage of absorbed Se was at 5 mg L^{-1} (53.25%) Se concentration in the medium, there was a slight decrease in the % absorption at 10 (43.58%) and 20 mg L^{-1} (41.31%) Se concentration and drastic reduction at 50 (11.17%) and 100 mg L^{-1} (3.60%) Se concentration. Muszyńska *et al* (2020) studied the Se content in Se-enriched *Lentinula edodes*

mycelium and reported that Se content was 0.01 mg/100 g dw in non Se-enriched (control) mycelium of *Lentinula edodes*. Whereas, Se content of selenite triglycerides supplemented (25 and 50 mg L^{-1}) *Lentinula edodes* mycelium were 192.6 and 532.3 mg/100 g dw respectively.

Table 4.4 Estimation of selenium content in selenium enriched *Pleurotus* spp. mycelium using Inductively Coupled Plasma Mass Spectrometry/Atomic Absorption Spectroscopy (ICP-MS/AAS)

Se supplementation (mg L^{-1})	Total selenium (µg g^{-1} dw)			
	P. florida	***P. eryngii***	***P. djamor***	***P. cornucopiae***
5	240.67±12.98	210.38±21.88	270.21±18.99	206.13±12.40
10	409.28±15.78	394.22±16.67	427.17±20.76	372.64±15.11
15	579.81±14.61	562.00±14.74	608.13±15.71	527.91±18.52
20	880.61±15.78	850.27±20.78	920.32±17.99	792.72±20.93
0 (Control)	2.20±0.46	0.21±0.04	0.39±0.06	1.90±0.26
CD (5 %)	41.79	52.81	51.98	48.04

No. of replicates-3

Medium used: Potato Dextrose broth (PDB)

Incubation temperature:25±1°C

± values indicated standard error

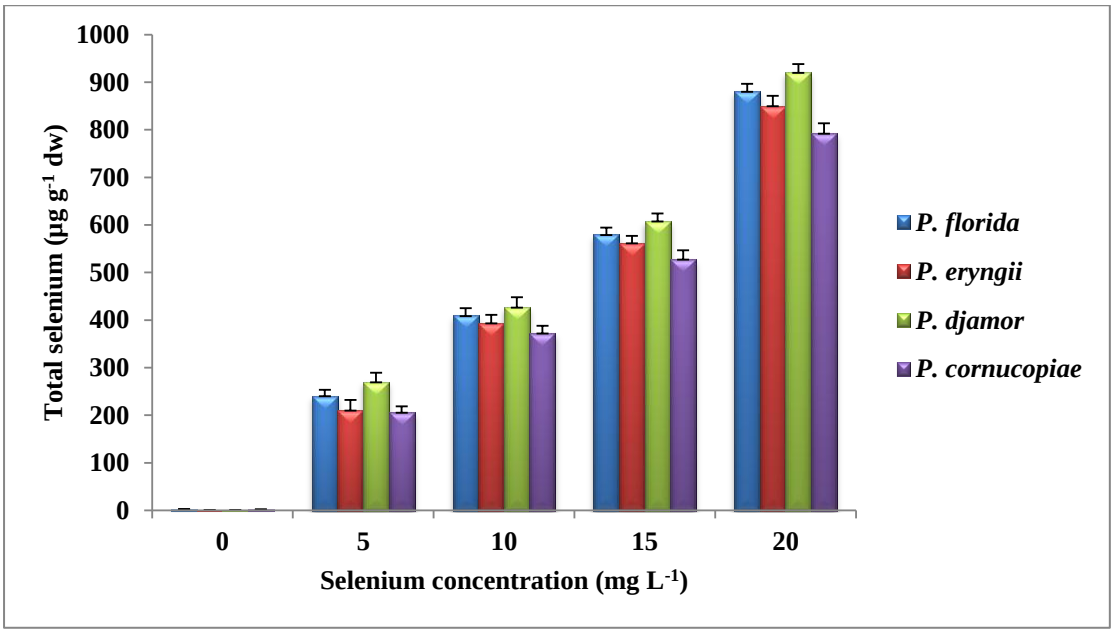


Fig. 4.4 Estimation of selenium content in selenium enriched *Pleurotus* spp. mycelium using Inductively Coupled Plasma Mass Spectrometry/Atomic Absorption Spectroscopy (ICP-MS/AAS)

4.4 ANALYSIS OF SELENIUM BIOFORTIFIED *Pleurotus* spp. HYPHAE USING SCANNING ELECTRON MICROSCOPY AND ENERGY DISPERSIVE SPECTROSCOPY (SEM AND EDS)

Scanning electron microscopy studies can help to study the morphological variations occurring in response to Se supplementation including hyphal thinning or thickening, changes in distance of the hyphal septa and the surface topography of the *Pleurotus* hyphae. The relative occurrence of different elements on hyphal surface was determined through SEM-EDS analysis. Various mycelial samples of *Pleurotus* spp. were processed for SEM-EDS as per the protocol given by Bozzola and Russell (1999). The processed sample was placed on aluminium stub using double-sided sticky carbon tape. The stubbed samples were sputtercoated with 2nm thick conductive gold layer using Ion sputter coater (model Jeol JFC-1100, USA). Coated samples were viewed in a Scanning Electron Microscope (model Jeol JSM-6100, USA) at an accelerating voltage of 10Kv.

Scanning Electron Microscopy showed that selenium stress led to a decrease in average hyphal diameter in all the four *Pleurotus* species i.e. *P. florida*, *P. eryngii*, *P. djamor* and *P. cornucopiae*. In all the four species the hyphal diameter increased @ 10 mg L^{-1} Se concentration as compared to control samples. At 20 mg L^{-1} Se concentration, the hyphal diameter decreased in all the four species. This shows that the *P. florida*, *P. eryngii*, *P. djamor* and *P. cornucopiae* had got a mechanism to tolerate selenium stress. This also shows that 10 mg L^{-1} Se concentration was best for the growth of *P. florida*, *P. eryngii*, *P. djamor* and *P. cornucopiae*. The mycelial texture became coarser and fragile in case of *P. florida*, *P. eryngii*, *P. djamor* and *P. cornucopiae* in response to increased concentration of Se (Plate 4.1). The numerous thin and long new hyphae appeared in control treatment as compared to 10 mg L^{-1} and 20 mg L^{-1} Se concentrations in all the four *Pleurotus* spp. namely *P. florida*, *P. eryngii*, *P. djamor* and *P. cornucopiae*. As the concentration of Se increased, the appearance of new hyphae decreased, besides exhibiting increase in thickness of the fungal or hyphal cell wall, and reduction in hyphal length. The number of septa and hyphal branching were increased, while the clamp connections decreased. The hyphal density increased in 10 mg L^{-1} and 20 mg L^{-1} Se concentration as compared to control sample in *P. florida*, *P. eryngii*, *P. djamor* and *P. cornucopiae* (Plate 4.1).

Similar results have been documented by Milovanović *et al* (2014) on Transmission Electron Microscopy analysis of mycelial growth of *P. ostreatus*. The researchers reported that mycelial growth was good in Se-enriched media of 5, 10, and 20 mg L^{-1}, 500 mg L^{-1} concentration greatly inhibited growth, and 1000 mg L^{-1} was the minimum inhibitory concentration. In contrast to hyaline, thin-walled, branched and anastomized hyphae with clamp-connections in the control, at 100 and 500 mg L^{-1} Se concentrations, they were considerably short, frequently septated, branched, with a more dense extracellular matrix and

without clamp connections. Goyal *et al* (2015) performed a Scanning Electron Microscopy (SEM) study on the radial growth of *Ganoderma lucidum* and showed Se concentration dependent gradual decrease in hyphal diameter with smaller internodes. At higher concentration, the mycelial network was immense which might be a phenomenon to overcome stress due to Se. At 10 ppm Se concentration no prominent stress due to Se was noticed and the mycelial culture grew rapidly without any remarkable changes in the structure and morphology.

Energy Dispersive Spectroscopy (EDS) is a useful technique for examination of concentration and distribution of different elements on the surface of samples. Elemental analysis of *Pleurotus* spp. mycelia supplemented with various concentrations of Se using SEM-EDS showed signals characteristic for selenium on the surface of *P. florida*, *P. eryngii*, *P. djamor* and *P. cornucopiae* mycelia, conforming that the selenium was incorporated into the cellwall components of fungal mycelia. The percentage weight and atomic percentage of selenium was highest in 10 mg L^{-1} Se supplementation treatment in all the four *Pleurotus* spp. The Se content in percentage weight and atom percentage increased at 10 mg L^{-1} Se concentration and then decreased at 20 mg L^{-1} Se concentration as compared to control samples which exhibited the lowest in Se concentration. Percentage weight and atom percentage of oxygen in *P. florida* and *P. cornucopiae* increased at 20 mg L^{-1} Se concentrations as compared to control and 10 ppm but it is inversely associated for *P. eryngii* and *P. djamor* showing decrease in the percentage weight and atomic percentage of oxygen at 10 mg L^{-1} and 20 mg L^{-1} Se concentration as compared to control (Table 4.5). The decrease in oxygen concentration may be due to the replacement of oxygen with selenium as both of these elements belong to the same group in the periodic table. Percentage weight of carbon slightly decreased and atomic percentage of carbon slightly increased with increase in Se concentration in case of *P. florida* and in case of *P. eryngii* the percentage weight of carbon is slightly increased and atomic percentage of carbon is slightly decreased (Fig. 4.5, 4.6). In case of *P. cornucopiae* percentage weight of carbon is slightly increased at 10 mg L^{-1} Se concentration and then decreased at 20 mg L^{-1} Se concentration, atomic percentage of carbon is slightly decreased at 10 mg L^{-1} Se concentration and then increased at 20 mg L^{-1} Se concentration (Fig. 4.7). In *P. djamor* percentage weight and atomic percentage of carbon increased with increase in concentration of Se (Fig. 4.8).

Similar results were obtained by Goyal *et al* (2015) in SEM-EDS study of hyphal mass of *Ganoderma lucidum*. It showed difference in the percentage weight and percentage atom C and O_2 composition. The results showed that there is an initial decrease in percentage C till 10 ppm followed by an increasing trend with respect to control with maximum percentage weight carbon @ 15 ppm. In contrast to this % weight oxygen exhibited a decreasing trend. Similar results were obtained in case of percentage atom of carbon and

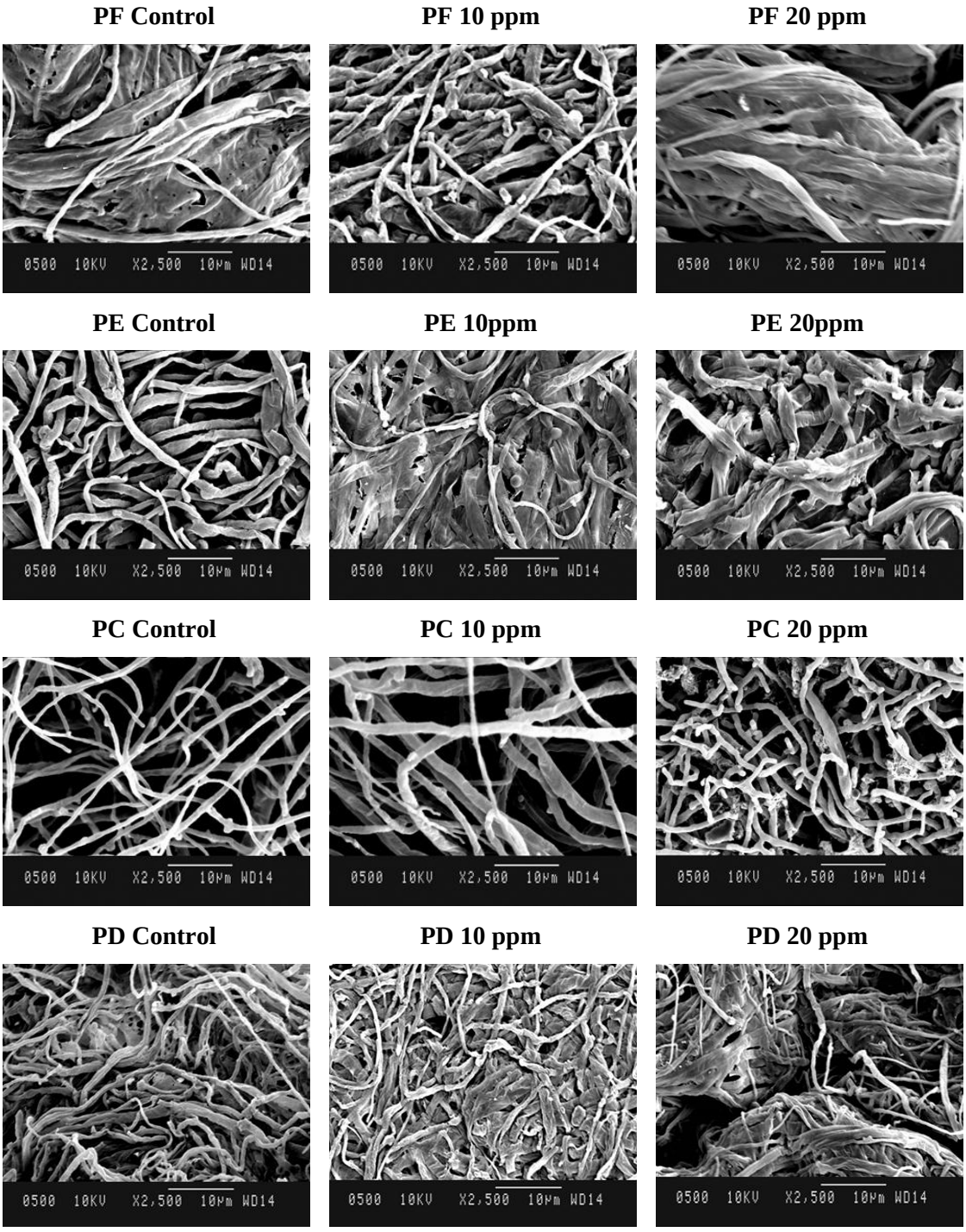


Plate 4.1 Morphological variations in hyphae of *P. florida, P. eryngii, P. cornucopiae* and *P. djamor* at 0, 10 and 20 mg L^{-1} sodium selenate concentration

oxygen. The crystalline structure of the silver nanoparticles (AgNPs) was determined by recording their elemental spectra by an EDX (Energy Dispersive X-ray Spectroscopy). The EDX analysis confirmed the presence of elemental silver. 13 percent of silver besides carbon and oxygen was found to be present in silver nanoparticles at 3 keV. The existence of carbon and oxygen atoms might be due to the presence of other active principles in the liquid extract of *P. ostreatus* (Al-Bahrani *et al* 2017). Our results also confirmed the presence of selenium on the surface of Se-enriched fruit bodies of *Pleurotus* spp. The Energy Dispersive X-ray Spectroscopy analysis of rice roots treated with (As-III) resulted in the loss of several elements like Si, S, K, Cl, Ca, Cu and Fe when compared with untreated root. In case of As-III treated shoot, changes in elemental composition was observed in terms of per cent atomic weight i.e. sodium (0.65-3.52%), potassium (1.17-0.90%), sulphur (0.49-2.52%) and chlorine (1.04-24.75%), when compared with the control shoot. Arsenic controls the concentration of

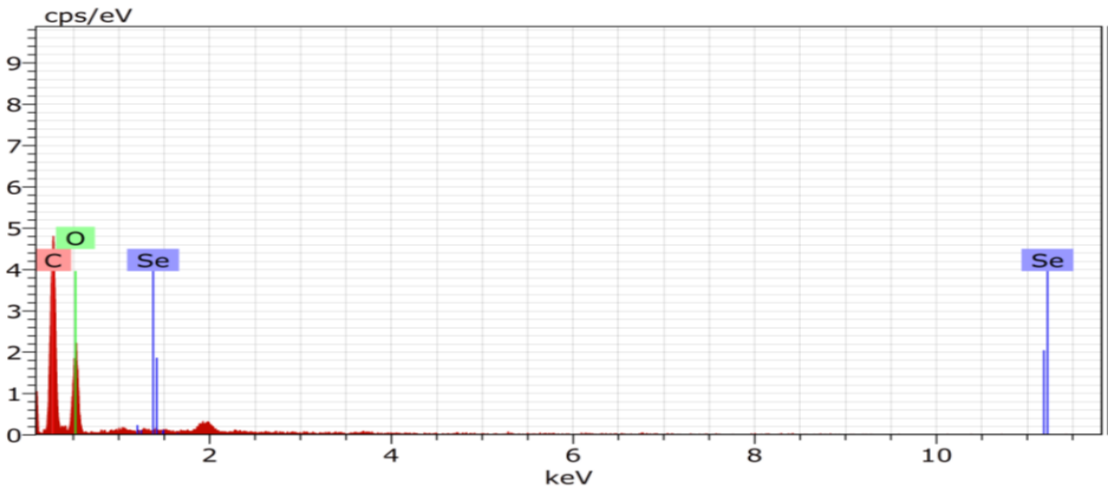
Table 4.5 Scanning Electron Microscopy-Energy Dispersive Spectroscopy (SEM-EDS) analysis of *P. florida, P. eryngii, P. cornucopia* and *P. djamor* at 0, 10 and 20 mg L^{-1} sodium selenate concentration

Species	Sodium selenate concentration in medium (mg L^{-1})	Weight%			Atom%		
		Carbon	Oxygen	Selenium	Carbon	Oxygen	Selenium
P. florida	0 (Control)	57.16	42.67	0.17	64.07	35.90	0.03
	10	56.72	42.57	0.71	63.88	36.00	0.12
	20	55.78	44.02	0.20	62.77	37.19	0.03
P. eryngii	0(Control)	48.95	50.60	0.45	42.04	57.89	0.08
	10	57.46	41.73	0.81	64.63	35.23	0.14
	20	49.33	50.23	0.44	56.63	43.29	0.08
P.cornucopiae	0 (Control)	54.53	45.40	0.07	61.53	38.46	0.01
	10	57.42	35.16	7.41	67.60	31.07	1.33
	20	52.99	46.89	0.12	60.07	39.91	0.02
P. djamor	0 (Control)	50.42	49.58	0.00	57.53	42.47	0.00
	10	54.44	44.77	0.79	61.74	38.12	0.14
	20	55.81	43.90	0.28	62.84	37.11	0.05

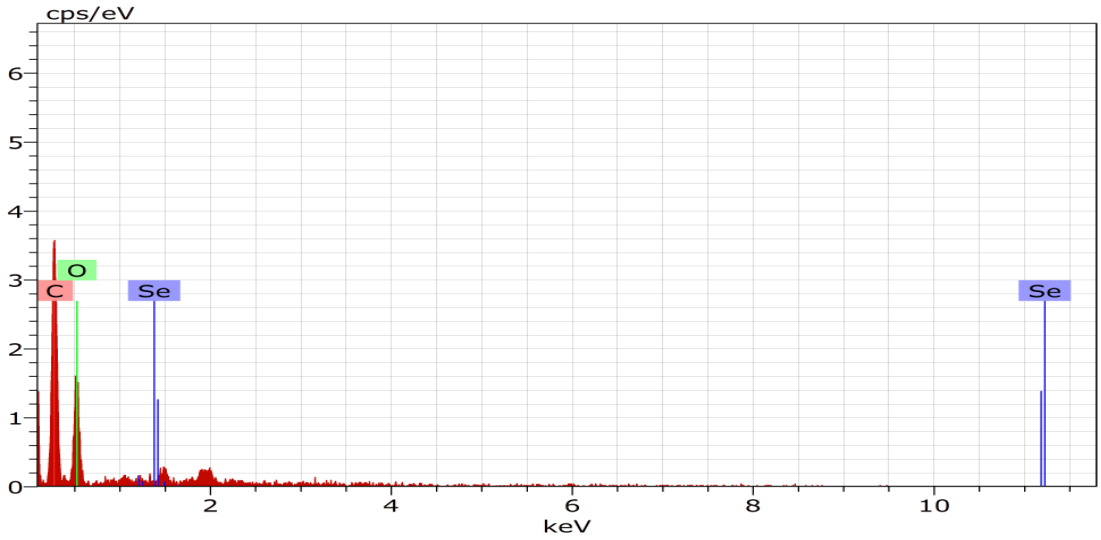
Medium used: Potato Dextrose Agar (PDA)

Incubation temperature -25±1°C

Incubation period-7 days



(a)



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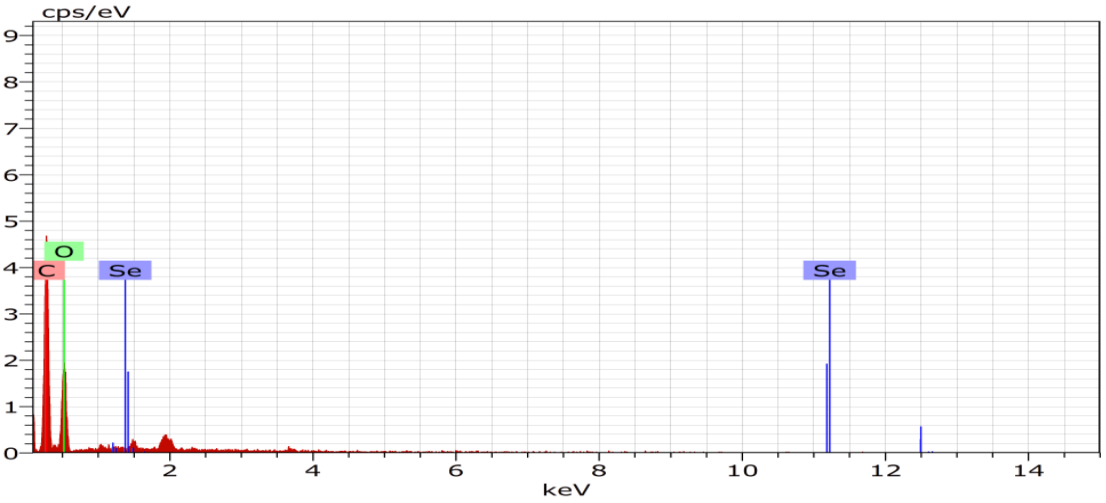
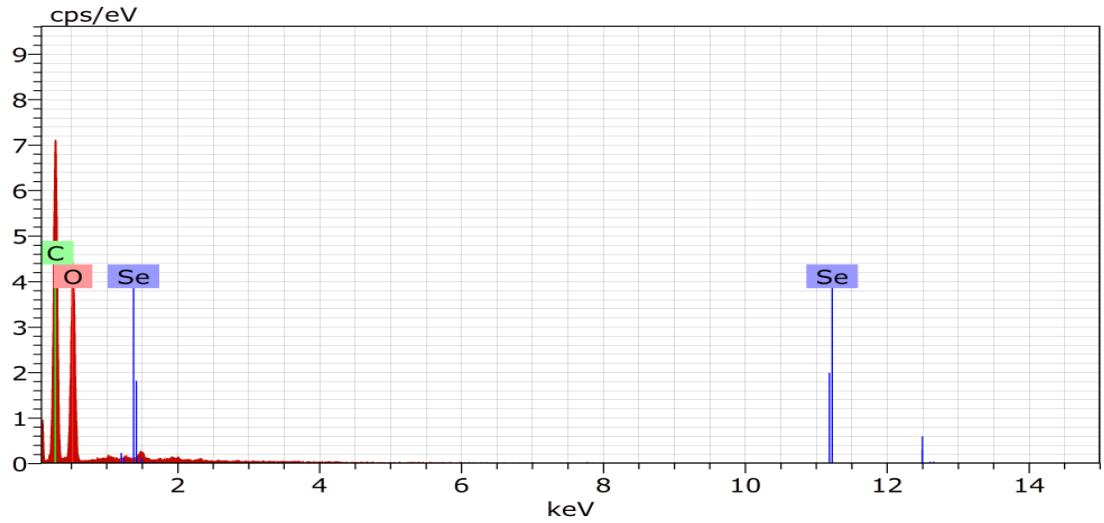
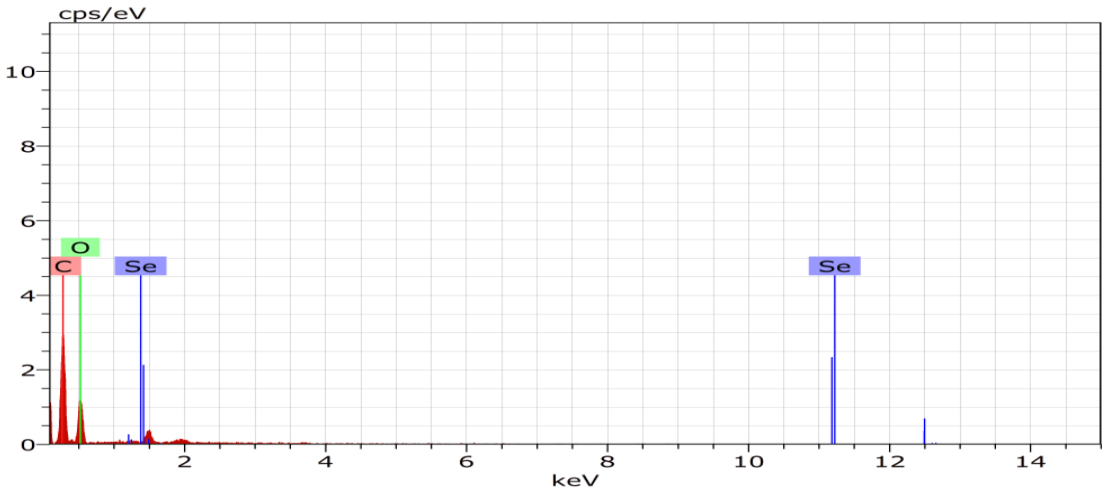


(c)

Fig. 4.5 Energy Dispersive X-ray Spectroscopy signals for *P. florida* at (a) 0, (b) 10 and (c) 20 mg L^{-1} Se concentration



(a)



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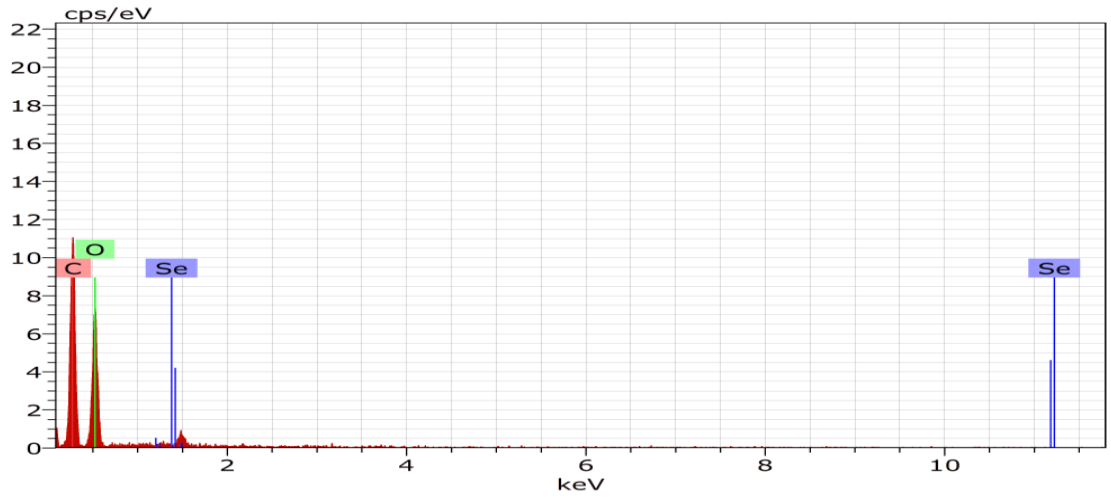
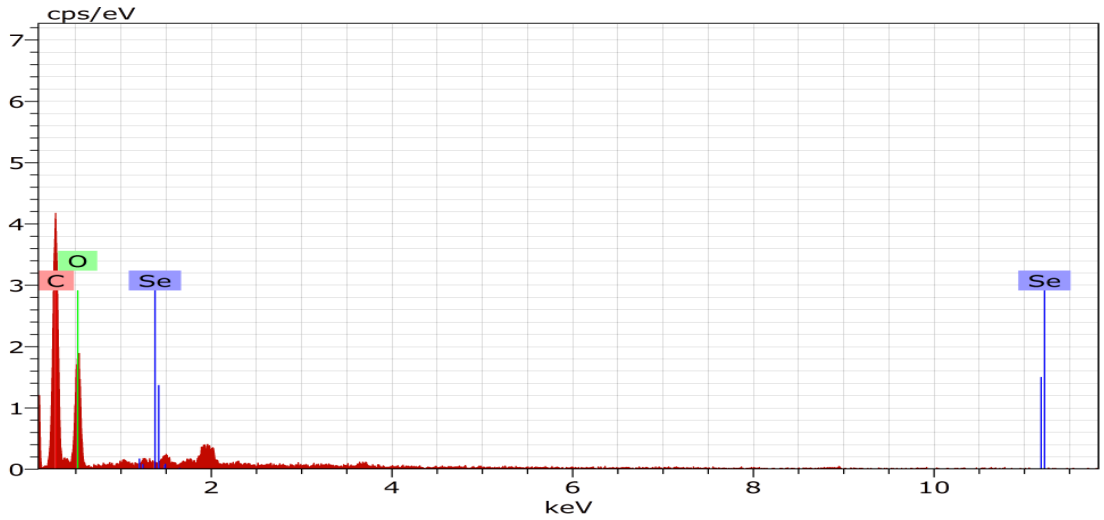
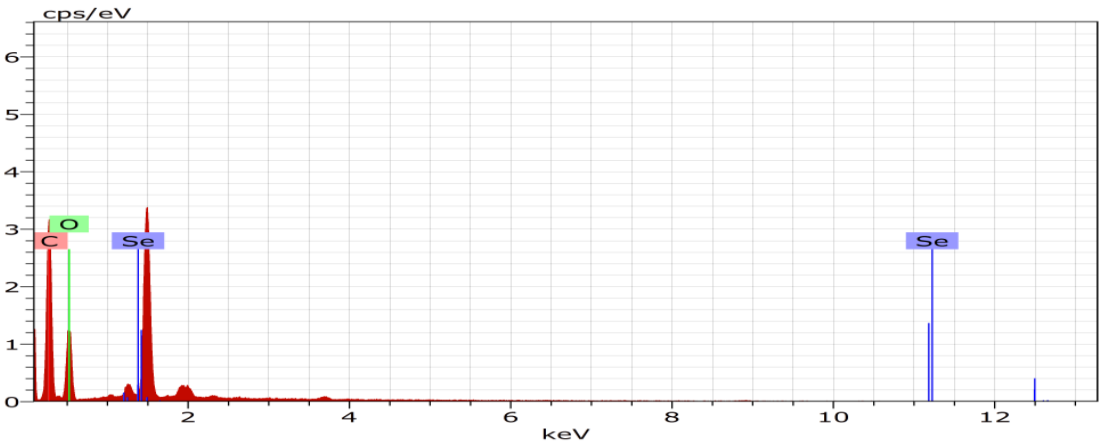


(c)

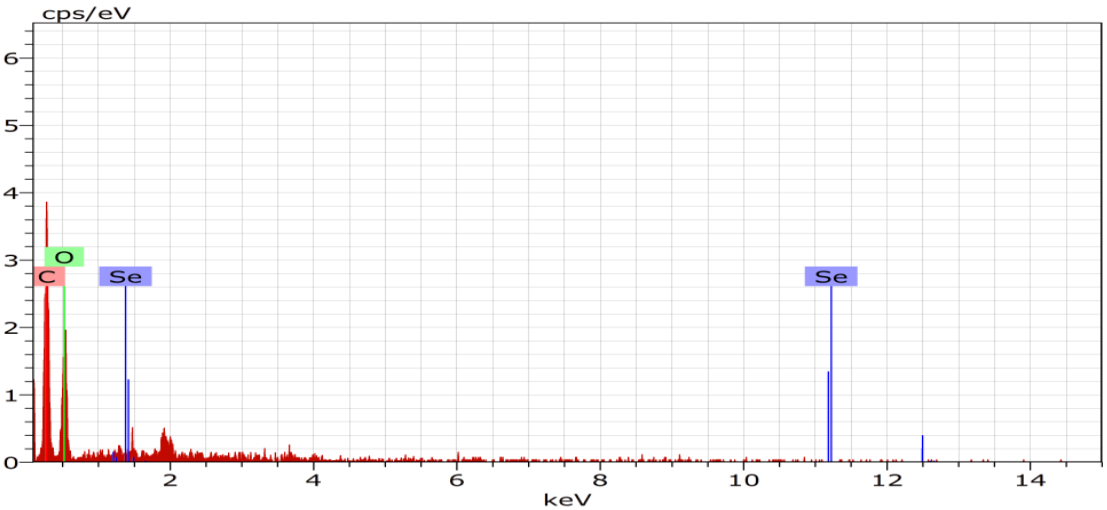
Fig. 4.6 Energy Dispersive X-ray Spectroscopy signals for *P. eryngii* at (a) 0, (b) 10 and (c) 20 mg L^{-1} Se concentration



(a)

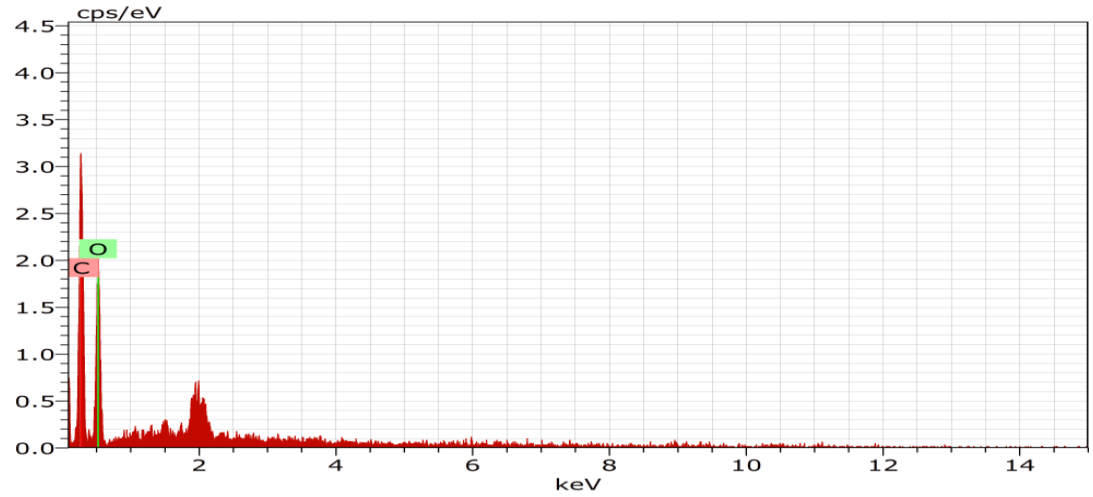


(b)

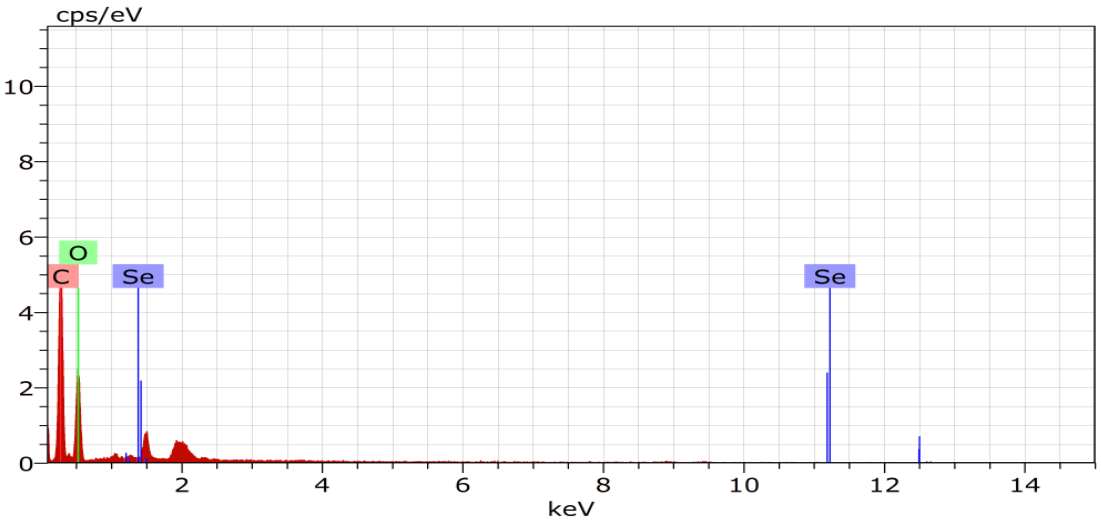


(c)

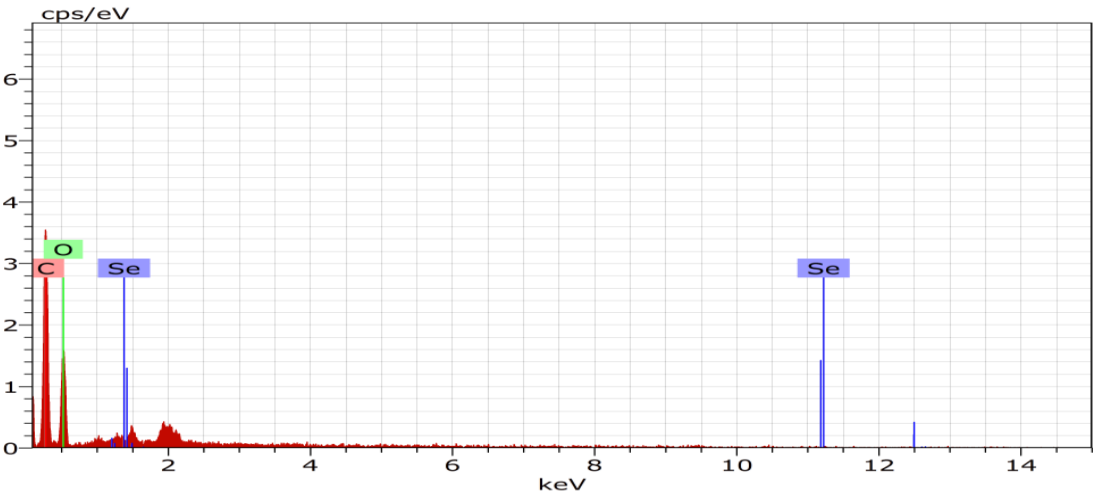
Fig. 4.7 Energy Dispersive X-ray Spectroscopy signals for *P. cornucopiae* at (a) 0, (b) 10 and (c) 20 mg L^{-1} Se concentration



(a)



(b)



(c)

Fig. 4.8 Energy Dispersive X-ray Spectroscopy signals for *P. djamor* at (a) 0, (b) 10 and (c) 20 mg L^{-1} Se concentration

major minerals present in the shoot and root of rice as observed by EDS analysis. Rice tillers treated with 10 μM of Se and sub lethal dose of As-III (60 μM) resulted in good growth response in terms of root (11.4%), shoot length (10.71%), biomass (11.7%), decreased content of malonyldialdehyde (35.14%) and improved the antioxidant activity indicating better arsenic tolerance potential. Besides, a selenium dependent reduction in arsenic accumulation was also observed in rice root (14.24%) and shoot (23.78%) when compared with rice plant treated with arsenic alone. Hence, revealed the capability of selenium in solving the ecotoxicological problems associated with the arsenic buildup in agricultural lands (Singh *et al* 2018). Our EDS results also showed changes in the elemental composition of oxygen, carbon and selenium in terms of percent weight and atom percentage in case of Se-enriched and non-enriched mycelium of *Pleurotus* spp.

4.5 CULTIVATION OF *Pleurotus* spp. ON SELENIUM ENRICHED AND NON ENRICHED WHEAT STRAW

Pleurotus florida, *P. eryngii*, *P. cornucopiae* and *P. djamor* mushrooms were cultivated on selenium enriched and non-enriched wheat straw (Plate 4.2 and 4.3). Observations on days for spawn run, number of fruiting bodies (no./q dry straw), average weight of a fruit body (g) and biological efficiency (%) were made. The Se content of selenium enriched wheat straw collected from seleniferous regions of Punjab were found to be 22.34 μg g^{-1} and in control it was found to be 0.059 μg g^{-1} respectively (Table 4.12). *Pleurotus florida*, *P. eryngii*, *P. cornucopiae* and *P. djamor* showed no significant difference in the days for spawn run, number of fruiting bodies (no./q dry straw), average weight of a fruit body (g) and biological efficiency (%) when cultivated on Se enriched wheat straw. *Pleurotus florida* showed biological efficiency (66.50%) on Se enriched wheat straw in comparison to their respective control (biological efficiency 64.57%). Spawn run was completed in 28 days in Se enriched wheat straw and in 31 days in their respective control sample. There was no significant difference in the number of fruiting bodies (no./q dry straw) in selenium enriched (6800) and non-enriched wheat straw (6712). The average weight of a fruiting body was found to be 9.78 g in Se enriched wheat straw and 9.62 g in control. The biological efficiency (%) of *P. eryngii* was found to be 30.41% on Se- enriched straw and 27.16% on non-enriched straw. Spawn run was completed in 39 days on Se enriched wheat straw while it took 37 days on non-enriched wheat straw. Number of fruiting bodies and average weight of a fruiting body on Se enriched wheat straw (4200 no./q dry straw and average weight of a fruiting body 7.24 g) were observed as compared to control (4000 no./q dry straw and average weight of a fruiting body 6.79 g). There was no significant difference in the biological efficiency (%) of Se-enriched (30.14%) and non-enriched *P. djamor* (29.16%). The spawn run took same days for both Se-enriched and control wheat straw (44 days). No significant difference was observed in the average weight of fruit bodies on

selenium enriched (3.50 g) and non-enriched wheat straw (3.43 g). The number of fruiting bodies (no./q dry straw) was 8612 in Se enriched wheat straw and 8500 in control. On Se-enriched wheat straw, *P. cornucopiae* showed 36.74% of biological efficiency as compared to control (33.11%; Fig. 4.9). The number of fruiting bodies (no./q dry straw) was 6631 in Se enriched wheat straw and 6544 in control. The average weight of fruit body was 5.54 g on Se-enriched wheat straw and 5.06 g on non-enriched straw (Table 4.6).

Mushrooms can accumulate Se in their fruiting bodies when cultivated on Se-enriched substrates or supplemented with organic or inorganic salts of Se. Similar results were obtained by cultivating five mushroom species belonging to different genera namely *P. sajor-caju*, *P. citrinopileatus*, *P. ostreatus*, *A. bisporus* and *V. volvaceae* on post agricultural residues belonging to both seleniferous and non-seleniferous (control) sites (Solovyev *et al* 2018). The average yield of all mushrooms belonging to both Se-rich and non-Se (control) on their respective substrates was recorded after a cycle of 3 flushes and observed that Se accumulation did not induce any significant difference on total yield and biological efficiency

Table 4.6 Yield potential of selenium enriched and non-enriched *Pleurotus* spp.

***Pleurotus* spp.**	**Treatment**	**Spawn Run (d)**	**NFB (no./q dry straw)**	**Av.wt. of a FB (g)**	**Biological efficiency (%)**	**Disease/Pest**
P. florida	Control	31	6712	9.62	64.57	-
	Se straw	28	6800	9.78	66.50	
CD (5%)	-	NS	NS	NS	NS	
P. eryngii	Control	37	4000	6.79	27.16	-
	Se straw	39	4200	7.24	30.41	
CD (5%)	-	NS	NS	NS	NS	
P. djamor	Control	44	8500	3.43	29.16	-
	Se straw	44	8612	3.5	30.14	
CD (5%)	-	NS	NS	NS	NS	
P. cornucopiae	Control	29	6544	5.06	33.11	-
	Se straw	32	6631	5.54	36.74	
CD (5%)	-	NS	NS	NS	NS	

No. of replicates=3

Days of spawn run=28-44

Temperature during the crop = 25±10ºC

Relative humidity during the crop = 80-85%

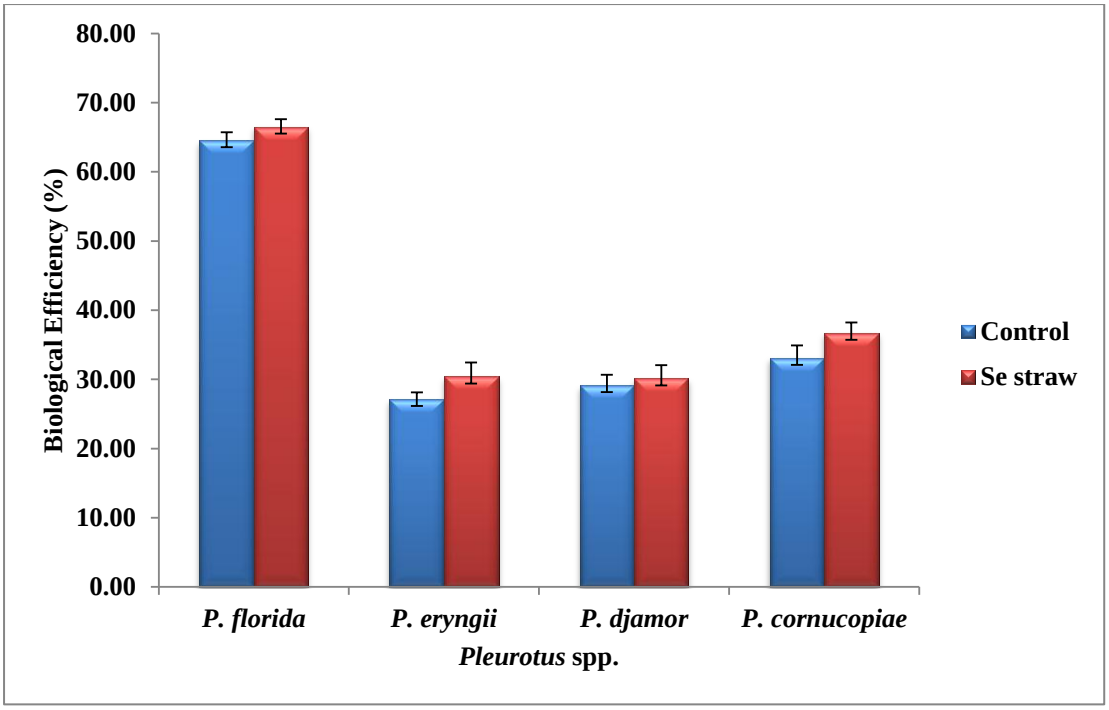


Fig. 4.9 Yield potential of selenium enriched and non-enriched *Pleurotus* spp.

of mushrooms over non-Se (control). Bhatia *et al* (2013) cultivated *Pleurotus* mushrooms on Se-hyperaccumulated wheat straw and non-accumulated (control) wheat straw. They also reported that mushrooms cultivated on naturally Se-enriched substrates did not differ from controls in terms of yield, biological efficiency, and production length. Ogidi *et al* (2017) displayed biological efficiency percentage (62.8) of *P. pulmonarius* (Pindo) from the Se-enriched substrate was found to be similar with its control i.e. without Se (70.6). This revealed that fungi possess some mechanism for metabolizing Se present in the straw. Zou *et al* (2018) reported that *P. tuoliensis* promoted fruiting bodies of good quality and had a low malformation rate at 10 mg kg^{-1} of Se concentration.

4.6 ANALYSIS OF SELENIUM BIOFORTIFIED *Pleurotus spp.* FRUIT BODIES

4.6.1 Total soluble protein content

The total soluble protein content was estimated from the fruit bodies of *Pleurotus* spp. grown on selenium enriched substrate. Among the control fruit bodies of *Pleurotus* spp., the total soluble protein content was found to be highest in *P. djamor* (3.60±0.24 mg g^{-1}) followed by *P. eryngii* (1.60±0.24 mg g^{-1}), *P. florida* (1.41±0.09 mg g^{-1}) and *P. cornucopiae* (1.38±0.11 mg g^{-1}). In case of selenium biofortified fruiting bodies of *Pleurotus* spp, *P. djamor* (4.56±0.31 mg g^{-1}) showed the most significant difference between the selenium biofortified and control followed by *P. eryngii* (2.55±0.24 mg g^{-1}), *P. cornucopiae* (2.16±0.15 mg g^{-1}) and *P. florida* (1.98±0.12 mg g^{-1}) (Fig. 4.10). There was a significant difference in the total protein content of selenium enriched and non-enriched fruit bodies of all the *Pleurotus* spp. except *P. djamor* (Table 4.7).

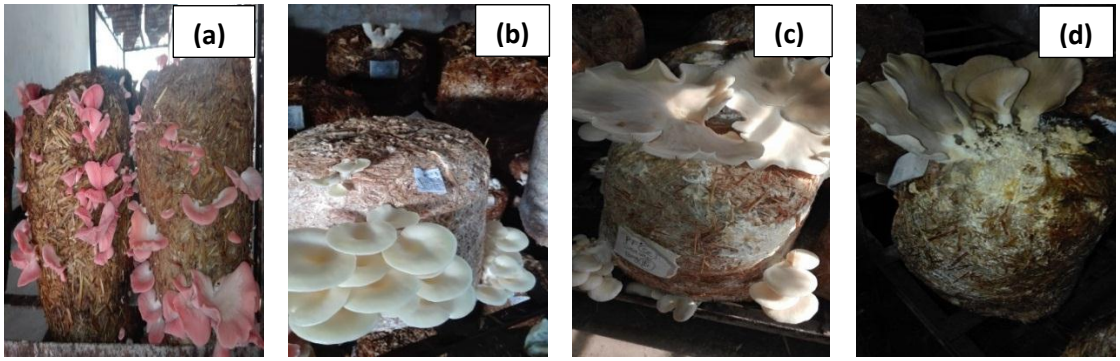


Plate 4.2 Fruiting bodies of different *Pleurotus* spp. cultivated on selenium rich wheat straw: (a) *P. djamor,* (b) *P. cornucopiae,* (c) *P. florida* and (d) *P. eryngii*

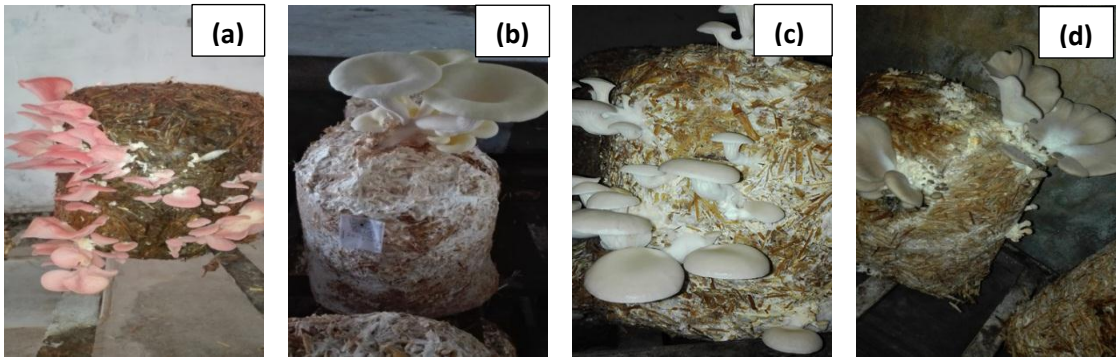


Plate 4.3 Fruiting bodies of different *Pleurotus* spp. cultivated on non-enriched wheat straw: (a) *P. djamor,* (b) *P. cornucopiae,* (c) *P. florida* and (d) *P. eryngii*

Table 4.7 Effect of Se enrichment on the total soluble protein content of *Pleurotus* spp. fruit bodies

Treatment	Total soluble protein content (mg g^{-1})			
	P. florida	***P. eryngii***	***P. djamor***	***P. cornucopiae***
Se straw	1.98±0.12	2.55±0.24	4.56±0.31	2.16±0.15
Control	1.41±0.09	1.60±0.24	3.60±0.24	1.38±0.11
CD 5 %	0.42	0.92	NS	0.51

No. of replicates-3

Temperature during the crop = 25±10°C

± values indicated standard error

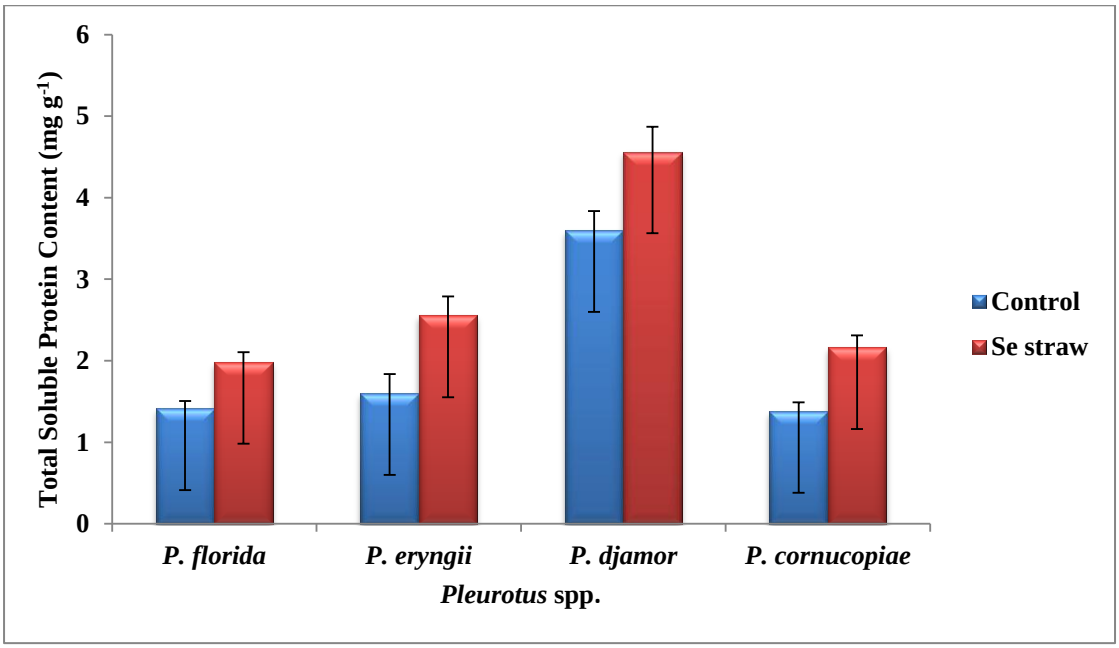


Fig. 4.10 Effect of Se enrichment on the total soluble protein content of *Pleurotus* spp. fruit bodies

Bhatia *et al* (2014) reported that the total protein content in methanolic extracts of Se enriched mushrooms was found to be significantly higher than that of control mushrooms ((307±4.5 vs 282±2.4 mg g^{-1}dw respectively). Crude protein content of the Se-enriched samples of Calocybe indica were found to be significantly higher than that of non-enriched fruit bodies (25.31 and 14.33 g/100 g on dry weight basis respectively; Rathore *et al* 2018). The total soluble protein content of the *Pleurotus* spp. fruit bodies increased with respect to selenium and *P. florida* showed the most significant difference between the selenium-enriched (2.492 mg g^{-1}) and control samples (1.644 mg g^{-1}) as reported by Kaur *et al* (2017). Higher protein contents of 16.35% and 18.21 % were found in *P. pulmonarius* and *P. ostreatus* fortified with selenium as compared to the fruiting bodies of control (Fasoranti *et al* 2019). Mushrooms possess large amount of high quality protein involved in the accumulation

of large amount of Se which in turn is involved in the biosynthesis of selenoenzymes and selenoproteins such as thioredoxin reductases, glutathione peroxidases, iodothyronine 5′-deiodases, selenoprotein W and selenoprotein P (Zięba *et al* 2020).

4.6.2 Total phenolic content

The total phenol content of methanolic extracts from dried fruiting bodies of selenium enriched and non-enriched samples of *P. florida*, *P. eryngii*, *P. djamor* and *P. cornucopiae* were determined by following the protocol given by Swain and Hillis (1959). Among the various Se-biofortied *Pleurotus* mushrooms, the phenolic content was highest in *P. djamor* (4.08±0.16 mg GAE/g dw) followed by *P. florida* (2.44±0.16 GAE/g dw), *P. cornucopiae* (2.00±0.15 GAE/g dw) and *P. eryngii* (1.28±0.10 GAE/g dw). Selenium enriched *Pleurotus* spp. showed an increase in the total phenolic content as compared to their non-enriched counterparts with *P. djamor* showing the most significant difference (1.16±0.10 GAE/g dw) followed by *P. florida* (0.42±0.06 GAE/g dw), *P. cornucopiae* (0.96±0.08 GAE/g dw) and *P. eryngii* (0.66±0.12 GAE/g dw; Table 4.8). There was a significant difference in the total phenolic content of selenium enriched and non-enriched fruiting bodies of all the four *Pleurotus* spp (Fig. 4.11). The phenolic compounds have been considered as a major antioxidants present in the mushrooms. Reis *et al* (2012) determined the total phenolic content in methanolic extracts from fruiting bodies (7.14 ± 2.01 mg GAE g^{-1}) in addition to mycelium (9.11 ± 0.23 mg GAE g^{-1}). Rathore *et al* (2018) analyzed the Se enriched *Calocybe indica* fruit bodies for the total phenolic content and found that the Se at the concentration of 5 mg mL^{-1} increased the total phenolic content (25.29 mg GAE g^{-1}) whereas, it decreased significantly with higher concentrations of Se in the substrate. Bhatia *et al* (2014) reported that Se-rich methanolic extracts of fruit bodies of both *P. sajar-caju* and *V. volvacea* showed significantly higher total phenolic content when compared with their respective non-enriched mushrooms. The amount of total phenolic content in the methanol extracts of Se-enriched

Table 4.8 Effect of Se enrichment on the total phenolic content of *Pleurotus* spp. fruit bodies

Treatment	Phenolic content (mg GAE/g)			
	P. florida	***P. eryngii***	***P. djamor***	***P. cornucopiae***
Se straw	2.44±0.16	1.28±0.10	4.08±0.16	2.00±0.15
Control	0.42±0.06	0.66±0.12	1.16±0.10	0.96±0.08
CD 5 %	0.48	0.42	0.53	0.47

No. of replicates-3

Temperature during the crop = 25±10ºC

± values indicated standard error

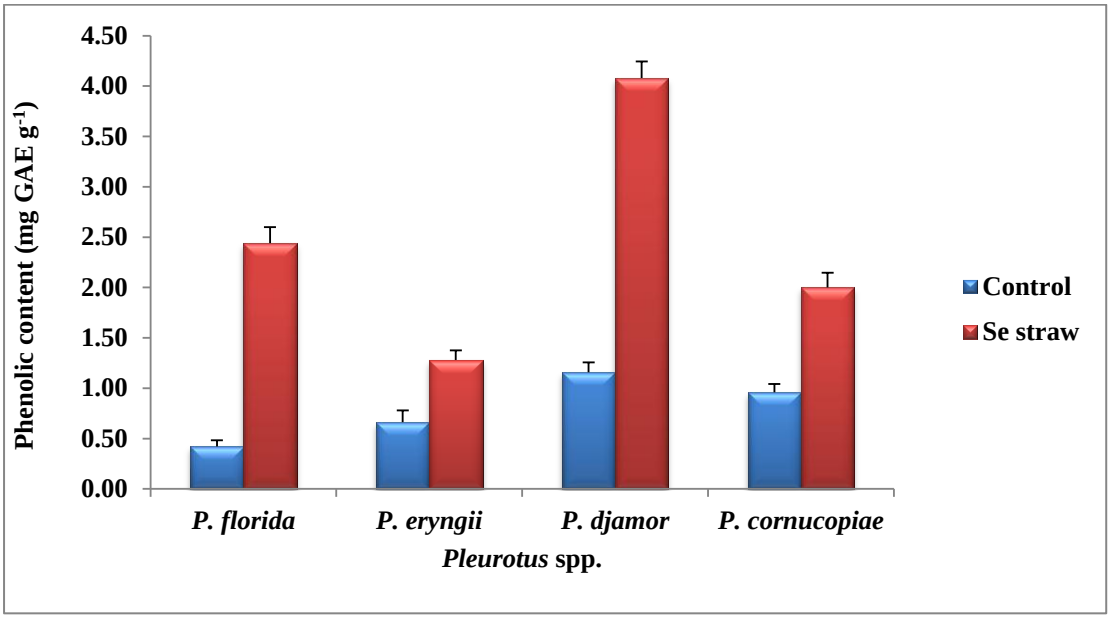


Fig. 4.11 Effect of Se enrichment on the total phenolic content of *Pleurotus* spp. fruit bodies

PSC (8.39 ± 0.6 mg GA g^{-1} dw) was significantly higher than the control (6.37± 0.2 mg GA g^{-1} dw) as observed in our results. Zięba *et al* (2020) reported that the total phenolics in the fruiting bodies of *P. eryngii* supplemented with selenite (226.44 ± 25.03 mg 100 g^{-1} dw), zinc sulfate (263.04 ±10.29 mg 100 g^{-1} dw), zinc hydroaspartate (183.72 ± 14.04 mg 100 g^{-1} dw) and control (212.76 ± 14.04 mg 100 g^{-1} dw). This difference may be due to growing conditions, type of medium used and type of extraction solvents used.

4.6.3 Free radical scavenging activity (%)

The hydrogen atom or electron donating ability of methanolic extracts of Se biofortified mushrooms and control mushrooms was measured from the bleaching of the purple coloured DPPH methanol solution. Among the various control fruiting bodies of *Pleurotus* spp., the % radical scavenging activity (expressed as 0.1 mL methanolic extract $0.5g^{-1}$ dw) was found to be highest in *P. florida* (8.98±0.24 %), followed by *P. cornucopiae* (7.56±0.20 %), *P. djamor* (7.53±0.19%) and *P. eryngii* (2.01±0.12%). Supplementation of selenium was found to have positive effect in case of all the four *Pleurotus* spp. (Table 4.9). The % radical scavenging activity among the Se enriched fruiting bodies was found to be highest in *P. cornucopiae* (10.72±0.26%) followed by *P. florida* (10.54±0.45%), *P. djamor* (9.16±0.28%), and *P. eryngii* (4.40±0.20%; Fig. 4.12). There was a significant difference in the radical scavenging activity (percentage) of selenium enriched and non-enriched fruiting bodies of all the four *Pleurotus* spp. Zhao *et al* (2006) reported that protein extracts from Se-enriched *Ganoderma lucidum* exhibited strong DNA protective effects from oxidative damage which increased with increase in Se content as observed in chemiluminescence

analysis. This indicated that Se indirectly played an important role in increasing the antioxidant activity of protein extracts. Our results were similar to the findings of Bhatia *et al* (2014) in which they reported that Se-enriched methanol (20%) extracts showed significantly higher scavenging activity (33.6±1%) than non enriched extracts (21.5±0.4%) in *P. fossulatus*. Rathore *et al* (2018) reported that the DPPH radical scavenging activity of the *Calocybe indica* extracts were found to be increased in response to increased Se concentration. This increase showed significantly higher scavenging effects when compared with the control. Hu *et al* (2019) reported that the scavenged DPPH radicals of both water and ethanol extract (12.5-100 mg mL^{-1}) from Se-biofortified *Cordyceps militaris* were significantly higher than that of control. The ability of methanol extracts of mushrooms to reduce DPPH radicals depends on the concentration of sample as reported by Miletić *et al* (2019). It was found that the *Coriolus versicolor* mycelial extract enriched with sodium selenite to be the most efficient with scavenging ability of 99.00% followed by mycelial extract enriched with selenourea (95.3%) and control (93.37%).

Table 4.9 Effect of Se enrichment on the Radical scavenging activity (%) of *Pleurotus* spp. fruit bodies

Treatment	Radical scavenging activity (%)			
	P. florida	***P. eryngii***	***P. djamor***	***P. cornucopiae***
Se straw	10.54±0.45	4.40±0.20	9.16±0.28	10.72±0.26
Control	8.98±0.24	2.01±0.12	7.53±0.19	7.56±0.20
CD 5 %	1.41	0.64	0.93	0.92

No. of replicates-3

Temperature during the crop = 25±10ºC

± values indicated standard error

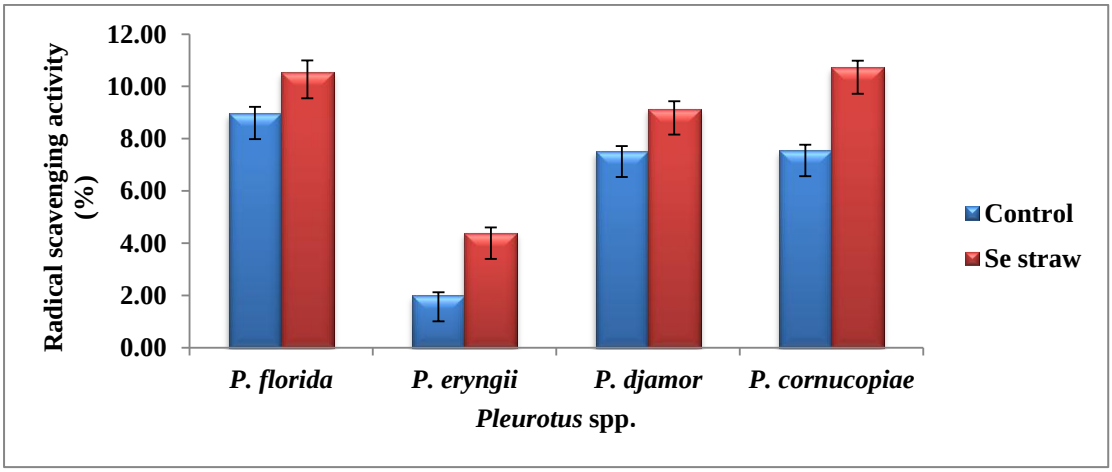


Fig. 4.12 Effect of Se enrichment on the Radical scavenging activity (%) of *Pleurotus* spp. fruit bodies

4.6.4 Total flavonoids

The flavonoid content of distilled water extracts from dried selenium enriched and control fruit bodies of various *Pleurotus* spp. was measured spectrophotometrically and expressed as Quercetin Equivalents per g of dried sample. Among the control fruit bodies, highest flavonoid content was found in *P. cornucopiae* (275±14.74 µg QE g^{-1}) followed by *P. eryngii* (250±20.60 µg QE g^{-1}), *P. florida* (225±10.15 µg QE g^{-1}) and *P. djamor* (50±1.53 µg QE g^{-1}). Garlic, which is considered as the richest source of flavonoids was found to contain 470 µg QE g^{-1} which were found to be quite close to the flavonoid content of mushroom samples as reported by Chun *et al* (2005). Biofortification of *Pleurotus* spp. with Se positively affected the total flavonoid content of *P. djamor*, *P. cornucopiae*, *P. eryngii* and *P. florida* (Table 4.10). The flavonoid content of Se enriched fruit bodies was found to be highest in case of *P. florida* (512.5 ±10.03 µg QE g^{-1}), followed by *P. cornucopiae* (500.0±19.55 µg QE g^{-1}), *P. eryngii* (425.0±16.17 µg QE g^{-1}) and *P. djamor* (250.0±13.75 µg QE g^{-1}; Fig. 4.13). There was a significant difference in the flavonoid content of selenium enriched and non-enriched fruiting bodies of all the four *Pleurotus* spp.

Our results are in agreement with the findings of Milovanović *et al* (2014) who studied the *P. pulmonarius* for total flavonoid content and found that Se enriched medium contains two folds higher flavonoid content than that of non-enriched medium (5.4 and 2.3 µg QE mg^{-1} of dried extract). They also observed that there was no significant difference in the flavonoid content of Se-amended and non-amended extracts of *P. eryngii* (2.9 and 2.8 µg QE mg^{-1} of dried extract, respectively). Gąsecka *et al* (2016) reported that the total flavonoid content for *Hericium erinaceus*, *Ganoderma 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 have superior antioxidant properties after Se addition because the scavenging ability on 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals was improved.

Table 4.10 Effect of Se enrichment on the total flavonoid content of *Pleurotus* spp. fruit bodies

Treatment	Flavonoid content (µg QE/g)			
	P. florida	***P. eryngii***	***P. djamor***	***P. cornucopiae***
Se straw	512.5±10.03	425.0±16.17	250.0±13.75	500.0±19.55
Control	225.0±10.15	250.0±20.60	50.0±1.53	275.0±14.74
CD 5 %	39.62	72.71	38.41	68.00

No. of replicates-3

Temperature during the crop = 25±10ºC

± values indicated standard error

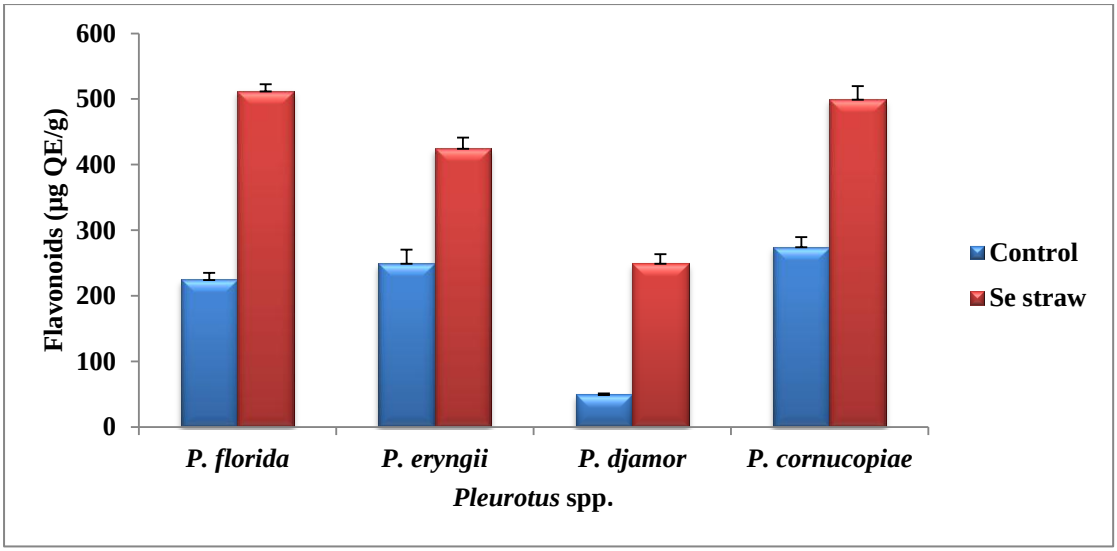


Fig. 4.13 Effect of Se enrichment on the total flavonoid content of *Pleurotus* spp. fruit bodies

4.7 ESTIMATION OF SELENIUM CONTENT IN SELENIUM BIOFORTIFIED *Pleurotus* spp. FRUITING BODIES BY ENERGY DISPERSIVE SPECTROSCOPY (EDS)

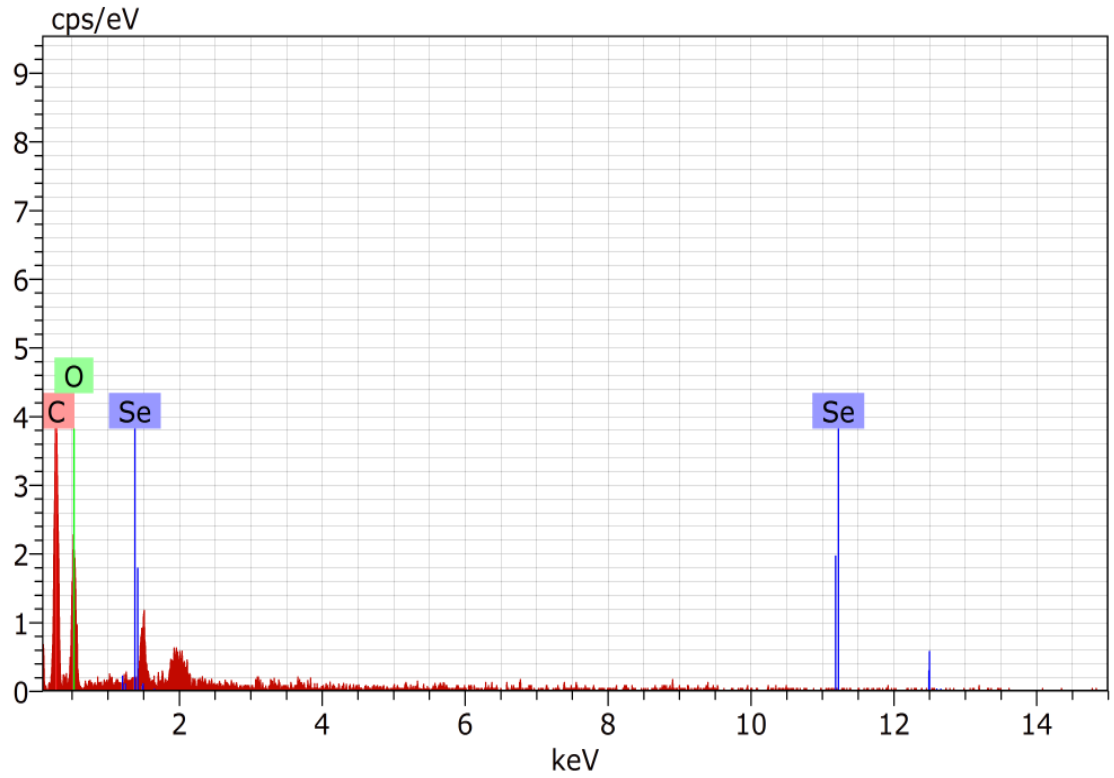
Energy Dispersive Spectroscopy (EDS) is a useful technique for examination of concentration and distribution of different elements on the surface of samples and to reveal the presence of seleno-proteins or organic selenium on the surface of fruiting bodies of mushrooms. Elemental analysis of Se biofortified *Pleurotus* mushrooms using SEM-EDS showed signals characteristic for selenium on the surface of *P. florida* and *P. djamor* conforming that the selenium was incorporated into the cell wall of these mushroom fruiting bodies. On the contrary, no selenium was found on the surface of fruiting bodies of *P. eryngii* and *P. cornucopiae* indicating that the selenoproteins and other forms of selenium to be cytosolic moieties. The percentage weight and atom percentage of selenium was high in Se biofortified mushrooms of *P. florida* (0.72 and 0.12 respectively) and *P. djamor* (2.85 and 0.49 respectively) as compared to control mushrooms (0.22, 0.04 (*P. florida*) and 0.83, 0.14 (*P. djamor*) respectively; Table 4.11). Percentage weight and atom percentage of oxygen decreased for Se biofortified *P. florida* and *P. cornucopiae* mushrooms as compared to their control whereas it was increased in the Se biofortified fruiting bodies of *P. eryngii* and *P. djamor* mushrooms. The decrease in oxygen concentration may be due to the replacement of oxygen with selenium as both these elements belong to the same group in the periodic table. Percentage weight and atomic percentage of carbon is increased in case of Se biofortified fruiting bodies of *P. florida* and *P. cornucopiae* mushrooms as compared to their respective control samples, whereas it is decreased in case of Se biofortified fruiting bodies of *P. eryngii* and *P. djamor* mushrooms in comparison to their respective control samples (Fig. 4.14, 4.15,

4.16 and 4.17).

Similar results were obtained by Goyal *et al* (2015) in SEM-EDS study of hyphal mass of *Ganoderma lucidum*. It showed difference in the percentage weight and percentage atom C and O_2 composition. The results showed that there is an initial decrease in percentage C till 10 ppm followed by an increasing trend with respect to control with maximum percentage weight carbon @ 15 ppm. In contrast to this % weight oxygen exhibited a decreasing trend. Similar results were obtained in case of percentage atom of carbon and oxygen. The crystalline structure of the silver nanoparticles (AgNPs) was determined by recording their elemental spectra by an EDX (Energy Dispersive X-ray Spectroscopy). The EDX analysis confirmed the presence of elemental silver. 13 percent of silver besides carbon and oxygen was found to be present in silver nanoparticles at 3 keV. The existence of carbon and oxygen atoms might be due to the presence of other active principles in the liquid extract of *P. ostreatus* (Al-Bahrani *et al* 2017). Our results also confirmed the presence of selenium on the surface of Se-enriched fruit bodies of *Pleurotus* spp. The Energy Dispersive X-ray Spectroscopy analysis of rice roots treated with (As-III) resulted in the loss of several elements like Si, S, K, Cl, Ca, Cu and Fe when compared with untreated root. In case of As-III treated shoot, changes in elemental composition was observed in terms of per cent atomic weight i.e. sodium (0.65-3.52%), potassium (1.17-0.90%), sulphur (0.49-2.52%) and chlorine (1.04-24.75%), when compared with the control shoot. Arsenic controls the concentration of major minerals present in the shoot and root of rice as observed by EDS analysis. Rice tillers treated with 10 μM of Se and sub lethal dose of As-III (60 μM) resulted in good growth response in terms of root (11.4%), shoot length (10.71%), biomass (11.7%), decreased content of malonyldialdehyde (35.14%) and improved the antioxidant activity indicating

Table 4.11 SEM-EDS Analysis of Se enriched and non-enriched fruiting bodies of *P. florida, P. eryngii, P. cornucopiae* and *P. djamor*

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



(a)

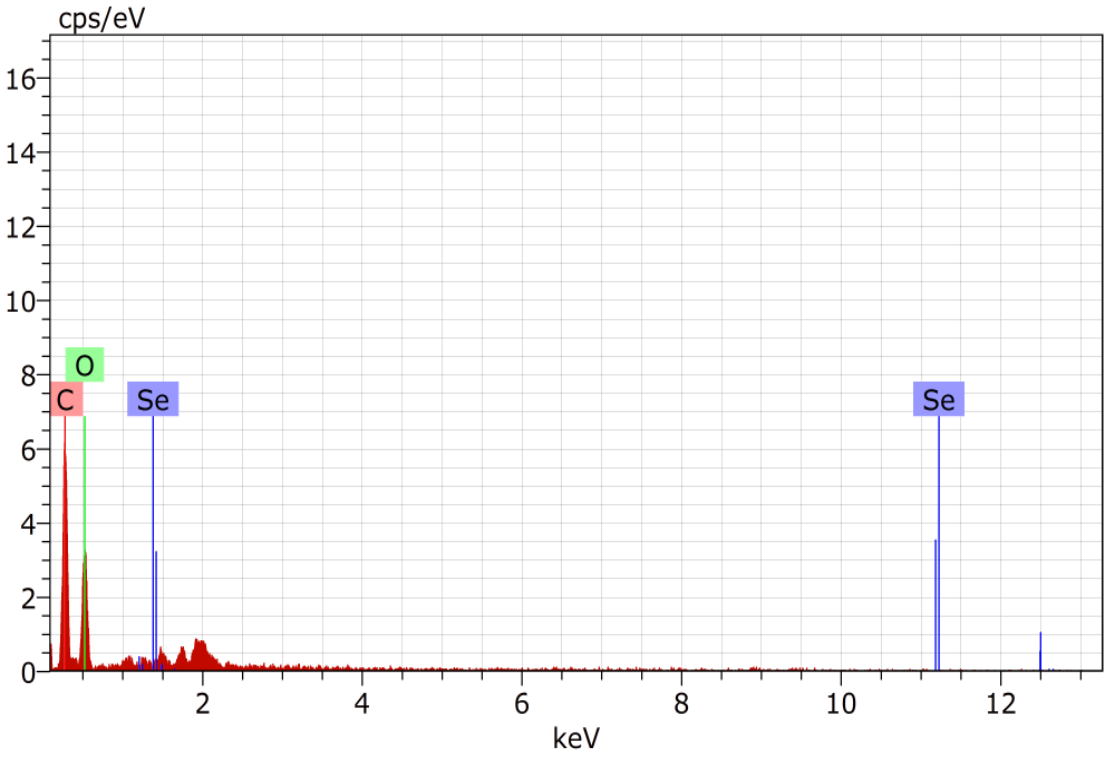


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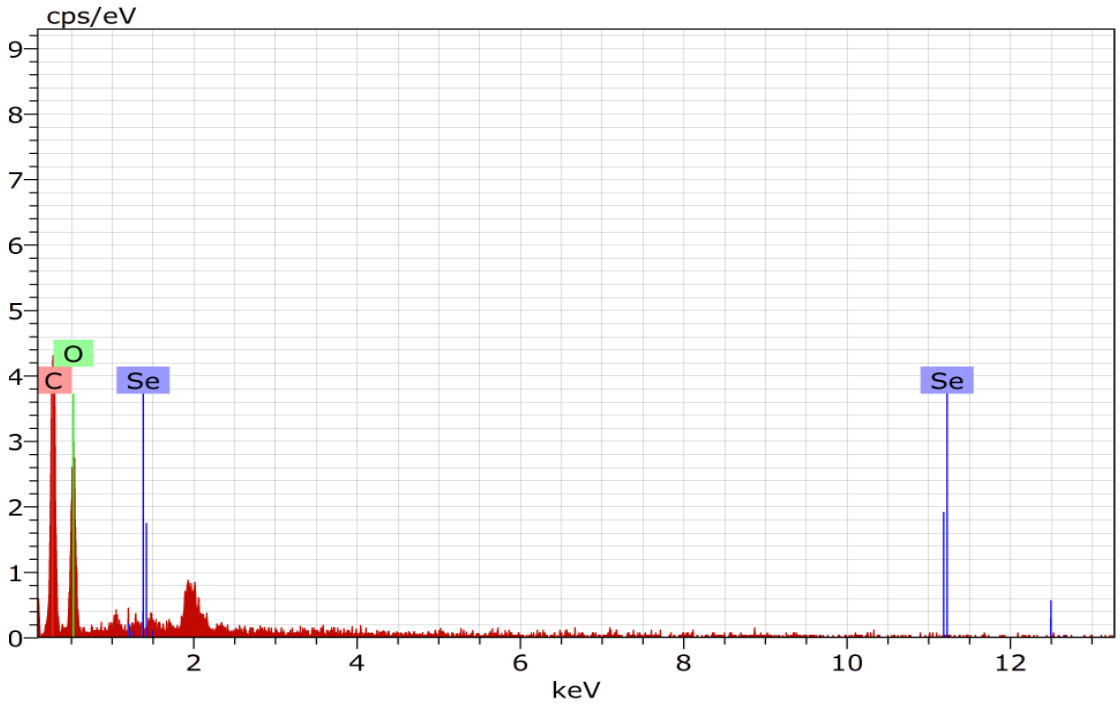
Fig. 4.14 Energy Dispersive X-ray Spectroscopy signals for (a) Se enriched fruiting bodies of *P. florida* (b) Non enriched fruiting bodies of *P. florida*



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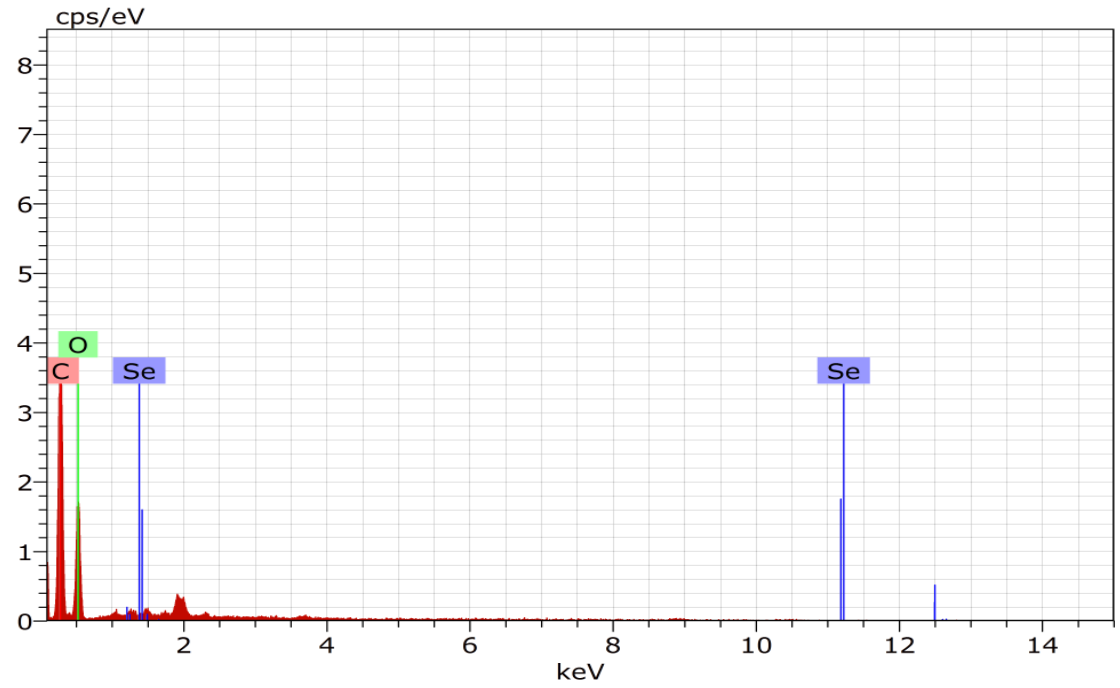
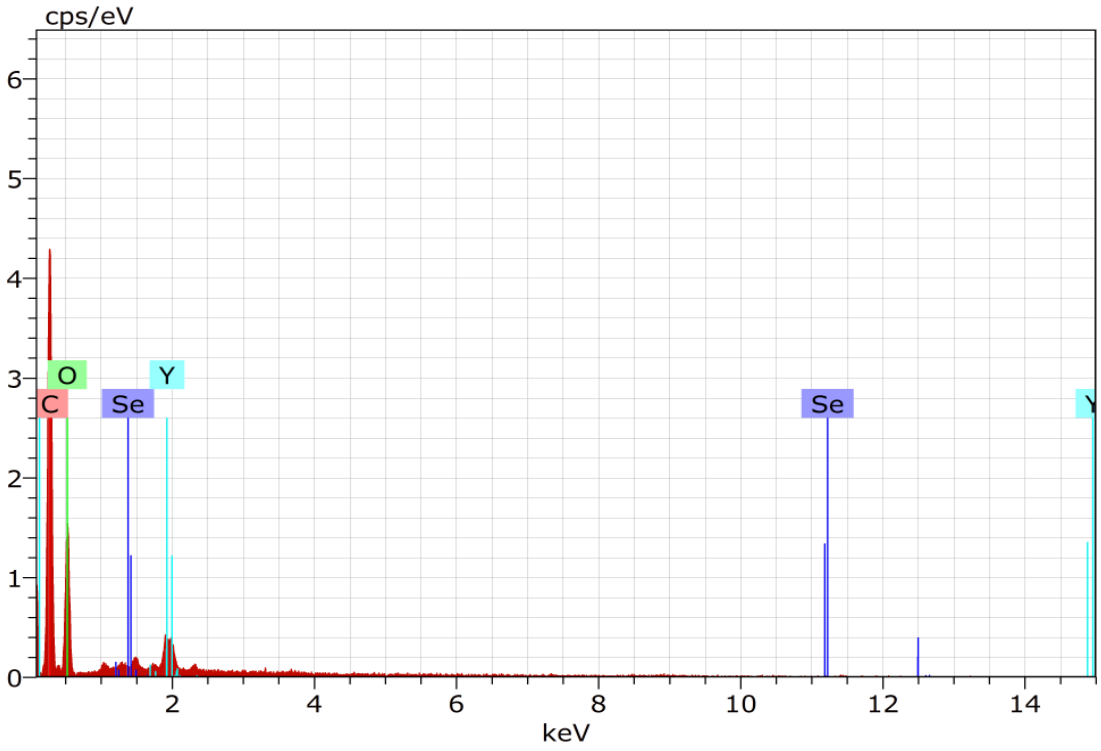


(b)

Fig. 4.15 Energy Dispersive X-ray Spectroscopy signals for (a) Se enriched fruiting bodies of *P. eryngii* (b) Non enriched fruiting bodies of *P. eryngii*



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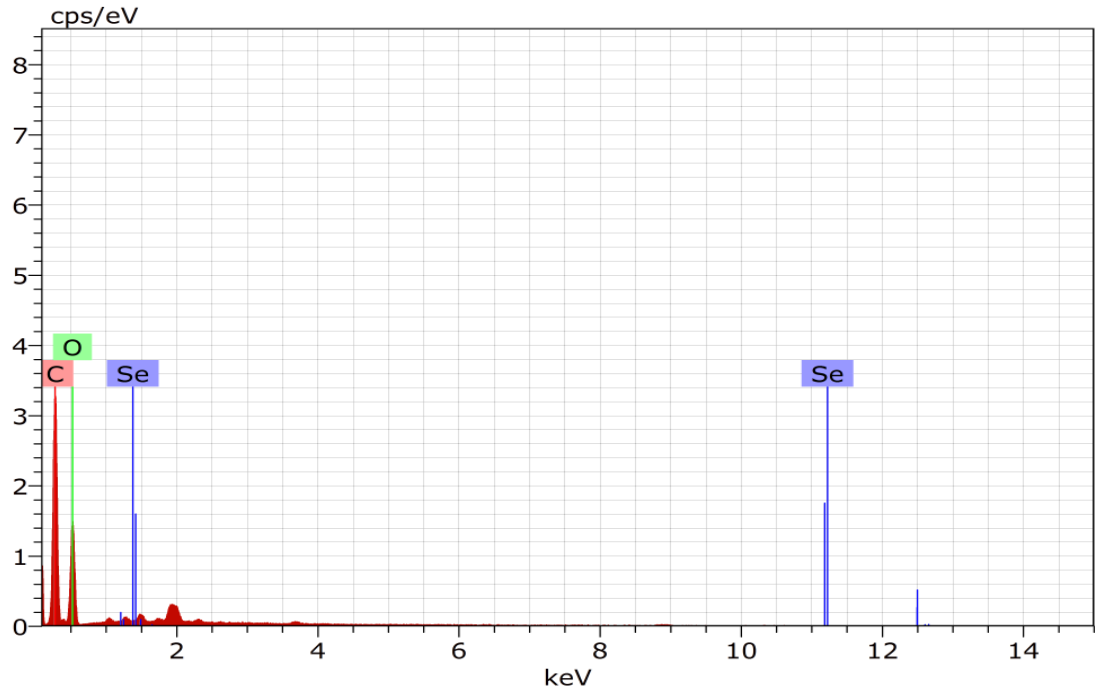
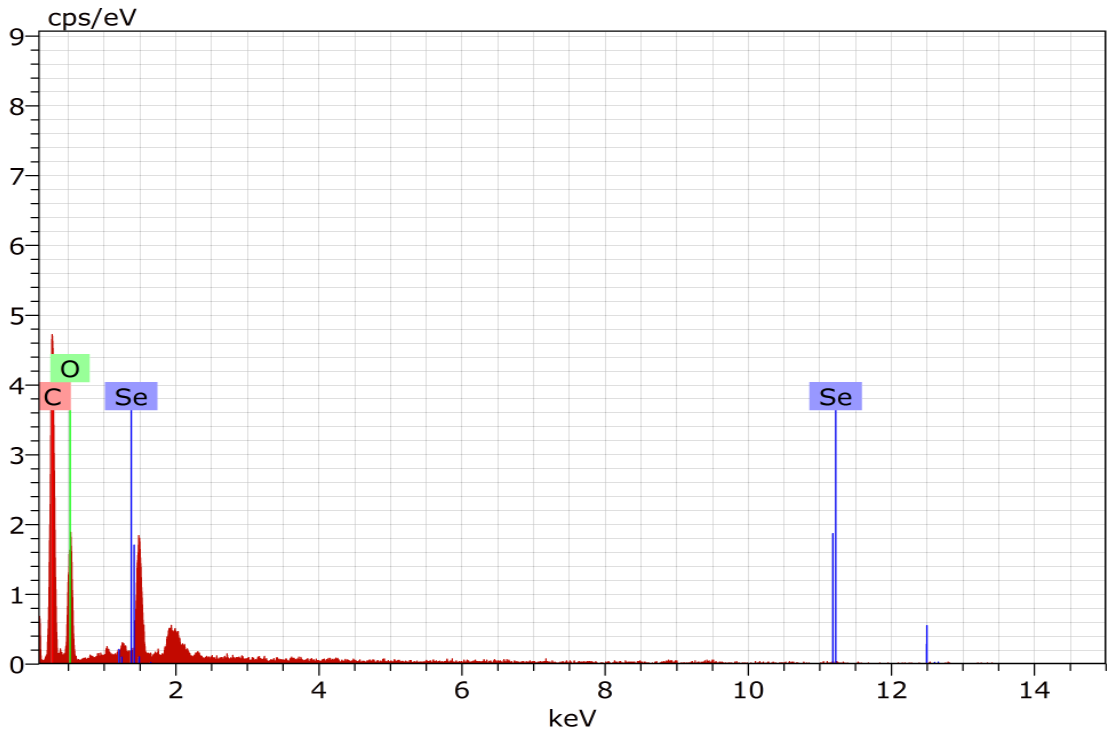
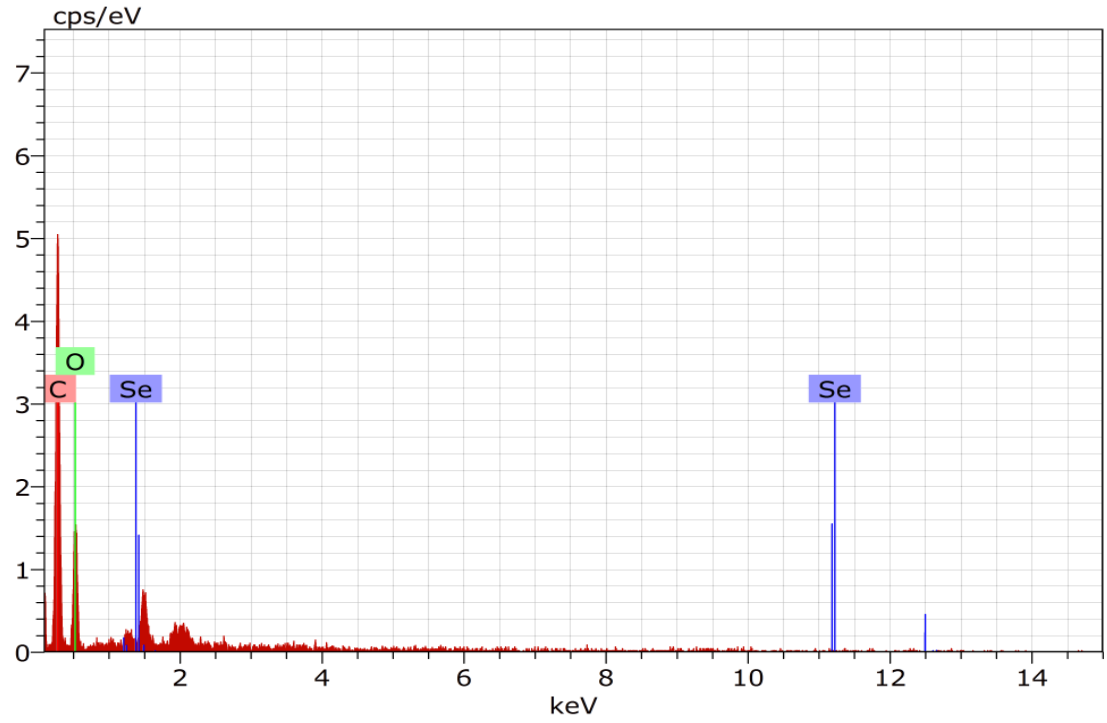


(b)

Fig. 4.16 Energy Dispersive X-ray Spectroscopy signals for (a) Se enriched fruiting bodies of *P. cornucopiae* (b) Non-enriched fruiting bodies of *P. cornucopiae*



(a)



(b)

Fig. 4.17 Energy Dispersive X-ray Spectroscopy signals for (a) Se enriched fruiting bodies of *P. djamor* (b) Non enriched fruiting bodies of *P. djamor*

better arsenic tolerance potential. Besides, a selenium dependent reduction in arsenic accumulation was also observed in rice root (14.24%) and shoot (23.78%) when compared with rice plant treated with arsenic alone. Hence, revealed the capability of selenium in solving the ecotoxicological problems associated with the arsenic buildup in agricultural lands (Singh *et al* 2018). Our EDS results also showed changes in the elemental composition of oxygen, carbon and selenium in terms of percent weight and atom percentage in case of Se-enriched and non-enriched fruit bodies of *Pleurotus* spp.

4.8 ESTIMATION OF SELENIUM CONTENT IN SELENIUM BIOFORTIFIED *Pleurotus* spp. FRUITING BODIES USING INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY/ATOMIC ABSORPTION SPECTROSCOPY (ICP-MS/AAS)

Selenium content was estimated from the wheat straw (substrate) and fruit bodies of *Pleurotus* spp. using ICP-MS/AAS and expressed as µg Se g^{-1} dw. The Se content was found to be 22.34±2.84 µg g^{-1} dw in Se enriched wheat straw and 0.06±0.01 µg g^{-1} dw in respective non-enriched wheat straw. There was a significant difference in the Se content of Se-enriched and non-enriched fruit bodies of all the four *Pleurotus* spp. (Fig. 4.18). Among the Se-enriched fruit bodies, highest Se content was found in *P. djamor* (156.60±7.66 µg g^{-1} dw), followed by *P. florida* (124.00±7.94 µg g^{-1} dw), *P. eryngii* (57.25±7.33 µg g^{-1} dw) and *P. cornucopiae* (46.25±6.59 µg g^{-1} dw). Among the control (non Se-enriched) fruit bodies, highest Se content was found in *P. djamor* (4.33±0.45 µg g^{-1} dw), followed by *P. florida* (2.48±0.39 µg g^{-1} dw), *P. eryngii* (0.49±0.03 µg g^{-1} dw) and *P.cornucopiae* (0.16±0.03 µg g^{-1} dw) (Table 4.12).

Bhatia *et al* (2013) studied selenium bioaccessibility and speciation in Se-enriched *Pleurotus* mushrooms cultivated on selenium-hyperaccumulated agricultural residues. They reported that Se content in the Se-enriched and control substrate used for cultivating mushrooms were 27.9 and 0.026 µg g^{-1} dw respectively. They also found that selenium concentration in biofortified mushrooms was 800 times higher in comparison with the control samples grown on wheat straw from non-selenium rich areas (141 vs 0.17 µg g^{-1} dry weight). Similar results were obtained by Bhatia *et al* (2012) when they analyzed the total Se content in the Se-hyperaccumulated wheat straw and fruit bodies. They reported that Se content in the Se-enriched and control substrate used for cultivating mushrooms was 24.0 and 1.9 µg g^{-1} dw respectively. They even reported that among the five *Pleurotus* spp. cultivated, highest Se content was accumulated by *P. djamor* as compared to control samples (145.4 vs 5 µg g^{-1} dw), followed by *P.ostreatus* (44.3 vs 3.4 µg g^{-1} dw), *P. sojorcaju* (43.5 vs 5.2 µg g^{-1} dw), *P. fossulatus* (37.2 vs 3.5 µg g^{-1} dw) and *P. citrinopileatus* (26.1 vs 2.9 µg g^{-1} dw).

Table 4.12 Estimation of Se content in Se biofortified fruiting bodies of *Pleurotus* spp. using ICP-MS/AAS

Treatment	Total selenium (µg Se g^{-1} dw)				
	Wheat straw	***P. florida***	***P. eryngii***	***P. djamor***	***P. cornucopiae***
Se-enriched	22.34±2.84	124.00±7.94	57.25±7.33	156.60±7.66	46.25±6.59
Control	0.06±0.01	2.48±0.39	0.49±0.03	4.33±0.45	0.16±0.03
CD (5 %)	7.8	22.07	20.34	21.30	18.29

No. of replicates-3

Temperature during the crop = 25±10°C

± values indicated standard error

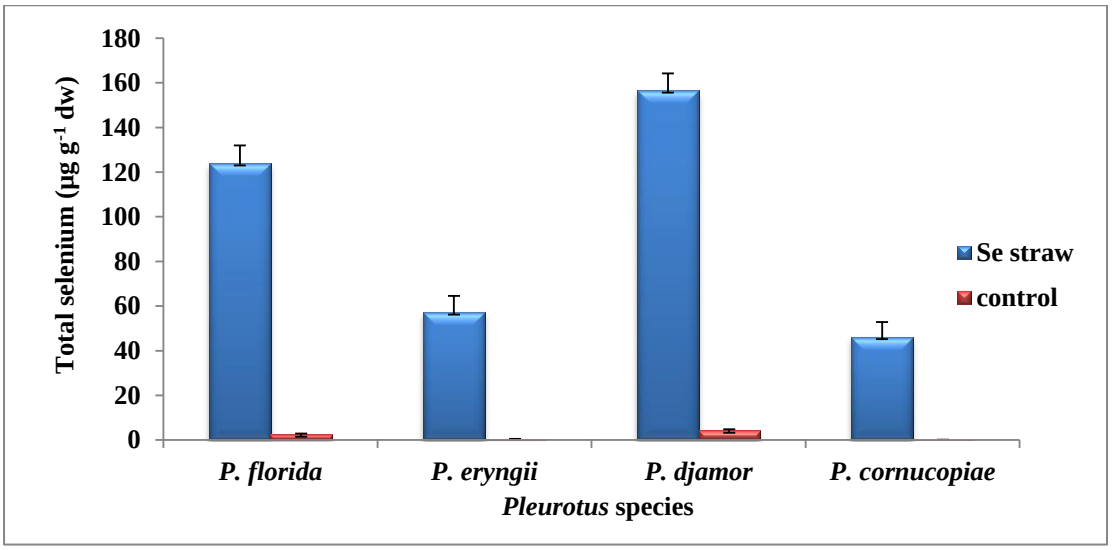


Fig. 4.18 Estimation of Se content in Se biofortified fruiting bodies of *Pleurotus* spp. using ICP-MS/AAS

4.9 CHARACTERIZATION OF SELENIUM BIOFORTIFIED *Pleurotus* spp. FRUITING BODIES BY FOURIER TRANSFORM INFRA RED SPECTROSCOPY (FT-IR SPECTROSCOPY)

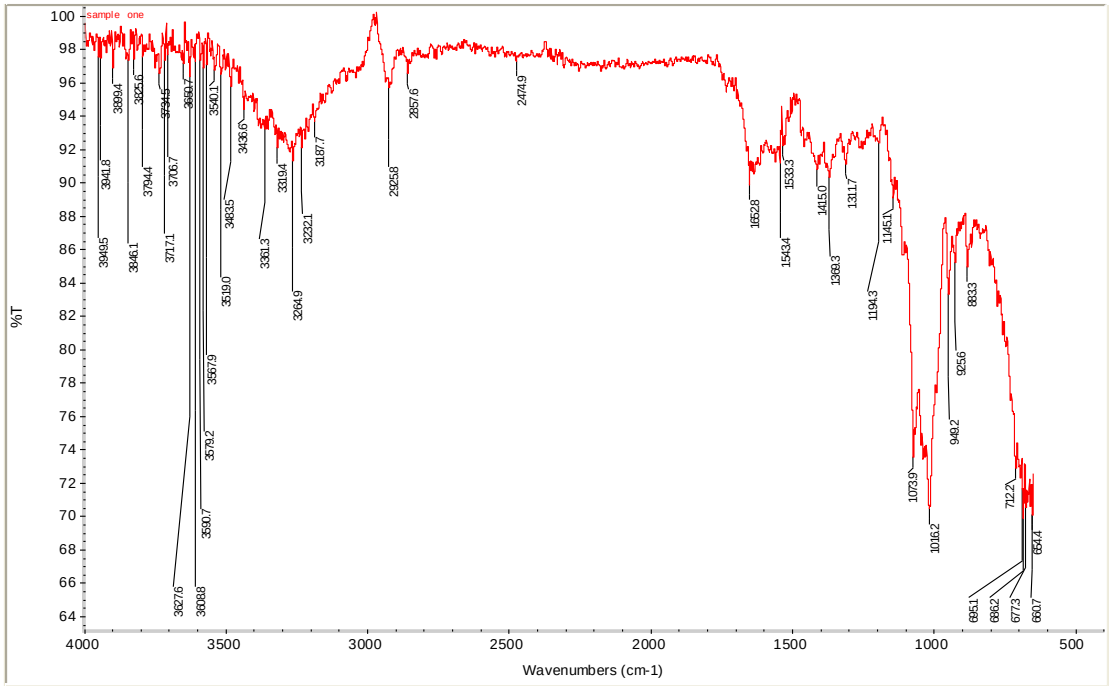
Since the selenium biofortification of *Pleurotus* spp. was found to be associated with the proteins and polysaccharides, the samples were subjected to the FT-IR spectroscopy analysis to identify the functional group and conformational changes in mushroom polysaccharides and proteins as a result of Se supplementation. The FT-IR spectra of Se enriched fruiting bodies of *P. florida*, *P. eryngii*, *P. djamor* and *P. cornucopiae* showed broad peaks between 4000-3000 cm^{-1} which were due to stretching vibrations of O-H functional group in sugar residues. The peaks in range of 2300-3000 cm^{-1} appeared due to the stretching of C-H bonds in carbohydrates. The peaks in range of 1200-900 cm^{-1} characteristic of carbohydrates corresponded to the stretching vibrations of C-C, C-O-C and C-O bonds

occurring in glucopyranose structure. The absorption peaks in the 950-750 cm^{-1} region represents the anomeric configuration of polysaccharides. Peaks representing glucan band (α-anomer C-H deformation) were observed in Se enriched fruit bodies of *P. florida* (at 925.6 cm^{-1} and 949.2 cm^{-1}), *P. eryngii* (at 944.7 cm^{-1}), *P. djamor* (at 943.8 cm^{-1}) and *P. cornucopiae* (at 933.6 cm^{-1}), while the glucan band due to β-anomer C-H deformation were observed in Se enriched fruit bodies of *P. florida* (at 883.3 cm^{-1}), *P. eryngii* (at 887.7 cm^{-1}), *P. djamor* (at 893.2 cm^{-1}) and *P. cornucopiae* (at 889.4 cm^{-1}). Peaks representing polysaccharide linked proteins were more in number in Se-enriched fruit bodies of *P. 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)], whereas, they were less in number in the Se-enriched fruit body of *P. eryngii* [at 1639.2 cm^{-1} (Amide-I)] and no change in their number in the Se-enriched fruit body of *P. cornucopiae* [at 1652.6 cm^{-1} (Amide-I) and 1559.7 cm^{-1} (Amide-II)]. Peaks representing β-glycosidic bonds were observed in Se-enriched fruit bodies of *P. 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 and 1368.7 cm^{-1}) and *P. cornucopiae* (at 1380.3 cm^{-1}). Peak corresponding to the C=O stretching of phospholipids were present in Se-enriched fruit bodies of *P. eryngii* (at 1743.3 cm^{-1}), *P. djamor* (at 1748.7 cm^{-1}) and *P. cornucopiae* (at 1746.6 cm^{-1}). In *P. florida*, *P. eryngii*, *P. cornucopiae* control fruit bodies, the number of peaks between 4000-3000 cm^{-1} due to stretching vibrations of O-H functional groups were more in number as compared to Se-enriched counterpart. Whereas, these bands were less in number in the control fruit body of *P. djamor*. There was a slight change in the wavenumbers due to stretching of C-H bonds in carbohydrates in the control fruit bodies of *P. florida*, *P. eryngii*, *P. djamor* and *P. cornucopiae* i.e. in between 2300 to 3000 cm^{-1}. The number of peaks between 1200-900 cm^{-1} due to stretching vibrations of C-C, C-O-C and C-O occurred in glucopyranose structure with slight change in the wavenumbers of control fruit bodies of *P. florida*, *P. eryngii*, *P. djamor* and *P. cornucopiae*. Peaks representing polysaccharide linked proteins were less in number in control fruit bodies of *P. florida* and *P. djamor* [at 1652.6 (Amide-I) and 1652.4 (Amide-I), 1542.5 cm^{-1} (Amide-II) respectively] whereas, they were more in number in the control fruit body of *P. eryngii* [at 1652.6, 1639.2 (Amide-I) and 1560 cm^{-1} (Amide-II)] and no change in their number in control fruit body of *P. cornucopiae* [at 1652.5 (Amide-I) and 1571.9 cm^{-1} (Amide-II)]. Peaks representing β-glycosidic bonds varied slightly in wave numbers in control fruit bodies of *P. florida* (at 1364.1 and 1403.8 cm^{-1}), *P. eryngii* (at 1380.5, 1396.5 and 1458.4 cm^{-1}), *P. djamor* (at 1380.2 and 1395.9 cm^{-1}) and *P. cornucopiae* (at 1377.3 and 1397.2 cm^{-1}). Peak (at 1748.4 cm^{-1}) can be attributed to the C=O stretching of phospholipids was present only in the control fruit body of *P. florida* and absent in their respective Se enriched fruit body. Whereas, it occurred with slight change in wave numbers in the control fruit bodies of *P. eryngii* (at 1746.1 cm^{-1}), *P. cornucopiae* (at 1734.0 cm^{-1}) and was absent in

P. djamor (Fig. 4.19, 4.20, 4.21 and 4.22). Mohaček-Grošev *et al* (2001) analyzed the polysaccharide extracts from wild growing mushrooms and toadstools by FT-IR spectroscopy. They have reported that the peaks formed between 750–950 cm^{-1}, corresponds to α or β anomer C_1-H deformation. The band at 890 cm^{-1} was attributed to a β-1,3-glucan, while the bands at 850 and 929 cm^{-1} were assigned to a α-1,6-glucan, and the band at 822 cm^{-1} to α-1,3-glucan. Bekiaris *et al* (2020) studied the *Pleurotus* mushrooms glucans and ergosterol content by ATR-FTIR Spectroscopy. They assigned the peak at 1745 cm^{-1} in *P. eryngii* mushroom to the C=O stretching of phospholipids. Rodrigues *et al* (2015) studied the FT-IR spectra of mushroom truffles and various *Amanita* species. They reported that bands occurring in the range of 4000-1800 cm^{-1} were due to the stretching vibrations of O-H and C-H and bands in the range of 2900-2880 cm^{-1} were due to the stretching vibrations of CH_2 and CH_3 fatty acids. Peaks occurring at 1650 and 1560 cm^{-1} were due to Amide I and Amide II of proteins; a band at 1740 cm^{-1} was due to carbonyl stretching vibration of alkyl-esters; peaks occurring in the range of 1500 to 750 cm^{-1} were due to vibrations of proteins, lipids and also polysaccharides. Bands around 1077 and 1042 cm^{-1} were due to C-O stretching of polysaccharides; 950-750 cm^{-1} region corresponds to the anomeric configuration of polysaccharides. Band occurring at 890 cm^{-1} was assigned to β-glycosides and 860 to 810 cm^{-1} for α-glycosides. Our results showed that the polysaccharides of Se-enriched fruit bodies of *Pleurotus* spp. structurally belong to β-glucans (majorly 1, 3 and 1, 6 β-D-glucans) with strong antioxidant activity.

FT-IR spectra of Se enriched fruit bodies of *P. florida* (at 3232.1, 3264.9 and 3319.4 cm^{-1}), *P. eryngii* (at 3275 cm^{-1}) and *P. djamor* (at 3211.1, 3231.9, 3252.6, 3268.9 and 3292.2 cm^{-1}) showed peaks which corresponds to NH stretching of Amide A region and were absent in *P. cornucopiae*. There was a slight change in the wave numbers corresponding to Amide A region in their respective control samples i.e. *P. florida* (at 3220.0 and 3265.3 cm^{-1}), *P. eryngii* (at 3257.0 cm^{-1}), *P. djamor* (at 3312.1 cm^{-1}) and *P. cornucopiae* (at 3222.5 and 3311.9 cm^{-1}) due to absence of selenium. Peaks at 3077.3 and 3098.9 cm^{-1} corresponds to Amide B region due to NH stretching were observed only in the Se enriched fruit body of *P. djamor* and absent in all other Se-enriched as well as non-enriched fruit bodies. Peaks corresponding to Amide-I region due to C=O stretching were observed in Se-enriched fruit bodies of *P. 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}), which occurred with slight change in wavenumbers in their respective control fruit bodies i.e. *P. florida* (at 1652.6 cm^{-1}), *P. eryngii* (at 1639.2 and 1652.6 cm^{-1}), *P. djamor* (at 1652.4 cm^{-1}) and *P. cornucopiae* (at 1652.5 cm^{-1}). Peaks (at 1543.4 and 1533.3 cm^{-1}) corresponding to Amide II region due to CN stretching and NH bending were recorded in Se enriched fruit body of *P. florida* but absent in their respective control sample. In *P. eryngii* (at 1560.0 cm^{-1}) it was observed only in control fruit body and

was absent in Se-enriched treatment. 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). Peaks corresponding to amide III region due to CN stretching and NH bending was present only in control fruit body of *P. florida* (1243.2 cm^{-1}) and absent in their respective Se-enriched fruit body. Whereas, these peaks occurred with slight change in wavenumber in the Se-enriched and control fruit bodies of *P. eryngii*, *P. djamor* and *P. cornucopiae* respectively. Peaks corresponding to Amide IV region due to OCN bending were observed in Se-enriched fruit bodies of *P. florida* (at 654.4, 660.7, 677.3, 686.2, 695.1 and 712.2 cm^{-1}), *P. eryngii* (at 701.9, 743.2 and 767.3 cm^{-1}), *P. djamor* (at 670.7, 712.9, 723.1, 736.8 and 761.9 cm^{-1}) and *P. cornucopiae* (at 760.7 cm^{-1}). Whereas, they occurred with slight change in wavenumbers in their respective control samples. The peaks assigned to Amide V region due to out of plane NH bending were observed only in the control fruit body of *P. florida* (at 773.1, 783.5 and 798.8 cm^{-1}) and were absent in their respective Se-enriched fruit bodies. Whereas, in *P. eryngii* (784.7 cm^{-1}) it was present in Se-enriched fruit body and absent in their respective control fruit body. In *P. cornucopiae* and *P. djamor* the peaks occurred with slight change in wave numbers in Se-enriched and non-enriched fruit bodies. 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. florida* respectively. In both control and Se enriched fruit bodies of *P. eryngii* Amide-I region showed β sheet at 1639.2 cm^{-1}. In their respective control sample peak at 1652.6 cm^{-1} corresponding to Amide-I region showed α helix structure of protein which was absent in Se enriched sample. Amide-I region showed peaks for α helix of protein 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. 4.19, 4.20, 4.21 and 4.22). FT-IR spectra of proteins from all the four *Pleurotus* spp. showed an increase in the flexibility and unfolding upon Se supplementation. Higher H-bonding was observed in proteins from Se-enriched fruit bodies which indicate an increase in hydrophilicity of proteins. Kaur *et al* (2017) studied the FT-IR spectra of mycelial proteins of Se enriched *Pleurotus* spp. and found that Se caused significant conformational changes in the protein structure. They further reported that peaks corresponding to Amide-I region in selenium-rich *Pleurotus* spp. samples represented reduced flexibility and unfolding of proteins. Peak at 1637 cm^{-1} in se-enriched *P. florida* indicated β-sheet conformation of protein whereas, in non-enriched proteins peak at 1654 cm^{-1} indicated α-helical structure of protein. *Pleurotus ostreatus* also showed peaks for β-sheet and α-helical structure of protein while their respective control proteins resulted in peaks for β-turns. The control sample proteins of *P. sajor-caju* showed peaks for β-sheet, α-helical and β-turn conformations whereas β-turns were absent in their respective selenium



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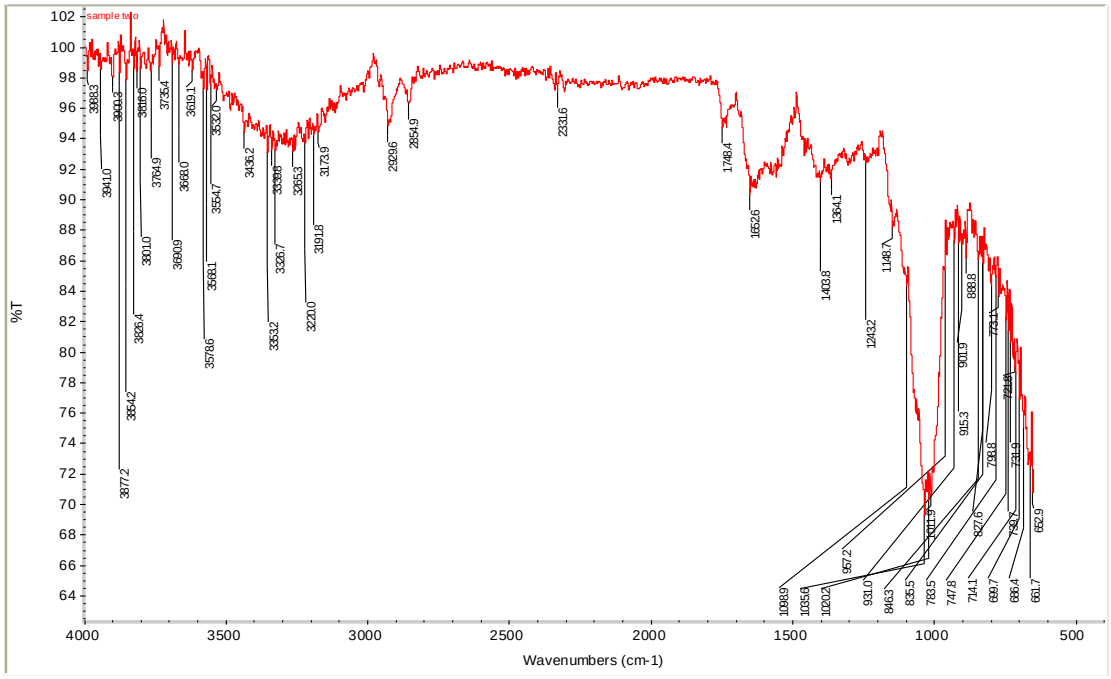
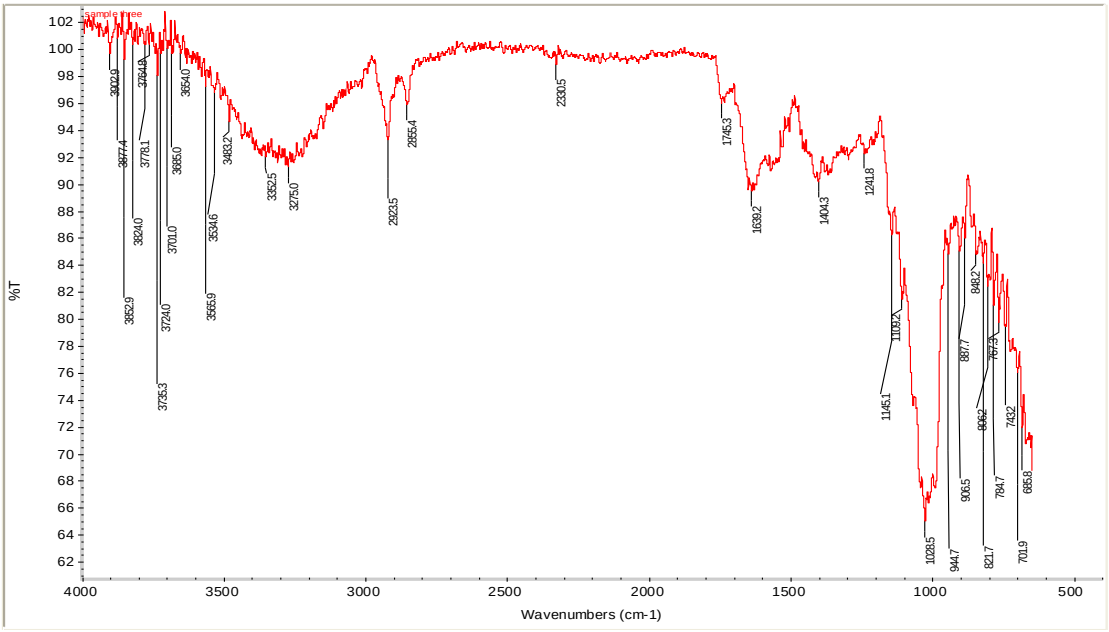


(b)

Fig. 4.19 FT-IR spectra of (a) Se enriched fruiting body of *P. florida* (b) Non-enriched fruiting body of *P. florida*



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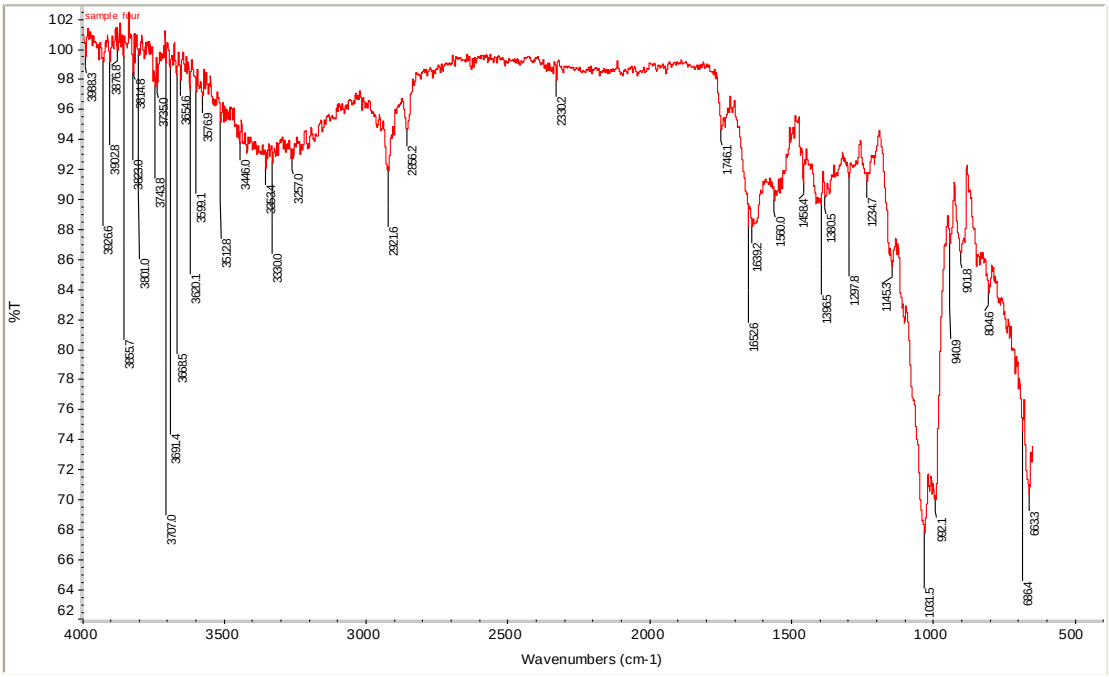
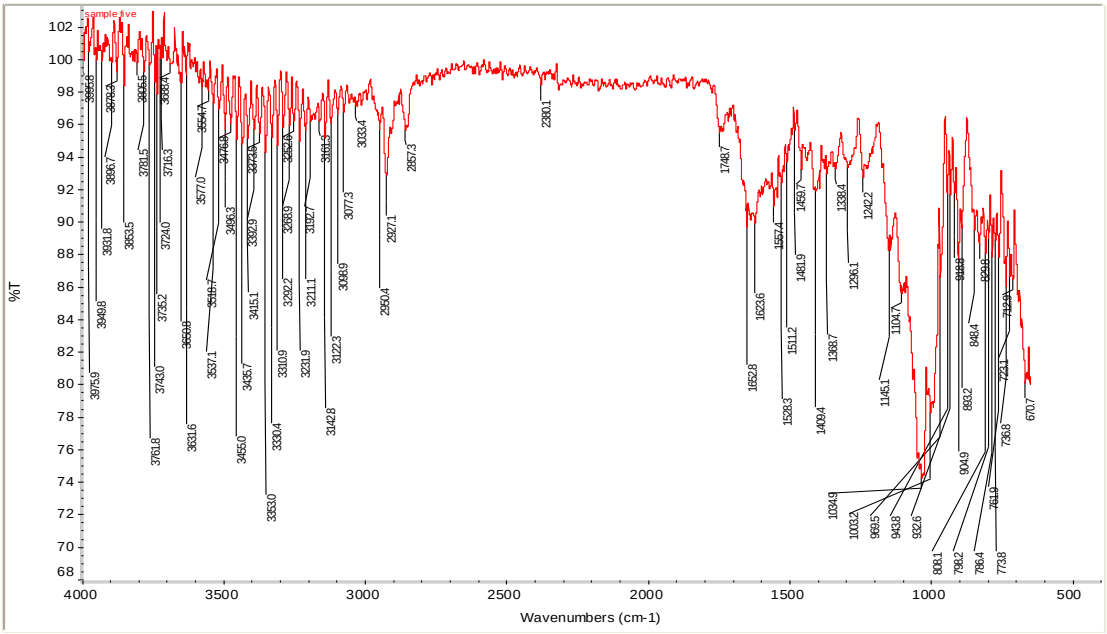
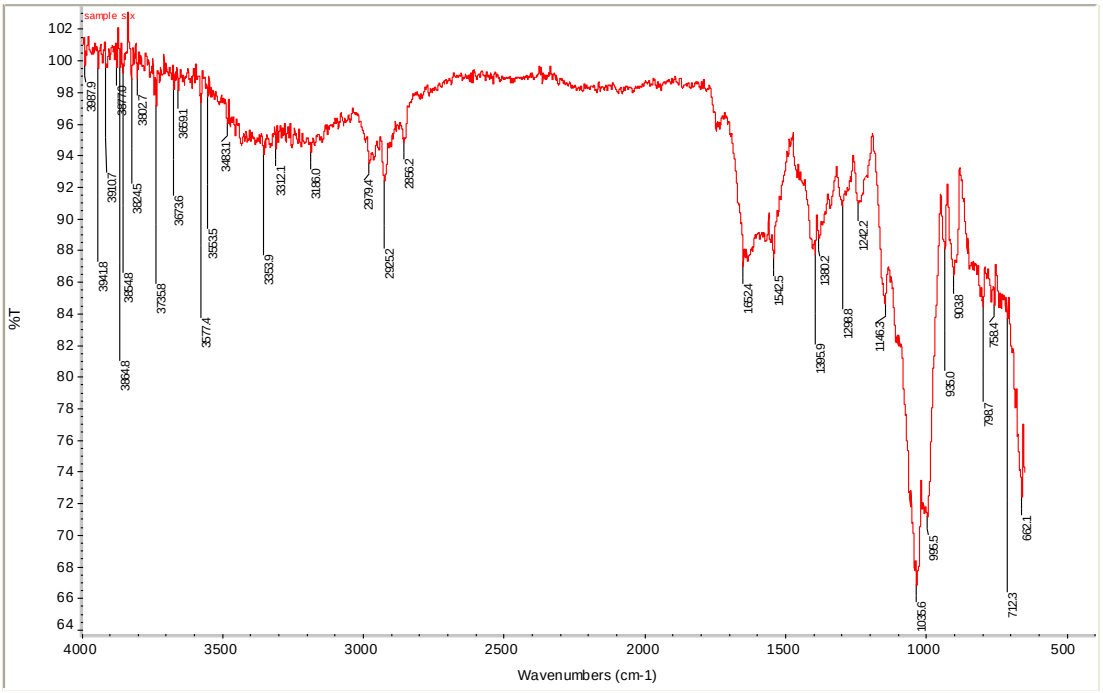


(b)

Fig. 4.20 FT-IR spectra of (a) Se enriched fruiting body of *P. eryngii* (b) Non-enriched fruiting body of *P. eryngii*

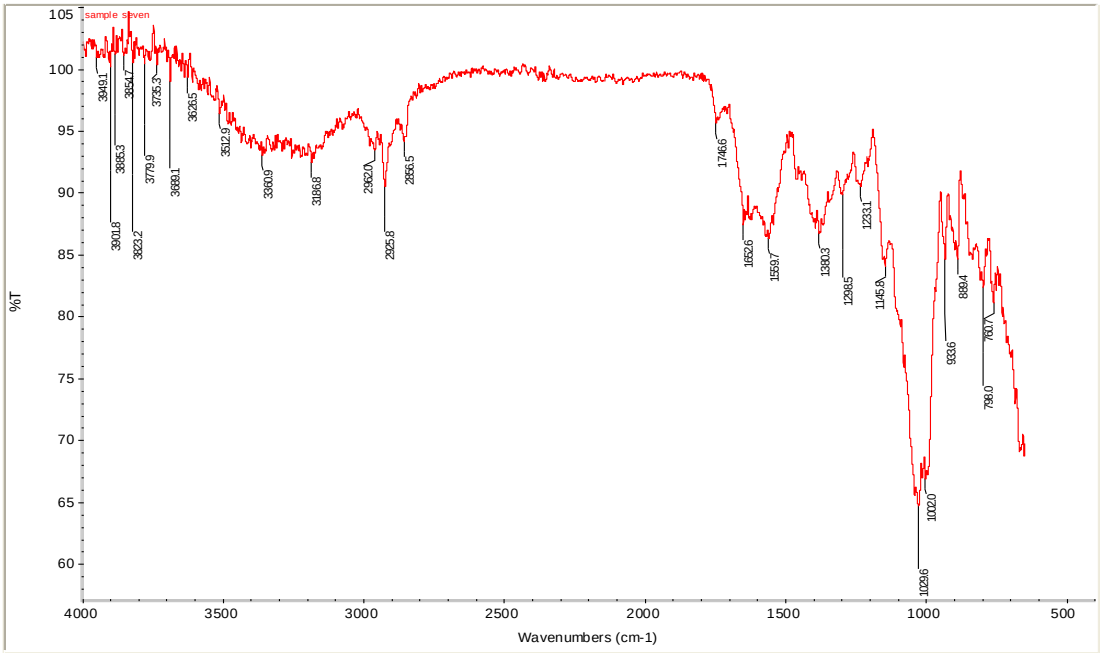


(a)

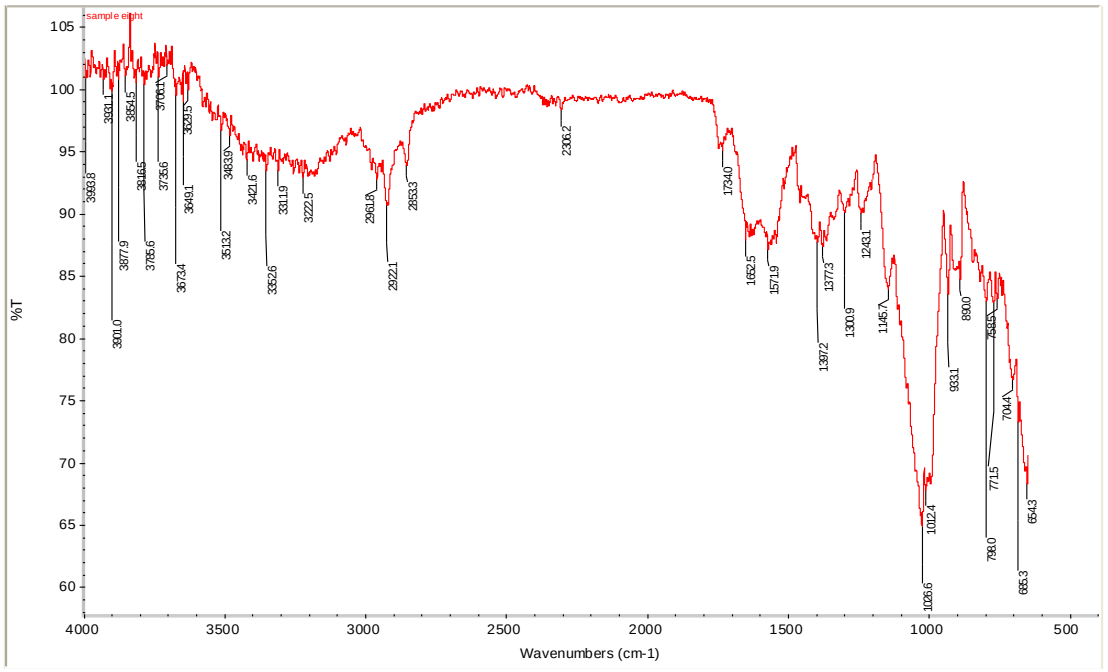


(b)

Fig. 4.21 FT-IR spectra of (a) Se enriched fruiting body of *P. djamor* (b) Non-enriched fruiting body of *P. djamor*



(a)



(b)

Fig. 4.22 FT-IR spectra of (a) Se enriched fruiting body of *P. cornucopiae* (b) Non-enriched fruiting body of *P. cornucopiae*

rich samples. Selenium supplementation increased the bending frequencies indicating increased H-bonding of proteins in case of *P. florida* and *P. sajor-caju*. Peaks corresponding to proline side chain vibrations were also observed in case of Se-enriched *P. florida* and *P. sajor-caju*. Bekiaris *et al* (2020) studied the *Pleurotus* mushroom glucans and ergosterol content by ATR-FTIR Spectroscopy. They have assigned the peaks around 1650 and 1560 cm^{-1} to Amide I and Amide II regions of proteins.

CHAPTER-V

SUMMARY

Pleurotus commonly known as oyster mushroom is an edible mushroom of the tropical and temperate climates throughout the world known for its nutritional values. The present study compared the effect of selenium biofortification on antioxidant activity of four different selenium biofortified *Pleurotus* spp. i.e. *P. florida*, *P. eryngii*, *P. cornucopiae* and *P. djamor*. Effect of selenium supplementation @ 5, 10, 15, 20 mg L^{-1} and control on the radial hyphal growth, biomass production and the total soluble protein content of mycelium were observed. The Se content of the Se biofortified mycelium and fruit bodies were estimated using ICP-MS/AAS, Scanning Electron Microscopy and Energy Dispersive Spectroscopy (EDS) techniques. The four *Pleurotus* species were cultivated on selenium enriched and non-enriched wheat straw. Fruit bodies for total soluble protein content, total phenol and flavonoid content, and percent radical scavenging activity were analyzed. Se biofortified *Pleurotus* spp. fruit bodies were also characterized by FT-IR Spectroscopy.

For *in-vitro* studies, maximum radial growth was observed in *P. djamor* (7.11±0.19 mm/day) and *P. cornucopiae* (8.02±0.16 mm/day) @ 10 mg L^{-1}sodium selenate supplementation while in *P. florida* maximum radial growth (9.00±0.25 mm/day) was found @ 5 mg L^{-1}. The *P. eryngii* showed maximum radial growth (8.56±0.22 mm/day) @ 20 mg L^{-1} sodium selenate supplementation. Maximum biomass was obtained by *P. djamor* (88.5±3.0 g L^{-1}) and *P. cornucopiae* (58.3±1.7 g L^{-1}) @ 10 mg L^{-1} Se concentration. In case of *P. florida* (88.0±3.2 g L^{-1}) maximum biomass was obtained @ 5 mg L^{-1} and in *P. eryngii* (77.0±2.3 g L^{-1}) @ 20 mg L^{-1} Se concentration. The total soluble protein content of mycelium was found to be highest at 10 mg L^{-1} Se concentration in *P. djamor* (1.97±0.15 mg g^{-1}) and *P. cornucopiae* (1.94±0.10 mg g^{-1}). In *P. florida* the highest protein content (2.28±0.16 mg g^{-1}) was found at 5 mg L^{-1} of Se concentration and in *P. eryngii* showed highest protein content (2.55±0.13 mg g^{-1}) @ 20 mg L^{-1} of Se concentration. Se content was estimated from the Se-enriched biomass of *Pleurotus* spp. using ICP-MS/AAS and expressed as µg Se g^{-1} dw. The highest Se content was observed at 20 mg L^{-1} of Se concentration in all the four *Pleurotus* spp. i.e. in *P. djamor* (920.32±17.99 µg g^{-1} dw), followed by *P. florida* (880.61±15.78 µg g^{-1} dw), *P. eryngii* (850.27±20.78 µg g^{-1} dw) and *P. cornucopiae* (792.72±20.93 µg g^{-1} dw). Highest percentage of absorbed selenium was found at 5 mg L^{-1} Se concentration in broth in all the four *Pleurotus* spp. namely *P. djamor* 57.27% (920.32±17.99 µg g^{-1} dw), followed by *P. florida* 51.02% (880.61±15.78 µg g^{-1} dw), *P. eryngii* 44.60% (850.27±20.78 µg g^{-1} dw) and *P. cornucopiae* 43.70% (792.72±20.93 µg g^{-1} dw).

Scanning Electron Microscopy studies showed that Se concentration @ 10 mg L^{-1} was found to be the best for the growth of *P. florida*, *P. eryngii*, *P. djamor* and *P. cornucopiae*.

The mycelial texture became coarser and fragile in case of *P. florida*, *P. eryngii*, *P. djamor* and *P. cornucopiae* in response to increased concentration of Se. Elemental analysis of *Pleurotus* spp. mycelia supplemented with various concentrations of Se using SEM-EDS showed signals characteristics for selenium on the surface of *P. florida*, *P. eryngii*, *P. djamor* and *P. cornucopiae* mycelia, conforming that the selenium was incorporated into the cell wall components of fungal mycelia. The percentage weight and atomic percentage of selenium was highest in 10 mg L^{-1} Se supplementation treatment in all the four *Pleurotus* spp.

Pleurotus florida, *P. eryngii*, *P. cornucopiae* and *P. djamor* mushrooms were cultivated on selenium enriched and non-enriched wheat straw. There was no significant difference in terms of spawn run, number of fruit bodies, average weight of fruit body and biological efficiency (%). Highest biological efficiency (%) was observed in *P. florida* (66.50 % in Se-enriched wheat straw and 64.57% in non-Se enriched wheat straw), followed by *P. cornucopiae* (36.74 % in Se-enriched wheat straw and 33.11 % in non-Se enriched wheat straw), *P. eryngii* (30.41 % in Se-enriched wheat straw and 27.16 % in non-Se enriched wheat straw) and *P. djamor* (30.14 % in Se-enriched wheat straw and 29.16 % in non-Se enriched wheat straw).

The total soluble protein content was estimated from the fruit bodies of *Pleurotus* spp. grown on selenium enriched substrate. Among the control fruit bodies of *Pleurotus* spp., the total soluble protein content was found to be highest in *P. djamor* (3.60±0.24 mg g^{-1}) followed by *P. eryngii* (1.60±0.24 mg g^{-1}), *P. florida* (1.41±0.09 mg g^{-1}) and *P. cornucopiae* (1.38±0.11 mg g^{-1}). In case of selenium biofortified fruiting bodies of *Pleurotus* spp. *P. djamor* (4.56±0.31 mg g^{-1}) showed the most significant difference between the selenium biofortified and control followed by *P.eryngii* (2.55±0.24 mg g^{-1}), *P. cornucopiae* (2.16±0.15 mg g^{-1}) and *P. florida* (1.98±0.12 mg g^{-1}). Among the various Se-biofortied *Pleurotus* mushrooms, the phenolic content was highest in *P. djamor* (4.08±0.16 mg GAE/g dw) followed by *P. florida* (2.44±0.16 GAE/g dw), *P. cornucopiae* (2.00±0.15 GAE/g dw) and *P. eryngii* (1.28±0.10 GAE/g dw). Selenium enriched *Pleurotus* spp. showed an increase in the total phenolic content as compared to their non-enriched counterparts with *P. djamor* showing the most significant difference (1.16±0.10 GAE/g dw) followed by *P. florida* (0.42±0.06 GAE/g dw), *P. cornucopiae* (0.96±0.08 GAE/g dw) and *P. eryngii* (0.66±0.12 GAE/g dw). Among the various control fruiting bodies of *Pleurotus* spp., the % radical scavenging activity (expressed as 0.1mL methanolic extract $0.5g^{-1}$ dw) was found to be highest in *P. florida* (8.98±0.24 %), followed by *P. cornucopiae* (7.56±0.20 %), *P. djamor* (7.53±0.19%) and *P. eryngii* (2.01±0.12%). Supplementation of selenium was found to have positive effect in case of all the four *Pleurotus* spp. The % radical scavenging activity among the Se enriched fruiting bodies was found to be highest in *P. cornucopiae* (10.72±0.26%) followed by *P. florida* (10.54±0.45%), *P. djamor* (9.16±0.28%), and *P. eryngii* (4.40±0.20%). Among the

control fruit bodies, highest flavonoid content was found in *P. cornucopiae* (275.0±14.74 µg QE g^{-1}) followed by *P. eryngii* (250.0±20.60 µg QE g^{-1}), *P. florida* (225.0±10.15 µg QE g^{-1}) and *P. djamor* (50.0±1.53 µg QE g^{-1}).The flavonoid content of Se enriched fruit bodies were found to be highest in case of *P. florida* (512.5±10.03 µg QE g^{-1}), followed by *P. cornucopiae* (500.0±19.55 µg QE g^{-1}), *P. eryngii* (425.0±16.17 µg QE g^{-1}) and *P. djamor* (250.0±13.75 µg QE g^{-1}). The flavonoid content was found to be highest in Se-enriched fruit bodies of all the four *Pleurotus* spp. when compared to control.

The elemental analysis of Se biofortified *Pleurotus* spp. fruiting bodies using SEM-EDS showed signals characteristic for selenium on the surface of *P. florida* and *P. djamor* conforming that the selenium was incorporated into the cellwall of these mushroom fruiting bodies. On the contrary, no selenium was found on the surface of fruiting bodies of *P. eryngii* and *P. cornucopiae* indicating that the selenoproteins and other forms of selenium to be cytosolic moieties. The percentage weight and atom percentage of selenium was high in Se biofortified mushrooms of *P. florida* (0.72 and 0.12 respectively) and *P. djamor* (2.85 and 0.49 respectively) as compared to control mushrooms (*P. florida*-0.22, 0.04 and *P. djamor*-0.83, 0.14 respectively). The Se content was found to be 22.34±2.84 µg g^{-1} dw in Se enriched wheat straw and 0.06±0.01 µg g^{-1} dw in respective non-enriched wheat straw. Among the Se-enriched fruit bodies, highest Se content was found in *P. djamor* (156.60±7.66 µg g^{-1} dw), followed by *P. florida* (124.00±7.94 µg g^{-1} dw), *P. eryngii* (57.25±7.33 µg g^{-1} dw) and *P. cornucopiae* (46.25±6.59 µg g^{-1} dw). Among the control (non Se-enriched) fruit bodies, highest Se content was found in *P. djamor* (4.33±0.45 µg g^{-1} dw), followed by *P. florida* (2.48±0.39 µg g^{-1} dw), *P. eryngii* (0.49±0.03 µg g^{-1} dw) and *P. cornucopiae* (0.16±0.03 µg g^{-1} dw). FT-IR spectra of Se-enriched fruit bodies of all the four *Pleurotus* spp. showed that the polysaccharides of Se-enriched fruit bodies of *Pleurotus* spp. structurally belong to β-glucans (majorly 1, 3 and 1, 6 β-D-glucans) with strong antioxidant activity. FT-IR spectra of proteins from all the four *Pleurotus* spp. indicated towards an increase in flexibility and unfolding upon Se supplementation. The higher H-bonding was observed in proteins from Se-enriched fruit bodies which indicated an increase in hydrophilicity of proteins.

Thus, this study helps us to make potential use of Se-rich agricultural residues for the production of Se biofortified mushrooms which serves as an excellent nutraceutical with respect to meeting daily Se requirement of population thereby, combating malnutrition. The enrichment of media with Se leads to high content of these metals in mushroom mycelium and fruiting bodies with diverse effects. Consumption of Se-enriched fruit bodies of *Pleurotus* spp. helps in combating malnutrition by providing various major and minor nutrients, so it can be treated as a functional food. Hence, production of Se-biofortified mushrooms using Se-hyperaccumulated straw can be considered as a natural and ecofriendly way of producing biofortified food.

In addition to wheat straw, the feasibility of producing Se-biofortified mushrooms on other Se hyperaccumulated agricultural residue as a substrate can be tested. Besides the antioxidant potential of Se-biofortified *Pleurotus* species, further we have to analyze antihypertensive, anti-inflammatory, antinociceptive, antimicrobial, hypolipidemic, immunomodulatory, cytoprotective, neuritogenic, antiaging activities, it's effective absorption, physiological effects and influences of Se-enriched mushrooms in promoting and maintaining human health. Therefore, *Pleurotus* spp. can be used as an excellent nutraceutical with numerous health benefits

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ANNEXURE-I

RADIAL GROWTH

PE SE RG

NR = 3 NT = 5 NE = 1

Analysis of CRD with equal no. of replications

REATMENT MEANS

1 5.5600000 6.1000000 6.9700000 8.3200000 8.5600000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 4 5.2563630 49.44 .592921

Error 10 .10631710 7.1020000 4.59

PF SE RG

NR = 3 NT = 5 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 8.2000000 9.0000000 7.6999990 7.2000000 6.4000000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 4 2.9100040 13.27 .851474

Error 10 .21925660 7.7000000 6.08

PD SE RG

NR = 3 NT = 5 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 5.9800000 5.7800000 7.1100000 5.0400000 4.1500000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 4 3.6542210 38.04 .563586

Error 10 .96057130E-01 5.6120000 5.52

PC SE RG

NR = 3 NT = 5 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 5.8000010 5.3700000 8.0200000 6.8700000 6.6300000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 4 3.1679080 21.62 .696058

Error 10 .14652100 6.5380000 5.85

BIOMASS PRODUCTION

PF BIO

NR = 3 NT = 5 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 63.500000 88.000000 74.703330 58.883330 58.753340

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 4 473.14260 21.08 8.61580

Error 10 22.449220 68.767990 6.89

PE BIO

NR = 3 NT = 5 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 43.200000 57.500000 60.900000 65.300000 77.000000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 4 452.50980 36.54 6.39920

Error 10 12.383980 60.780000 5.79

PD BIO

NR = 3 NT = 5 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 85.000000 83.000000 88.500000 71.000000 49.800000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 4 747.17770 30.65 8.97789

Error 10 24.375780 75.459990 6.54

PC BIO

NR = 3 NT = 5 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 49.500000 47.200000 58.300000 33.000000 28.900000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	4	444.05030	56.71	5.08837		
Error	10	7.8300780		43.380000	6.45	

MYCELIAL PROTEIN CONTENT

PF mycelial protein

NR = 3 NT = 5 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 1.2060000 1.2930000 2.2800000 2.0800000 1.8300000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	4	.67518040	17.56	.356536		
Error	10	.38442990E-01		1.7378000	11.28	

PE mycelial PROTEIN

NR = 3 NT = 5 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 1.5180000 1.5680000 2.0300000 2.0436670 2.5530000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	4	.53328800	7.51	.484466		
Error	10	.70980070E-01		1.9425330	13.72	

PC mycelial protein

NR = 3 NT = 5 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 .99000000 1.6753330 1.9433330 1.1760000 1.1100000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 4 .50338410 11.25 .384602

Error 10 .44733430E-01 1.3789330 15.34

PD mycelial protein

NR = 3 NT = 5 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 1.0233330 1.1900000 1.9746670 1.9200000 1.9000000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 4 .62505630 16.03 .359127

Error 10 .39003750E-01 1.6016000 12.33

MYCELIAL SELENIUM CONTENT

PFMSE

NR = 3 NT = 5 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 2.2000000 240.66670 409.28000 579.81000 880.61000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 4 333376.10 631.24 41.7892

Error 10 528.12500 422.51330 5.44

PEMSE

NR = 3 NT = 5 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 .21000000 210.38000 394.22000 562.00000 850.27000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 4 318562.50 377.66 52. 8134

Error 10 843.52500 403.41600 7.20

PCMSE

NR = 3 NT = 5 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 1.9000000 206.13000 372.64000 527.91000 792.72000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	4	274094.70	392.80			48.0353
Error	10	697.80000		380.26000		6.95

PDMSE

NR = 3 NT = 5 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 .39000000 270.21000 427.17000 608.13000 920.32000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	4	360815.90	441.53			51.9828
Error	10	817.20000		445.24400		6.42

SPAWN RUN

PF spawn run

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 31.000000 28.000000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	1	13.500000	.64	NS		
Error	4	21.000000		29.500000		15.53

PE spawn run

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 37.000000 39.000000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	1	6.0000000	.46	NS		

Error 4 13.000000 38.000000 9.49

PC spawn run

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 29.000000 32.000000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 1 13.500000 .79 NS

Error 4 17.000000 30.500000 13.52

PD spawn run

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 44.000000 44.000000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 1 .00000000 .00 NS

Error 4 14.000000 44.000000 8.50

NUMBER OF FRUIT BODIES

PF no FB

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 6712.0000 6800.0000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 1 11616.000 .12 NS

Error 4 94816.000 6756.0000 4.56

PE no FB

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 4000.0000 4200.0000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 1 60000.000 .60 NS

Error 4 100000.00 4100.0000 7.71

PC no FB

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 6544.0000 6631.0000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 1 11344.000 .12 NS

Error 4 91540.000 6587.5000 4.59

PD no FB

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 8500.0000 8612.0000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 1 18816.000 .16 NS

Error 4 121288.00 8556.0000 4.07

AVERAGE WEIGHT OF FRUIT BODY

PF avg wt

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 9.6200000 9.7800000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 1 .38452150E-01 .04 NS

Error 4 .92860410 9.7000000 9.93

PE avg wt

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 6.7900000 7.2400000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	1	.30374150	1.60	NS		
Error	4	.18979640		7.0150000	6.21	

PC avg wt

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 5.0600000 5.5400000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	1	.34561160	1.57	NS		
Error	4	.21969600		5.3000000	8.84	

PD avg wt

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 3.4300000 3.5000000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	1	.73547360E-02	.04	NS		
Error	4	.16474910		3.4650000	11.71	

BIOLOGICAL EFFICIENCY (%)

PF BE

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 64.570000 66.504000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 1 5.6074220 1.45 NS

Error 4 3.8706050 65.537000 3.00

PE BE

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 27.160000 30.408000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 1 15.824710 2.07 NS

Error 4 7.6402590 28.784000 9.60

PC BE

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 33.110000 36.735000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 1 19.710940 2.38 NS

Error 4 8.2696530 34.922500 8.23

PD BE

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 29.155000 30.142000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 1 1.4614260 .16 NS

Error 4 8.9367680 29.648500 10.08

TOTAL PROTEIN CONTENT OF FRUIT BODIES

PF fb protein

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 1.4133330 1.9833330

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	1	.48735050	13.87	.424953		
Error	4	.35133360E-01		1.6983330	11.04	

PE fb protein

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 1.6000000 2.5533330

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	1	1.3632680	8.15	.927504		
Error	4	.16736650		2.0766670	19.70	

PC fb protein

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 1.3800000 2.1633330

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	1	.92041780	17.92	.513836		
Error	4	.51367280E-01		1.7716670	12.79	

PD fb protein

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 3.6000000 4.5633340

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	1	1.3920140	6.16	NS		
Error	4	.22586820		4.0816660	11.64	

TOTAL PHENOLIC CONTENT OF FRUIT BODIES

PF phenol

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 .42333330 2.4400000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	1	6.1004160	138.75	.475383		
Error	4	.43966770E-01		1.4316670	14.65	

PE phenol

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 .66333340 1.2800000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	1	.57041740	16.79	.417837		
Error	4	.33966540E-01		.97166660	18.97	

PC phenol

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 .96000000 2.0000000

ANOVA TABLE

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)	G.M.	C.V.
Treatments	1	1.6223990	38.17	.467386		
Error	4	.42500020E-01		1.4800000	13.93	

PD phenol

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 1.1600000 4.0800000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 1 12.789600 235.97 .527815

Error 4 .54200170E-01 2.6200000 8.89

FLAVONOID CONTENT OF FRUIT BODIES

PF flavo

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 225.00000 512.53330

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 1 124013.00 406.04 39.6216

Error 4 305.42190 368.76670 4.74

PE flavo

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 250.00000 425.00000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 1 45937.500 44.66 72.7083

Error 4 1028.5000 337.50000 9.50

PC flavo

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 275.00000 500.00000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 1 75937.500 84.42 67.9958

Error 4 899.50000 387.50000 7.74

PD flavo

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 50.000000 250.00000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 1 60000.000 209.06 38.4081

Error 4 287.00000 150.00000 11.29

RADICAL SCAVENGING ACTIVITY (%)

PF dpph

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 8.9782330 10.543330

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 1 3.6742550 9.48 1.41120

Error 4 .38745120 9.7607830 6.38

PE dpph

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 2.0080000 4.4003330

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 1 8.5848920 107.19 .641600

Error 4 .80087660E-01 3.2041670 8.83

PC dpph

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 7.5600000 10.720000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 1 14.978360 90.59 .921884

Error 4 .16534420 9.1400000 4.45

PD dpph

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 7.5313330 9.1553340

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 1 3.9560850 23.58 .928623

Error 4 .16777040 8.3433330 4.91

SELENIUM CONTENT OF FRUITBODIES

Stawse

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 .59000000E-01 22.340000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 1 744.66450 61.60 7.88235

Error 4 12.087830 11.199500 31.04

PFFBSE

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 2.4800000 124.00000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 1 22150.670 233.83 22.0659

Error 4 94.728520 63.240000 15.39

PEFBSE

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 .49000000 57.250000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 1 4832.5460 60.04 20.3394

Error 4 80.484620 28.870000 31.07

PCFBSE

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 .16000000 46.250000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 1 3186.4320 48.95 18.2918

Error 4 65.095340 23.205000 34.77

PDFBSE

NR = 3 NT = 2 NE = 1

Analysis of CRD with equal no. of replications

TREATMENT MEANS

1 4.3333330 156.60000

ANOVA TABLE

SOURCE d.f. M.S. F-Ratio CD(5%) G.M. C.V.

Treatments 1 34777.700 394.00 21.3001

Error 4 88.267580 80.466670 11.68

VITA

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