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GROWTH AND FRUIT BODY FORMATION OF *PLEUROTUS OSTREATUS* ON MEDIA SUPPLEMENTED WITH INORGANIC SELENIUM

ABSTRACT: Selenium is a trace mineral chemically related to sulfur and tellurium. In the body selenium combines with protein molecules to form selenoproteins and it is distributed in low concentrations and unequally in air, soil and water all over the world. Edible mushrooms are known to be selenium accumulators. Since mushrooms contain relatively high protein levels, and they can accumulate large amounts of selenium, it is reasonable to expect that selenium could be incorporated into proteins. The growth of mycelia and fruit body formation of different medicinal mushroom strains of *Pleurotus ostreatus* (Hk-35 and P_{70}) over the wide range of concentrations of inorganic form of selenium were examined. Mushrooms were cultivated on agar base media and on substrates based on sawdust. Vegetative growths of mycelium were measured as colony diameter in pure cultures supplemented with inorganic form of Se supplements, prepared as Na_2SeO_4 and Na_2SeO_3 in concentrations of: 1, 10, 25, 50, 75, 100 and 150 mg/l. Inorganic form of Se supplements, showed stimulation effects (in concentration of 1—50 mg/l) and toxic effects in higher concentration. On the standard industrial sawdust based substrate, supplemented with 100 mg/kg Na_2SeO_4 and Na_2SeO_3, accumulation of Se in fruit bodies was determined by the method of flameless atomic absorption spectrophotometer. The readings were performed on Varian SpectrAA-10 spectrophotometer equipped with VGA-76. Se as Na_2SeO_4 and Na_2SeO_3 was effectively taken up from substrates and accumulated in fruit bodies. Mushrooms accumulated selenium between 120 and 250 mg/kg dry weight. In mushrooms cultivated without Se supplement, Se contents were only about 1 mg/kg and in substrate about 0.1 mg/kg.

KEY WORDS: *Pleurotus ostreatus*, Selenium, Spectrophotometer, Substrate

INTRODUCTION

Selenium is a trace mineral chemically related to sulfur and tellurium. In the body selenium combines with protein molecules to form selenoproteins. Selenium deficiency is linked with numerous diseases: cancer, muscular dystrophy, malaria and cardiovascular disease including endemic cardiomyophaty

in selenium deficient regions (Nassir et. al., 1997). Selenium is distributed in low concentrations and unequally in air, soil and water all over the world (Barceloux, 1999). The soil in some parts of China and Russia has scant amounts of selenium. The amount of Se in soil, which varies by region, determines the amount of Se in the plant foods and meat. The daily value recommended for selenium by the FDA is 70 μg. When supplements are used, 100—200 μg daily of selenomethionine is recommended. Amounts greater than 200 μg should be avoided.

Fungi are known as accumulators some chemical elements, like Zn, Cd, As etc. Edible mushrooms are known to be selenium accumulators. Since mushrooms contain relatively high protein levels, and they can accumulate large amounts of selenium, it is reasonable to expect that selenium could be incorporated into the proteins (Gergely, 2006). The genus *Pleurotus* (higher *Basidiomycetes*) includes edible and medicinal species, some of them are cultivated and have important economical value and some have important medicinal properties: reduction of cholesterol levels, antitumor, antiviral, antibacterial and immunomodulating activity. *Pleurotus* species may present an excellent dietary source of some microelements because of their ability to absorb them from medium (Stajić, 2001).

The aim of the study was to determine the contents of mineral element (Se) in the cultivated mushrooms *Pleurotus ostreatus*.

MATERIAL AND METHODS

Growth and fruit body formation on media supplemented with selenium was investigated on mushroom strains (Faculty of Agriculture collection): *Pleurotus ostreatus* (Hk-35, P_{70}). Cultures were maintained at 4°C on malt agar base contained in screw slants with periodic transfers to fresh agar base (pH = 5.4 ± 0.2).

The response of fungi to inorganic selenium supplements (Faculty of Chemistry, University of Belgrade) was examined. Inorganic form of selenium supplements were prepared as Na_2SeO_4 and Na_2SeO_3 (1 mg/l, 10 mg/l, 25 mg/l, 50 mg/l, 75 mg/l, 100 mg/l, 150 mg/l) in Petri dishes with three replicates per treatment.

After agar sterilization at high preasure in an autoclave for 15 minutes at 121°C, the fragments of mycelia were inoculated and incubated at 25°C in the dark. The diameters of the formed colonies were determined 9 days after inoculation in order to assess the effects of Se on mycelial growth.

Seed for bags inoculation were prepared in glass jar, on wheat grain. Grains were cooked in water and dried with filter paper. To adapt pH at 6—7, CaCO3 was added into wheat. The prepared grains were put into jams and sterilized in autoclaves for 40 minutes at 121°C. After cooling, grains were inoculated with fragments from mycelia and incubated at 25°C in the dark for about 4 weeks, depending on the mushroom species and strains.

The substrates for *P. ostreatus* cultivation were prepared with sawdust (42%) as basic substratum (100%) and straw (29%), chopped oik (24%) and

gypsum (5%) were added to the basic substratum. The moisture content of substrate was adjusted to about 60%. Polipropylen bags contained substrate (1.5 kg/bag).

Selenium as Na_2SeO_3 and Na_2SeO_4 solution was supplemented at 100 mg/kg into 5 bags. The bags were sterilized, inoculated with seed and incubated at 26°C under 85% humidity in the dark for 20 days to allow full colonization of mycelia. All bags were then transferred to the green house for fruiting under daily irrigation and attenuated illumination. The substrate blocks were exposed to the temperature of 21—23°C and humidity 60—80%. The fruit bodies of both strains, developed completely and discharged spores from 50—60 days after the inoculation. The fresh mushrooms were stored frozen in boxes and kept at –20°C for a while. Melt fruitbodies of mushrooms were cut into pieces, dried at hot air (80°C, for few days) and then ground into a consistent powder.

The total selenium content in soil samples was determined by the method of flameless atomic absorption spectrophotometer, after its digestion with acids (HNO_3 and $HClO_4$) and reduction of Se^{6+} to Se^{4+} with 6MHCl. The readings were performed on Varian SpectrAA-10 spectrophotometer equipped with VGA-76.

RESULTS AND DISCUSSION

The effects of inorganic supplements on mycelial growth of mushroom species on agar base media and sawdust substrates were examined. The obtained results showed that the investigated *Pleurotus* strains have different abilities to absorb selenium by mycelia from medium where it is present in the form of Na_2SeO_3, Na_2SeO_4 and in different concentrations. They also differ in their ability to retain selenium in fruit body.

The investigated selenium sources and concentrations had different effect on the production of mycelial biomass in the investigated *Pleurotus* strains. In some cases, selenium did not show any effects on production and it was the same as in the control medium. However, in some cases, the presence of selenium in medium caused either an increase or a decrease of mycelial biomass production in comparison with the control.

— Mycelial growth on agar base media was measured as colony diameter in pure cultures supplemented with Na_2SeO_3 and Na_2SeO_4. Na_2SeO_4 (1 mg/l, 10 mg/l and 25 mg/l) stimulated the growth of *P. ostreatus*, strain Hk-35 (Fig. 1).

Mycelia at both strains showed slight inhibition at 150 mg/l. Growth of strain P_{70} was inhibited with all tested concentrations (Fig. 2).

Na_2SeO_3 stimulated mycelial growth of *P. ostreatus* Hk-35 at 1 mg/l and 10 mg/l. Not any of tested concentrations (1—150 mg/l) showed inhibition by 50? for *P. ostreatus* strains.

— Selenium as Na_2SeO_4 was effectively taken up from the substrates and accumulated in fruit bodies. The mycelia grew normally and young primordia of *P. ostreatus* formed well. There was no difference in morphology of fruit bodies grown on substrates with and without selenium supplements. Accumu-

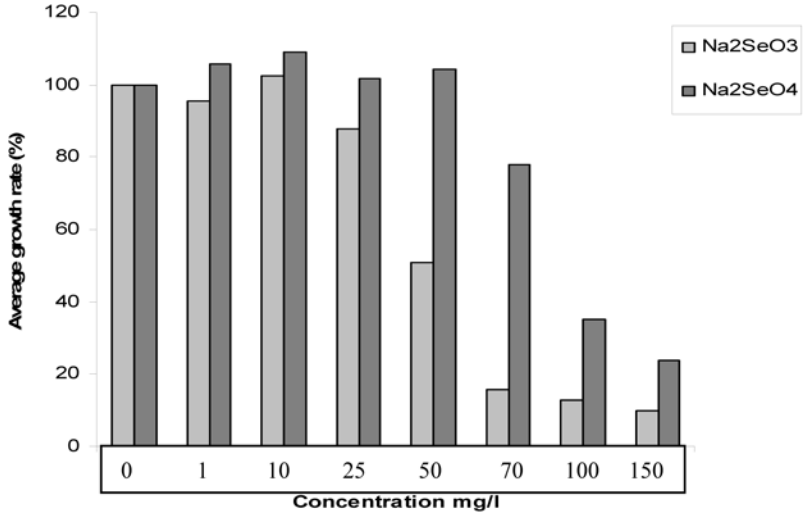

Fig. 1 — Mycelial growth of *Pleurotus ostreatus Hk-35* on agar base supplemented with Na_2SeO_3 and Na_2SeO_4

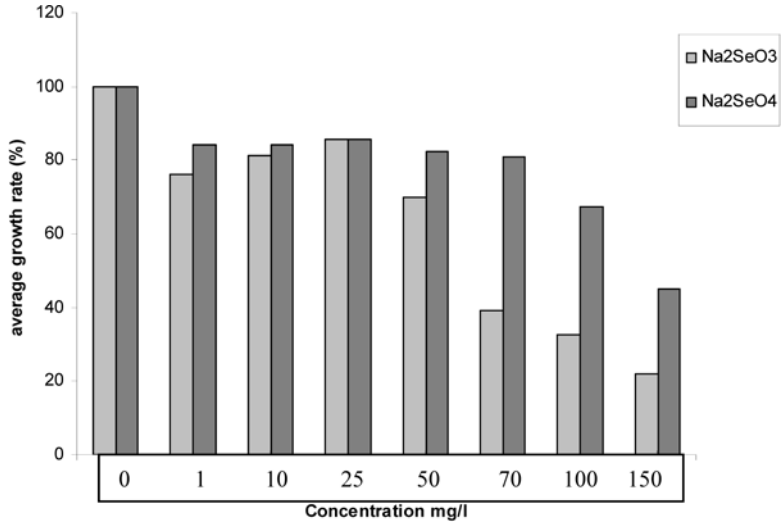

Fig. 2 — Mycelial growth of *Pleurotus ostreatus* P_{70} on agar base supplemented with Na_2SeO_3 and Na_2SeO_4

lation of selenium in fruit bodies was determined by electrochemical atomic absorbtion methods. The results showed that selenium as Na_2SeO_3 and Na_2SeO_4 was effectively taken up from the substrates and accumulated in fruit bodies. (Tab. 1)

Tab. 1 — Average content of selenium in mushroom fruit bodies (mg/g) grown on Se enriched substrate with 100 mg/kg d.w.

Mushroom strain	Natrium selenat	Natrium selenit	Control
Pleurotus ostreatus P70	169.3	213.0	0.85
Pleurotus ostreatus Hk-35	154.7	205.2	1.1

The total selenium content of the frozen and dried mushrooms depends on the mushroom strains and form of supplemented selenium. *P. ostreatus* accumulated selenium better from Na_2SeO_3 than from Na_2SeO_4. *P. ostreatus* accumulated selenium between 120 and 250 mg/kg. In mushrooms cultivated without Se supplement, Se content was 1 mg/kg, and in the control substrat it was 0.1 mg/kg.

CONCLUSION

Selenium enriched mushrooms are an excellent dietary source. Selenium as Na_2SeO_4 and Na_2SO_3 was effectively taken up from the substrates and accumulated in fruit bodies. Considering the foregoing results, further investigations can be made in the following areas:

— The study of selenium metabolic pathway and its forms, which are presented in *Pleurotus* species;

— Analyzing other cultivation media and finding the best one for maximum Se absorption by mycelia and the incorporation of organic selenium compounds in *P. ostreatus*;

— Considering both the medicinal properties of the *Pleurotus* species, and selenium antioxidative, antimutagenic and anticarcinogenic features, and the investigation of the effect of using Se-enriched fruit bodies in nutrition as well as their extracts.

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ПОРАСТ И ФОРМИРАЊЕ ПЛОДОНОСНИХ ТЕЛА ГЉИВЕ *PLEUROTUS OSTREATUS* НА СУПСТРАТУ ОБОГАЋЕНОМ НЕОРГАНСКИМ ЈЕДИЊЕЊИМА СЕЛЕНА

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Резиме

Селен је есенцијелни микроелемент, неопходан у малим количинама. Земљиште на територији Европе има низак садржај селена. Печурке садрже релативно висок ниво протеина, те стога могу да усвоје високе концентрације селена, који се инкорпорира у протеине формирајући селенопротеине. У раду је испитиван утицај већег броја различитих концентрација неорганских једињења селена на пораст и формирање плодоносних тела медицински значајне гљиве *Pleurotus ostreatus* (комерцијални сојеви Hk-35 и P_{70}). Пораст мицелијума на сладном агару праћен је мерењем пречника колоније чисте културе обогаћене неорганским једињењима селена. Коришћена неорганска једињења селена, у облику Na_2SeO_4 и Na_2SeO_3 (1—50 mg/l), стимулисала су пораст мицелијума, док су веће концентрације показале различит токсичан ефекат у зависности од концентрације додаваног једињења и соја гљиве. Печурке су затим гајене на хранљивом супстрату обогаћеном неорганским једињењима селена концентрације до 100 mg/kg. Садржај селена у плодоносним телима одређен је помоћу AAS (хидридни метод) након влажне дигестије и резултати су очитани на VarianSpectarAA-10 спектрофотометру са VGA-76 (помоћни апарат за испаравање) LSD тестом. Анализе су

показале да су гљиве упешно усвојиле селен у плодоносном телу. Тотални садржај селена у плодоносном телу печурака зависио је од испитиваног соја и врсте додаваног једињења. *Pleurotus ostreatus* је боље усвојила селен из Na_2SeO_3 него из Na_2SeO_4. Концентрација усвојеног селена се кретала између 120 и 250 mg/kg суве масе. У печуркама које су гајене на супстрату без додатка селена, садржај селена је износио свега око 1 μg/g, док се ова вредност за чист супстрат кретала око 0.1 mg/kg.