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THE EFFECT OF SODIUM SELENATE APPLICATION ON GROWTH OF THE FRUITING BODIES IN THE FIRST FLUSH OF MUSHROOM *PLEUROTUS OSTREATUS* (JACQ.) P. KUMM

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Pleurotus spp. is in the top three of the most widely grown mushrooms in the world production. It is known that mushrooms are able to accumulate most of the substances found in the culture medium very well. Selenium is a significant antioxidant and plays a very important role in the prevention of various types of diseases. This paper points to the possibility of enriching the culture medium of *Pleurotus ostreatus* (Jacq.) P. Kumm. with inorganic selenium. The aim is to obtain a biologically active food applicable to wide population nutrition. Using such foods as nutraceuticals can make a significant contribution to the positive influence of human health.

Keywords: *Pleurotus ostreatus*, nutraceutical, selenium, fortification

Selenium (Se) is an essential element with antioxidant effects. It is an important component of several major metabolic reactions, including synthesis of thyroid hormones, antioxidant defense systems and immune functions (Kohrle and Gartner, 2009; Gandhi et al., 2013). The essentiality of selenium was demonstrated in 1957 (Hegedűs et al., 2006; Hegedűs et al., 2007). In 1976, the necessity of selenium for humans was proven, despite the fact that its negative effects were highlighted previously (Hegedűs et al., 2008; Jakabová et al., 2008; Jakabová et al., 2009). Selenium is effective at low concentrations in heavy metal poisoning such as mercury and arsenic (Ralston and Raymond, 2010). The World Health Organization's report recommends taking 55–65 μg selenium per day. This dose is necessary to ensure the optimal functioning of the adult human organism. The upper limit of the daily dose for adults is 400 μg (FAO/WHO, 2002). Mushrooms as a part of food can have antioxidant effects related to the selenium and phenolic compounds content (Werner and Beelman, 2002; Beelman and Royse, 2006; Wong and Chye, 2009). Cultivation of saprophytic fungi on substrates rich in selenium may be an effective means of producing food with built-in selenium. Selenium plays an important role in the functioning of the catalytic center of several selenoproteins (Rayman, 2000). It is the only trace element identified in the genetic code as selenocysteine (Sec), which was recognized as the 21th amino acid (Rayman, 2002).

Oyster mushroom occurs mainly in European forests, where it is completely autochthonous. Production period is from the end of summer, through autumn till winter. These edible fungi can sometimes be found through to cold months of January and February. Oyster mushroom is a pleasant, slightly spicy mushroom (O'Reilly, 2011). Oyster mushroom was first scientifically described in 1775 by the Dutch naturalist Nikolaus Joseph Freiherr von Jacquin (1727–1817), under the name Agaricus ostreatus. It is a fungus with variable size, shape and colour. There are a lot of strains in the world; therefore, the identification of this species is relatively difficult. In practice, there are most common cross-species and bred strains of native species, which differ from wild strains mainly in the growth rate of fruiting bodies, yields, and other specific properties. For successful mass production, it is essential for a farmer himself/herself to realize that oyster mushroom grows in situ on wood, above ground, in damp and light conditions, predominantly in deciduous forests (Kurtzman, 2014).

Material and methods

Biological material

Oyster mushroom strain KRYOS B was provided from the Department of Horticulture, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences in Prague. Nowadays, this model strain is commonly used in mass production.

Selection of selenium concentration levels

Concentrations of selenium were derived from the results of the research tasks, which were solved at the Department of Vegetables Production of the Horticulture and Landscape

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Engineering Faculty of the Slovak University of Agriculture in Nitra. The required dose of sodium selenate for the preparation of the fortification solutions with 0.5 mg.dm^{-3} Se, 1.0 mg.dm^{-3} Se and 2.0 mg.dm^{-3} Se was calculated. Growth of oyster mushroom at selected selenium concentrations was verified in partial experiments on agar nutrient medium before the main experiment was established. The ability of radial growth of mycelium was evaluated optically.

Establishment of experiments to produce oyster mushroom fruiting bodies

Mushroom production was based on controlled cultivation conditions in the premises of The AgroBioTech Research Centre, in the Laboratory of Explant Cultures headed by Ing. Filová, PhD., in two cultivation periods. In accordance with existing knowledge, the production of mushrooms proceeded in the following phases:

- inoculum preparation,
- preparation of the substrate pasteurization, 48 hours at 25 °C, then 24 hours at 60 °C),
- inoculation by inoculum and incubation (mycelium substrate growth) at 25 °C about 2 weeks,
- initiation of the fruiting bodies at 11 °C for one day,
- fructification (12 hours, 16 °C in dark and 12 hours, 16 °C under light).

During all phases there was ensured optimal cleanliness of production premises, tools and equipment, personal hygiene, optimal relative air humidity and CO_2 content. The experiment consisted of 4 variants, each variant had 10 repetitions. The whole experiment was carried out in two cultivation periods in the following terms:

1. First cultivation period – from 4 April 2016 to 26 May 2016.
2. Second cultivation period – from 9 June 2016 to 27 July 2016

Created variants of experiments:

Preparation of all substrates consisted of wetting of dry straw pellets in water in a weight ratio of 1 : 2.6.

C – control – 0.0 mg.dm^{-3} Se.
X – with addition of 0.5 mg.dm^{-3} Se in the form of sodium selenate aqueous solution.
Y – with addition of 1.0 mg.dm^{-3} Se in the form of sodium selenate aqueous solution.
Z – with addition of 2.0 mg.dm^{-3} Se in the form of sodium selenate aqueous solution.

The ratios of the components in the growing substrates are given in Table 1.

Growing of fruiting bodies: The first cycle conditions: duration 12 hours, temperature 16 °C, relative air humidity 90%, dark, intense ventilation. The second cycle conditions: duration 12 hours, temperature 16 °C, relative air humidity 90%, light, intense ventilation.

Determination of selenium content

The homogenate of the lyophilized samples of fruiting bodies was mineralized in a "CEM Mars X" (microwave digestion oven). The total selenium content was determined by electrothermal atomisation (ET-AAS) with Zeeman correction on SpectrAA240FS (Varian, Mulgrave Virginia, Australia). Assay conditions: Selenium cathode lamp – current on the lamp 10 mA, wavelength 196 nm, slot width 1.0 nm. The atomizing medium was a graphite cuvette heated to 2,600 °C. The volume of the sample was 10 µl. The palladium modifier $Pb(NO_3)_2$ with a concentration of 0.1 mol.dm^{-3} was used and 1% ascorbic acid. The results were evaluated by the calibration curve method. CertiPur stock calibration solutions (Merck, Germany) had been prepared in advance with well-known concentrations of the heavy metals to be monitored. The final values of the measured parameters were subsequently obtained by software translating the calibration curve with the absorbance of the monitored analyte in the sample.

Statistical processing methods

Statgraphics Centurion XVII – multifactor analysis of variance (MANOVA, LSD test).

Results and discussion

Based on the experiments solved in collaboration with Ing. Jablonský from the Czech University of Life Sciences in Prague, we used a substrate consisting of a pelletized wheat straw, which is used as a litter for horses in practice. This substrate has a satisfactory quality and is easy to manipulate. The straw is pressed into the pellets without the addition of any binding agents. Selection of the substrate corresponds with authors such as Choi (2004), Lin (2004), Poppe (2004), Mandeel (2005), Yildiz et al. (2006) and others, who claim that wheat, barley straw and other lignocellulosic materials are ideal substrates for intensive production of oyster mushrooms.

Table 1 Ratio of components in the substrate for one growing container

Variant	Dry pellets : water : inoculum	Weight of substrate in kg	Weight of dry pellets in kg	Water volume in dm^{-3}	Weight of inoculum in kg
C	1 : 2.6 : 0.189	2	0.52	1.37	0.1
X	1 : 2.6 : 0.189	2	0.52	1.37	0.1
Y	1 : 2.6 : 0.189	2	0.52	1.37	0.1
Z	1 : 2.6 : 0.189	2	0.52	1.37	0.1

Source: author
Explanatory notes: C – 0.0 mg.dm^{-3} Se; X – 0.5 mg.dm^{-3} Se; Y – 1.0 mg.dm^{-3} Se; Z – 2.0 mg.dm^{-3} Se

Quantitative evaluation of fruiting bodies production

In the experiment there was evaluated the first flush of oyster mushroom on experimental substrates. These results were statistically processed, but did not reflect the total production potential of these substrates. The first harvests of the first flush of the first cultivation period were carried out 7 days after the start of the breeding phase, on four substrates of the variant Z (2.0 mg.dm^{-3} Se) and one substrate variant Y (1.0 mg.dm^{-3} Se). The last harvests of the first flush were carried out 14 days after the start of the breeding phase on the control variant C.

This phenomenon indirectly correlates with the findings of authors Upadhyay and Hofrichter (1993), Magae (1999), Sugimoto et al. (2001), Domondon et al. (2004), Berne et al. (2007), who worked on accelerating the formation of fruiting bodies germs and the subsequent fructification of substrates. The principle of accelerating fertility is based on the induction of a variety of stress types, which induce the mushroom to rapidly create a new population. It results from this, that by adding sodium selenate into the substrate, the fertility of the substrates may be accelerated, which agrees with the above-mentioned authors. After the first flush of the first cultivation period, substrates were removed from the cultivation boxes and moved to the botanical garden of the Department of Vegetables Production HLEF Horticulture and Landscape Engineering Faculty due to the time consuming of the experiment. The discarded substrates were regularly irrigated with a large dose of water, and the potential of the second and the third flush was monitored. It has been found that substrates are able to breed in other flushes. Parallel to the transfer of the first cultivation period substrates from the cultivation boxes, a second cultivation period was established.

In Table 2, we report the results fertility of substrates in terms of yields in the first flush per cultivation period and average for both cultivation periods.

In the first cultivation period of the experiment, it was statistically confirmed that in the control variant without selenium application the lowest average yield per substrate marked 217.46 g. The highest average yield of the first production period reached substrates in the variant Y with 1.0 mg.dm^{-3} Se, i.e. 261.44 g. In the second cultivation period, the highest yield was in the control variant C, i.e. 367.09 g of fresh fruiting bodies per substrate. The lowest yield was determined in the variant X with 0.5 mg.dm^{-3} Se (281.66 g). No statistically significant differences on average were found between the variants in both cultivation periods, the lowest yields were determined in the variant X with 0.5 mg.dm^{-3} Se (268.72 g) and the highest in the control variant C (292.28 g). If we consider the average yields without selenium application as 100% then the average yields in the variant X are lower by 16.33%, in the variant Y are lower by 3.24% and the yields in the variant Z are lower by 2.2%. On the basis of the above, we note that fortification with selenium does not have a significant effect on the height of the yield of fresh fruiting bodies in the first flush. For further research, it is advisable to verify the production potential of selenium enriched substrates under conditions of intensive production of edible mushrooms. Adebayoa et al. (2017) examined the flushes of the genus *Pleurotus*. In the case of *Pleurotus ostreatus* they found that the first flush represented 32.97% (121 g), the second 38.15% (140 g) and the third 28.88% (106 g) of the total yield 367 g. It is possible to assume that about 1/3 of the production potential was used in our conditions. In the next experiment, the production potential of the second and third flush should be evaluated.

Selenium content in the oyster mushroom fruiting bodies

The results of our experiments show that oyster mushroom is able to cumulate the inorganic form of sodium selenate, which was applied into the substrate in the form of a fortification solution. While lyophilized fruiting bodies in the control variant C in the first production period contained 0.116 μg.g^{-1} Se, in the variant Z fruiting bodies accumulated 699% more, i.e. 0.927 μg.g^{-1} Se. In each production period there was statistically confirmed the increasing accumulation of selenium in all variants, which was due to a higher concentration of fortification solution. The control variant C in the second production period contained 0.073 μg.g^{-1} Se, the variant Z accumulated 986% more Se (0.739 μg.g^{-1} Se). Similarly, on average, for both cultivation periods, the ability to accumulate selenium was statistically confirmed. We show in the Table 3 the average selenium content determined in lyophilized fruiting bodies of oyster mushroom in the first and the second production period.

The potential creation of a functional food by incorporating an inorganic selenium salt was described in 2008 by Falandysz. Our results are consistent with the

Table 2 Average yields of fresh fruiting bodies in the first flush in g

***Pleurotus ostreatus* KRYOS B**			
Variant	1. cultivation period	2. cultivation period	average
C	217.46 ±37.48a	367.09 ±46.36c	292.28 ±105.80a
X	255.78 ±43.84b	281.66 ±54.42a	268.72 ±18.30a
Y	261.44 ±31.05b	304.19 ±22.14ab	282.82 ±30.22a
Z	240.83 ±25.66ab	330.85 ±41.01bc	285.84 ±63.64a

Source: author
P <0.05 by LSD ANOVA
Explanatory notes: C – 0.0 mg.dm^{-3} Se; X – 0.5 mg.dm^{-3} Se; Y – 1.0 mg.dm^{-3} Se; Z – 2.0 mg.dm^{-3} Se according to Means and 95.0. Percent LDS Test. The values in the columns with different letters are significantly different from each other

Table 3 Average selenium content in lyophilized fruiting bodies in μg.g^{-1}

Pleurotus ostreatus KRYOS B			
Variant	1st cultivation period	2nd cultivation period	average
C	0.116 ±0.041a	0.073 ±0.006a	0.094 ±0.036a
X	0.437 ±0.069b	0.198 ±0.030b	0.318 ±0.132b
Y	0.443 ±0.069b	0.536 ±0.096c	0.489 ±0.094c
Z	0.927 ±0.185c	0.658 ±0.108d	0.793 ±0.202d

Source: author
Explanatory notes: C – 0.0 mg.dm^{-3} Se; X – 0.5 mg.dm^{-3} Se; Y – 1.0 mg.dm^{-3} Se; Z – 2.0 mg.dm^{-3} Se according to Means and 95.0. Percent LDS Test. The values in the columns with different letters are significantly different from each other

findings of the author. The ability of oyster mushroom to cumulate selenium was statistically demonstrated in both cultivation periods under model conditions. Falandysz (2008) reports that the concentration of selenium in fruiting bodies of frequently consumed edible mushrooms ranges from <1–20 μg Se.g^{-1} dry weight of fruiting bodies. However, this claim is inconsistent with our findings. The reality can be explained by the diverse chemical composition of the growing substrates. When cultivating mushrooms on crop residues from agricultural production from geographic locations with selenium contaminated soils, its built-up content is significantly higher in these substrates and hence also in mushrooms. The good availability of the thus incorporated selenium has been confirmed by Da Silva et al. (2012) and Rayman et al. (2007).

Bhatia et al. (2013) found that the content of selenium in saprophytic mushrooms grown on commonly available wheat straw with moderately low selenium content (according to Oldfield, 1999; Spadoni et al., 2007) is at the level of wild mushrooms, i.e. 0.12–3.4 μg Se.g^{-1} weight of the dried mushrooms *Pleurotus ostreatus*. The claim is consistent with our results. According to Kalac (2009) and Falandysz (2008), this value is similar to the selenium content of the commonly sold button mushrooms. Likewise, Da Silva et al. (2012), Estrada et al. (2009) and Wang et al. (2005) in their papers reported that mushrooms of the genus *Pleurotus* are able to accumulate selenium well. We fully agree with the statements, and the fact is confirmed in our model experiment.

Cubadda et al. (2010) investigated the ability of edible mushroom *Pleurotus florida* to cumulate selenium. They found that this mushroom is able to quickly mobilize and accumulate selenium from the substrate. Fruiting bodies yielded in the control variant without enrichment with selenium contained 0.17 μg Se.g^{-1} in the dry mass, while the fruiting bodies growing on the substrate enriched with large amount of inorganic selenium contained 800 times more Se (141 μg Se.g^{-1}) in a sample of dried mushrooms. The authors do not detail the concentration level of the used fortification solutions, but their findings correspond to the conclusions of our work.

Gąseckaa et al. (2015) reported that the concentration of selenium in the control samples of oyster mushroom fruiting bodies (without added selenium) was 2.54 ±0.22 μg Se.g^{-1} dry weight in their experiment. This assertion is inconsistent with our results, because the samples presented in the control variant of our experiment contain 2 602% less selenium (0.094 μg Se.g^{-1}) in the dry matter. This feature is probably caused by the diversity of the growing substrates, i.e. by growing on the substrates rich in selenium. The authors note that the next addition of selenium into the substrate led to an increase its accumulation in fruiting bodies. It follows that by increasing the selenium content in the substrate, the ability of its accumulation with an edible fungi increase. However, it should be pointed out that the accumulation potential can vary greatly between species and strains of mushrooms.

Conclusion

In this work there was verified the possibility of fortification of the world's third most-cultivated fungus with selenium, which is an essential element in small quantities. In the first step, an optimal lignocelluloses substrate for the production of oyster mushroom was chosen, which is suitable for easy handling, is well-storable and has a high sorption capacity. In the second step there was verified the ability of oyster mushroom to colonize the agar nutrient media at selected selenium concentration levels. It was confirmed that none of the selected solution concentrations was lethal to oyster mushrooms. The main experiment showed that the fortification of the cultivation substrate of oyster mushroom in the first flush had no negative effect on yields of the fresh fruiting bodies. On the contrary, it was found that fortified substrates initiated fruiting bodies production, thus shortening the time required for growing. Final analyzes confirmed that oyster mushroom has a high potential for selenium accumulation. It can therefore serve as a tasty, biologically active foodstuff with a positive effect on human health of wide population.

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