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Enrichment of *Pleurotus ostreatus* mushrooms with selenium in coffee husks

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ABSTRACT

Pleurotus ostreatus fungus forms an edible mushroom that possesses important nutritional and medicinal properties. Selenium (Se) is essential to human diets and it is in low concentration in the soil, and consequently in food. *P. ostreatus* was grown in coffee husks enriched with various concentrations of sodium selenite. The biological efficiency of *P. ostreatus* was affected by the addition of high concentrations of Se. The highest level of Se absorption was obtained by adding 51 mg kg^{1} of sodium selenite. The mushrooms from first flush contained more Se than the further flushes. These results demonstrate the great potential of coffee husks in the production of Se-enriched mushrooms and show the ability of this fungus to absorb and biomagnify Se.

1. Introduction

Mushrooms are highly appreciated for their flavour and have been well studied due to their nutritional and medicinal proprieties. *Pleurotus* mushrooms have high nutritional value and can be a good source of protein, carbohydrates, vitamins, calcium and iron (Schmidt, Wechsler, Nascimento, & Junior, 2003). Furthermore, these mushrooms have important medicinal properties, such as anti-tumour and immunostimulatory activity, as observed in rats (Sarangi, Ghosh, Bhutia, Mallick, & Maiti, 2006). The products derived from *Pleurotus* mycelia can promote biological responses during cancer treatment in humans and have been used as antitumourogenic drugs (Sarangi et al., 2006).

Pleurotus mushrooms have been grown in agro-industrial residues, such as banana waste (Reddy, Babu, Komaraiah, Roy, & Kothari, 2003), corn, bean and coffee (Dias, Koshikumo, Schwan, & Silva, 2003), and crop waste, such as soybean straw, cotton stalks, pigeon pea stalks and sugar cane remnants (Syed, Kadam, Mane, Patil, & Baig, 2009).

In Brazil, large amounts of agro-industrial residues are produced, including coffee husks, as Brazil is the top coffee producer in the world (~2800 million tonnes per year); coffee husks and peels comprise 50% of the grain (CONAB, 2010). This lignocellulolytic residue has been used as substrate in mushroom production (Dias et al., 2003). It is important to point out that the use of these residues in the production of mushrooms prevents their direct release into the environment, increases the producer's income and leads to food product with high nutritional quality.

Mushroom yields and their chemical composition can be affected by the substrates used in their growth (Shashirekha, Rajarathnam, & Bano, 2005). For instance, yields and chemical composition are enhanced by adding essential elements, such as Se, to the substrate. Addition of sodium selenite to the substrate used for growing *Ganoderma lucidum* resulted in a proportional increase of Se content in the mushrooms (Zhao et al., 2004).

Studies have revealed that Se is incorporated into the *P. ostreatus* biomass, as this element was found to be associated with the membrane (44%) and cell wall (56%). Se incorporation into fungal proteins reveals a great potential to improve the nutritional value of the mushroom (Munoz et al., 2006). In enriched mushrooms, the Se bioavailability was verified using *in vivo* methods. The higher levels of absorption of Se in rats fed with Se enriched mushrooms were verified by Silva et al. (2010), which compared these results with the ones achieved with rats with sodium selenate in their diets.

Due to the high demand of food across the world, its enrichment with essential micronutrients, such as Se, is crucial. However, Se can also be toxic when ingested in high concentrations (Gaso et al., 2000; Hartikainen, 2005). The recommended dose for an adult, male or female, is 55 μg day^{1} (IOM, 2000). Selenium has several physiological functions in protein activity, enhancing immune system function, reducing cancer risk (Finley, 2006), collateral effects of chemotherapy (Sieja & Talercszyk, 2004) and functional activity of cancer metastasis (Finley, Sigrid-Keck, Robbins, & Hintze, 2005). Thus, the aim of this work was to evaluate the use

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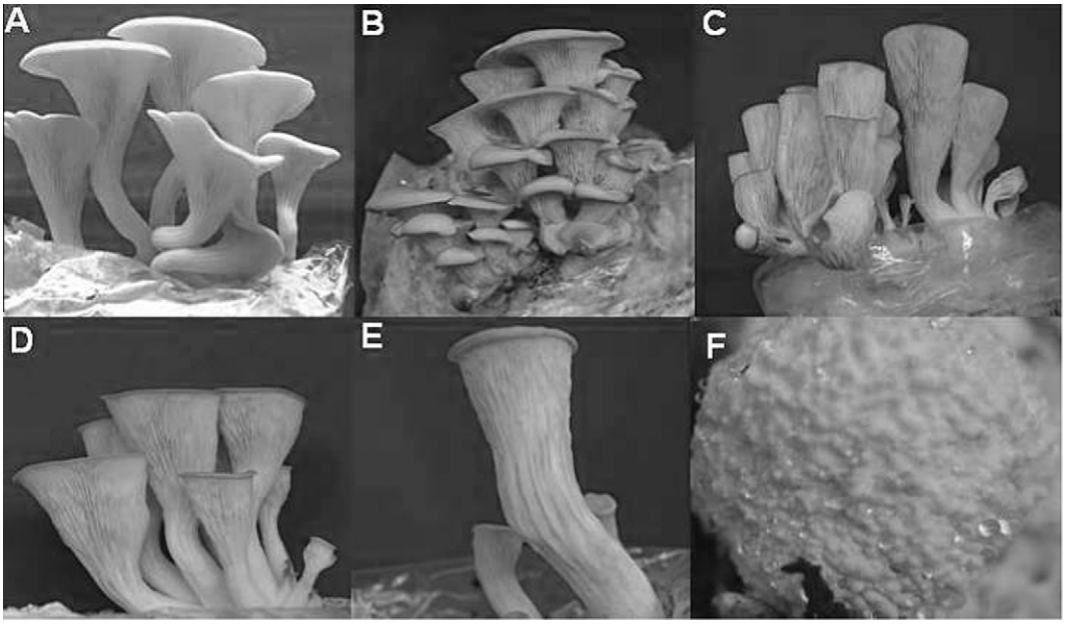

Fig. 1. Morphology of *Pleurotus ostreatus* mushrooms cultivated in coffee husk substrates in the absence (A) and presence of Se (6.4 mg kg^1) (B); 12.8 mg kg^1 (C); 25.4 mg kg^1 (D); 51 mg kg^1 (E); 102 mg kg^1 (F).

Table 1
Average incubation time for *Pleurotus ostreatus* in Se-enriched coffee husk substrates, with different Se concentrations, for three consecutive flushes.

Selenium (mg $kg^{1)}$	Flushes		
	1st	2nd	3rd
Days after inoculation			
0	23	35	43
3.2	26	48	60
6.4	23	49	60
12.7	28	52	60
25.4	39	54	65
50.9	40	54	61
76.4	36	52	66
101.8	36	56	79

of coffee husk in the production of *Pleurotus ostreatus* mushrooms enriched with selenium.

2. Materials and methods

2.1. Enrichment of the mushrooms

The fungus used was *P. ostreatus*, and inoculation was performed in rice cooked with water for 50 min and autoclaved at 121 °C for 2 h.

The coffee husk substrate was obtained from the Incofex Coffee Corporation, in Viçosa, Minas Gerais State, Brazil. The husk was boiled in water for 2 h, in order to reduce some compounds which could inhibit fungal growth and contaminants, and centrifuged at 1800 rpm for 5 min to remove excess water. Next, 1.5 kg of each sample were placed in polypropylene bags and autoclaved for 2 h. This procedure was repeated three times at 48-h intervals. The final humidity was 80%. At the end of the procedure, once the substrate reached room temperature, the inoculation with fungus spawn was performed.

For enrichment, a 5-mL volume of sodium selenite solution at various Se concentrations (3.2; 6.4; 12.8, 25.4; 51; 76.4; 102 mg kg^1) was added to packs containing coffee husks. A culture without Se was maintained for control purposes. The inoculated packs were incubated at 25 °C for 15 days. Fungi were placed at 20 °C and 90% air humidity, until mushroom formation. Mushrooms were collected during three flushing times, over a total period of 76 days.

2.2. Biological efficiency

The biological efficiency (BE) was calculated according to Wang, Sakoda, and Suzuki (2001):

$$BE = 100 \times (\text{fresh weight of harvested mushrooms}/\text{dry weight of the substrate}).$$

2.3. Sample preparation

Acid digestion was used to prepare the samples. Mushrooms were dried at 45 °C until they reached a constant weight and then were ground in a 2-mm sieve mill. A 200-mg mass of ground mushrooms was subjected to digestion in a microwave (model Microwave 3000, Anton Paar GmbH, Graz, Austria) oven in a diluted oxidant mixture (2.0 mL HNO_3 (Merck) + 1.0 mL H_2O_2 (Merck) + 3.0 mL H_2O). The microwave heating program includes four steps (temperature/°C; ramp/min; hold/min): 1 (140, 5, 1), 2 (180, 4, 5), 3 (200, 4, 10), 4 (0, 0, 20) (Naozuka & Oliveira, 2007; Naozuka, Vieira, Nascimento, & Oliveira, 2010). The coffee husks were also submitted to acid digestion using the procedure described above.

2.4. Nutrient contents

Ca, Pb, Cu, Fe, Mg, Mn, Zn, Cd, Cr and Ni determination in the digested solutions was performed by inductively-coupled plasma optical emission spectrometry (ICP-OES) using a Perkin Elmer Optima 3.300 DV™ spectrometer (Norwalk, CT). Solutions of each element were prepared from analytical reagent-grade chemicals (Merck), using high-purity water obtained from a Milli-Q water purification system (Millipore, Bedford, MA) (Naozuka et al., 2010).