Journal of Food Research; Vol. 5, No. 3; 2016
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Mycelial Growth, Biomass Production and Iron Uptake by Mushrooms of *Pleurotus* species Cultivated on *Urochloa decumbens* (Stapf) R. D. Webster

Olusola Clement Ogidi[1,2], Mateus Dias Nunes[2], Victor Olusegun Oyetayo[1], Bamidele Juliet Akinyele[1] & Maria Catarina Megumi Kasuya[2]

[1] Department of Microbiology, Federal University of Technology, PMB 704, Akure, Ondo State, Nigeria

[2] Departamento de Microbiologia/BIOAGRO, Universidade Federal de Viçosa, Viçosa, Minas Gerais 36570-000, Brazil

Correspondence: Maria Catarina Megumi Kasuya, Departamento de Microbiologia/BIOAGRO, Universidade Federal de Viçosa, Viçosa, Minas Gerais 36570-000, Brazil. E-mail: mkasuya@ufv.br

Received: February 28, 2016 Accepted: March 17, 2016 Online Published: May 11, 2016

doi:10.5539/jfr.v5n3p13 URL: http://dx.doi.org/10.5539/jfr.v5n3p13

Abstract

Mushrooms absorb minerals and are able to accumulate them as functional organic compounds during growth. Iron is one of the elements required for various metabolic processes but low intake has resulted to some nutritional deficiencies. This study investigated the effect of different iron concentrations in the culture media on mycelial growth, biomass production, hyphae diameter and distance between septa of six species of *Pleurotus,* namely *Pleurotus ostreatus; Pleurotus cornucopiae*; *Pleurotus djamor*; *Pleurotus pulmonarius*, and *Pleurotus djamor* v. *roseus*. These macrofungi were further grown on substrate based *Urochloa decumbens* with addition of Fe (0, 500 and 1000 mg Kg^{-1}). The mycelial growth rate and biomass produced by *Pleurotus* species decreased ($P<0.05$) as iron concentration increased from (0 - 100) mg L^{-1}. This shows an inhibitory interaction between the fungi and iron at higher concentration. The hyphae diameter and distance between septa of the examined fungi were respectively ranged from 45 μm to 80 μm and 40 μm to 119 μm at iron concentration of (0 -100) mg L^{-1}. The estimated iron uptake in cultivated *Pleurotus* species ranged from 37.8 μg g^{-1} to 96.6 μg g^{-1} when 500 mg Kg^{-1} and 1000 mg Kg^{-1} of Fe was added to the substrate. The cultivation of edible macrofungi enriched with iron could improve the socio economic status and play a nutritional role in decreasing anemia.

Keywords: agro wastes, mushroom cultivation, macronutrient, supplementation, anemia

1. Introduction

Iron is an essential element that is involved in synthesis of many important cell components such as adenine tri-phosphate (ATP), transport of oxygen, deoxyribonucleic acid (DNA) and electron transport (Abbaspour et al., 2014). In human, iron is a component of hemoglobin and myoglobin but when it is low in supply, it leads to nutritional deficiency called iron deficiency anemia (IDA). Iron deficiency anemia has been one of the major public health problems in under developing and developing countries, particularly among pregnant women, nursing mothers and the newborn babies (Kassebaum et al., 2014).

Iron is one of the most abundant metals on the earth but it is not readily available (Philpott, 2006). Although, most of the microbes have developed various regulated system for iron uptake, utilization and storage (Haas, 2004) but some food crops show low absorption capability for iron. The diminishing quantity of iron in food has tremendously contributed to the increase in malnutrition (Zhang et al., 2015), which therefore require a reliable and alternative source of iron supply in foods to enhance the promotion of good health.

Edible mushrooms are source of nutrient that enhances health benefits of individual. The ability of *Pleurotus* species to breakdown complex organic compounds and transform metals into biologically active compounds has significantly influenced their nutritional status, and also serves as complementary medicine to prevent degenerative diseases (Da Fonseca et al., 2015). Mushroom biotechnology is a renewed interest towards producing edible mushrooms fortified with minerals, contributing to the bioavailability of essential nutrients (De Assunção et al., 2012; Silva et al, 2012; Nunes et al., 2014) and thus, prevents the symptoms of micronutrient deficiencies.

Mushrooms of *Pleurotus* spp. can be easily cultivated on many cellulolytic substrates (Da Luz et al., 2012). Hence, *Pleurotus* spp grown on agro-waste residues with addition of minerals is not only an alternative means of recycling the wastes residues containing metals but of producing mineral-enriched mushrooms in an ecofriendly environment. The production of *Pleurotus* spp. mushrooms have been an integral part of food security due to the presence of essential food components such as proteins, carbohydrates, minerals; calcium, phosphorus, nitrogen, iron and vitamins (Ganeshpurkar et al., 2010). In essence, *Pleurotus* spp could be a source of macro and micronutrient that can be substituted for food deficient in some essential minerals (Falandysz & Borovička, 2013). The bioaccumulation of essential minerals in *Pleurotus* species had built a comprehensive understanding toward strengthening the bioavailability of nutrients in edible mushrooms. This study therefore investigates the effect of iron on the mycelial growth, biomass production and uptake of iron by six species of *Pleurotus* cultivated on iron-fortified substrate *Urochloa decumbens*.

2. Materials and Methods

2.1 Microorganism

The studied fungi were *Pleurotus djamor* (PLO13), *Pleurotus ostreatus* (PLO6), *Pleurotus ostreatus* (P93), *Pleurotus djamor* var. *roseus* (Psfarm), *Pleurotus pulmonarius* (Pindo) and *Pleurotus cornucopiae* (Plocor). These fungi belong to the Laboratório de Associações Micorrízicas, Bioagro, Departamento de Microbiologia, Universidade Federal de Viçosa, Minas Gerais, Brazil. The fungal isolates were cultivated on potato dextrose agar (PDA, Merck, Darmstadt, Germany) and incubated at 25±1 °C for seven days.

2.2 Effect of Iron on Mycelial Growth Rate and Biomass of Fungi

The fungi were exposed to iron at different concentrations. Briefly, one agar disc (8.0 mm) of each actively grown fungi was cut and transferred to the center of culture medium (PDA, Merck, Darmstadt, Germany) containing 0, 50.0 or 100.0 mg L^{-1} of iron in form of $FeSO_4$ .7 H_2O, pH 5.5 ± 0.1. The plates were incubated at 25 °C ± 1°C for seven days. The fungal growth rate was determined after seven days by measuring the colony's diameter in two directions that were perpendicular to each other (Kim et al., 2005). The biomass (dry mass) was determined by emptying the entire contents of Petri dish (mycelium + culture media) into a bottle with distilled water and heated in a water bath (5 - 10 min) to dissolve culture medium. Then, the solution was filtered and the mycelium was dried in an oven at 60 °C until a constant weight was reached (Reeslev & Kjøller, 1995).

2.3 Hyphae Diameter and Distance between Septa

The hyphae diameter and septa distance was determined. Briefly, a slide was inserted in a fungal plate at angle 45^o in order for the mycelia to spread on it. The slide was removed, stained with calcofluor (F3543 Tinopal UNPA-GX, Sigma-Aldrich, USA) and observed under epifluorescence microscopy (Olympus BX 50). Pictures were taken with a digital camera FUJIX HC-300Z, processed with the software Image Pro Plus to calculate hyphae diameter and distance between septa.

2.4 Mushroom Production and Iron Uptake

We investigated the effect of iron supplementation in substrate based *Urochloa decumbens* on iron level found in cultivated mushrooms. *Urochloa decumbens*; a grass-like plant was obtained from a farmland in Unai, Minas Gerais State, Brazil, which was dried at room temperature and milled into particle of different sizes, which passed through a sieve of 6.0 mm. The *U. decumbens* used in present assay have the following composition (mg Kg^{-1}): N (10.2), P (0.5), K (15.2), Ca (3.4), Mg (4.3), S (0.11), Zn (9.0), Fe (121.0), Mn (19.0), Cu (3.0) and B (6.5). The milled *U. decumbens* was soaked in lime water solution of 2% $Ca(OH)_2$ for 24 h and centrifuged at 1800 rpm for 5 min to remove excess water (Kasuya et al., 2014). Then, two experimental units of each fungus were cultivated on *U. decumbens* (150 g) supplemented with 0, 500 and 1000 mg Kg^{-1} of iron. The experimental units were incubated at 25 ± 3 ºC for 30 d. After this time, the bags were transferred to a room and incubated for fructification with light, at temperature of 25 ± 3 ºC in 90% air humidity for 10 to 20 d. The mushrooms produced were duly harvested and prepared for further analysis.

2.5 Determination of Iron Content in Cultivated Mushrooms

Dried mushroom samples (200 mg) were ground and subjected to digestion with a mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) (3:1, v:v) and 500 µL hydrogen peroxide (H_2O_2) at 200 °C (Tedesco et al., 1995). Complete oxidation of the organic matter occurred when no further darkening of the solution occurred upon continued heating and when a clear yellow or white solution was obtained. The mixture was cooled, quantitatively transferred to a volumetric flask and diluted with ultra-pure water (Milli-Q system, Millipore, USA) to a final volume of 15 mL. The resulting solutions were used for direct spectrophotometric analysis. Three blank digests were processed in the same way. The concentration of iron (Fe) was determined using an

atomic absorption spectrometer (Optima 3300 DV; Perkin Elmer, Waltham, MA). The Fe solution of atomic absorption spectrometer at concentration of 990 μg ml^{-1} in 1 % HCl (Sigma-Aldrich, St Louis, MO, USA) was used as standard. The solution was prepared with ultrapure water (Milli-Q system, Millipore, USA) in glassware washed with 2% v/v of HCl before use.

In order to calculate the efficiency of iron absorption by the fungi, we make a relation between the iron in the substrate and iron present in the mushrooms, using the following formula:

K_0 = total iron uptake by the mushrooms at 0 mg Kg^{-1}/ 121 (total iron content in the substrate)

K_{500} = total iron uptake by the mushrooms at 500 mg Kg^{-1}/ 621 (total iron content in the substrate)

K_{1000} = total iron uptake level in mushrooms at 1000 mg Kg^{-1}/1121 (total iron content in the substrate)

2.6 Statistical Analysis

The experiment used a randomized design. The results were expressed as the mean values with standard deviation. Test for differences in the mycelia growth rate, biomass and iron content of mushroom between fungi species *Pleurotus djamor* (PLO13), *Pleurotus ostreatus* (PLO6), *Pleurotus ostreatus* (P93), *Pleurotus djamor* v. *roseus* (Psfarm), *Pleurotus pulmonarius* (Pindo) and *Pleurotus cornucopiae* (Plocor) and iron concentrations (0, 50, 100 mg Kg^{-1}) were carried out using two-way ANOVA. The post-hoc HDS-Tukey tests were applied when differences were significant ($p < 0.05$).

3. Results

Iron supplementation reduced the mycelial growth of the tested *Pleurotus* spp (Table 1). This indicates that increasing the iron content makes the substrate toxic to the fungi. The two-way ANOVA showed significant effect of iron concentration [F (2, 36) = 28.55, P<0.01]. There was main effect of iron on the fungi [F (5, 36) = 28.55, P<0.01]. The fungi displayed varying growth rate at the same iron concentration. There was interaction between the iron concentrations and species of fungi F [(10, 36) = 28.55, P<0.01]. The two-way ANOVA of biomass production and iron concentration showed a similar pattern as two-way ANOVA of mycelia growth rate and iron concentration. This showed that iron has inhibitory effect on the fungi growth rate and biomass production. The hyphae diameter and distance between septa of the examined *Pleurotus* spp were respectively ranged from 45 to 80 μm and 40 to 119 μm at iron concentration from 0 mg L^{-1} to 100 mg L^{-1}. Figure 1 shows the effect of iron on the fungi hyphae and septa. Total iron uptake level was influenced by the iron concentration in the substrate and fungi species (Table 2).

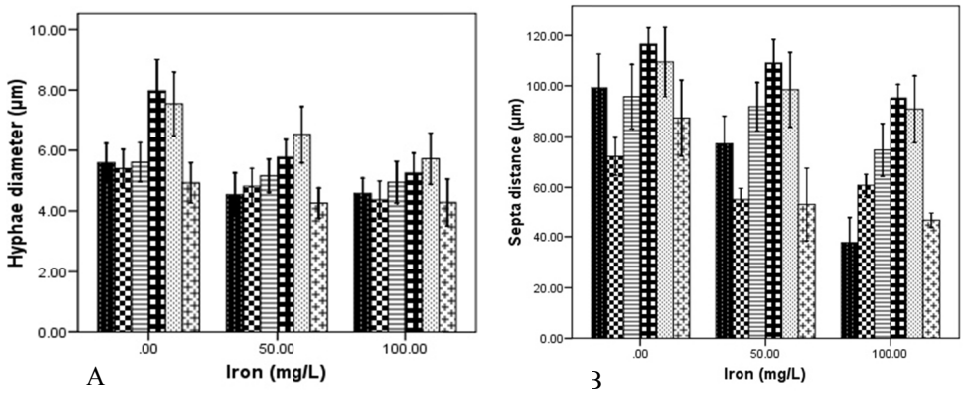

Figure 1. Hyphae diameter (A) and distance between septa (B) of *Pleurotus species* cultivated on Potato Dextrose Agar medium with different iron concentrations. Bar=standard deviation

Pleurotus djamor (PLO13), *Pleurotus ostreatus* (P. 93), *Pleurotus cornucopiae* (Plocor), *Pleurotus pulmonarius* (Pindo), *Pleurotus ostreatus* (PLO6) and *Pleurotus djamor* var. *roseus* (Psfarm)

Table 1. Mycelial growth rate (mm/day) and biomass (mg) of six fungi of genus *Pleurotus* cultivated on potato dextrose agar medium supplemented with different concentrations of iron, and results of two-way ANOVA evaluating the factors iron level and fungi MS = mean square; *F* = F statistic; *P* = significance level. (n = 3)

Iron concentration	Fungi						Factor	MS	*F*	*P*
	PLO 06	P. 93	Plocor	PLO 13	Pindo	Psfarm				
Mycelial growth rate										
0.0	8.42 ^{a} ± 0.14	8.40 ^{a} ± 0.10	5.85 ^{a} ± 0.15	6.11 ^{a} ± 0.10	6.40 ^{a} ± 0.18	6.56 ^{a} ± 0.13	Iron level	25.83	621.55	< 0.000
50.0	7.52 ^{b} ± 0.16	7.00 ^{b} ± 0.14	4.66 ^{b} ± 0.35	4.23 ^{b} ± 0.29	4.14 ^{b} ± 0.14	6.14 ^{b} ± 0.00	Fungi	11.67	280.91	< 0.000
100.0	6.00 ^{c} ± 0.29	4.47 ^{c} ± 0.36	4.24 ^{b} ± 0.08	3.52 ^{c} ± 0.08	3.47 ^{c} ± 0.29	5.80 ^{c} ± 0.16	Interaction	1.18	28.55	< 0.000
Biomass										
0.0	89.96 ^{a} ± 6.90	71.90 ^{a} ± 1.21	34.03 ^{a} ± 1.27	62.20 ^{a} ± 1.90	149.43 ^{a} ± 16.80	67.30 ^{a} ± 3.31	Iron level	10029.14	395.88	< 0.000
50.0	69.89 ^{b} ± 4.35	57.43 ^{b} ± 1.45	19.90 ^{b} ± 1.10	61.63 ^{a} ± 3.61	57.43 ^{b} ± 4.51	56.36 ^{b} ± 4.28	Fungi	3692.21	145.34	< 0.000
100.0	28.06 ^{c} ± 0.97	21.66 ^{c} ± 1.51	17.73 ^{b} ± 2.10	34.36 ^{b} ± 0.66	21.66 ^{c} ± 1.90	57.73 ^{b} ± 4.70	Interaction	1313.34	51.84	< 0.000

Means with different letters within a column, for each fungus and analyzes, are significantly different by Tukey test (P < 0.05).

PLO 06: *Pleurotus ostreatus*; P. 93: *Pleurotus ostreatus;* Plocor: *Pleurotus cornucopiae*; PLO 13: *Pleurotus djamor*; Pindo: *Pleurotus pulmonarius*, and Psfarm: *Pleurotus djamor* v. *roseus*

Table 2. Iron uptake level (μg g^{-1}) in *Pleurotus* species cultivated on substrate based *Urochloa decumbens* at different concentrations

Mushrooms	Iron level in substrate (mg Kg^{-1})					
	0	K_0	500	K_{500}	1000	K_{1000}
Pleurotus ostreatus (PLO 06)	32.30	0.26	37.80	0.06	50.50	0.04
Pleurotus ostreatus (P. 93)	60.40	0.49	55.59	0.08	88.54	0.07
Pleurotus pulmonarius (Pindo)	74.95	0.61	96.60	0.15	83.49	0.07
Pleurotus djamor (PLO 13)	50.30	0.41	59.60	0.09	74.20	0.06
Pleurotus cornucopiae (Plocor)	68.30	0.56	ND	ND	41.26	0.03
Pleurotus djamor var. *roseus* (Psfarm)	56.40	0.46	ND	ND	ND	ND

In order to calculate the efficiency of Iron absorption by the fungi, we make a relation between the iron in the substrate and iron present in the mushrooms, using the following formula:

K_0 = total iron uptake by the mushrooms at 0 mg Kg^{-1}/ 121 (total iron content in the substrate)

K_{500} = total iron uptake by the mushrooms at 500 mg Kg^{-1}/ 621 (total iron content in the substrate)

K_{1000} = total iron uptake level in mushrooms at 1000 mg Kg^{-1}/ 1121(total iron content in the substrate)

ND = not determined

4. Discussion

Edible fungi were cultivated on iron-fortified agro waste substrate to enrich the mineral content of the *Pleurotus* species. Microorganisms require some trace elements for their growth rate, metabolic processes and sustenance in other to overcome severe physiological consequences (Seth & Taga, 2014). The ability of an organism to survive in an environment with over accumulated or under secretion of metals gives competitive advantage in biotransformation. This has been the fundamental importance of *Pleurotus* in transforming organic and inorganic compound present in agro waste residues to various valuable metabolic products (Philippoussis & Diamantopoulou, 2011, Da Luz et al., 2014).

This study shows that the mycelial growth, hyphae diameter and distance between septa of tested *Pleurotus* spp was obviously reduced at higher concentration of iron 100 mg L^{-1} (Table 1). Optimum mycelial growth and biomass production of *P. ostreatus* had reported by Almeida et al. (2015) at 150 mg L^{-1} but reduced at 175 mg L^{-1} of iron concentration using a special medium at pH of 4.5 or 6.5. The varying response of fungi at different iron

concentrations could be the choice of medium and the growth factors. The reduction observed in mycelial growth indicates that iron displayed inhibitory action against the *Pleurotus* species. Fleurat-Lessard et al. (2011) had reported the inhibitory action of $FeSO_4$ on the mycelial growth of grapevine fungal pathogens such as *Botrytis cinerea, Eutypa lata*, *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum*, *Diplodia seriata*, and *Neofusicoccum parvum.* In essence, iron compounds could be suggested as source of preservatives and antimicrobial agents against some pathogenic fungi.

Despite the toxic action of iron at higher concentration, the ability of *Pleurotus* spp to survive and produce biomass indicated that these macrofungi possess extracellular enzymes, which can be recommended for the bioconversion of waste containing mineral residues. Therefore, the biotechnological applications and medicinal potentials of *Pleurotus* species could be attributed to their higher biomass production and presence of accumulated bioactive molecules, which are highly demanded and efficiently used for pharmaceutical and food products (Papaspyridi et al., 2011). Kim et al. (2002) had so far reported the mycelia growth and exo-biopolymer of some edible mushrooms such as *Pleurotus* spp*, Flammulina velutipes, Lentinus edodes, Grifola frondosa, Auricularia polytricha, Cordyceps militaris*. These are the medicinal mushrooms that are responsible for human health benefits by preventing diseases.

The iron contents obtained in cultivated *Pleurotus* species (Table 2) is slightly different from the findings of Vieira et al. (2013). These authors reported decrease in iron content due to low concentration of iron (0.8 mg Kg^{-1}) fortified with coffee husk during the cultivation of *Pleurotus ostreatus*. The low content of iron was also recovered from studied *Pleurotus* species when compared to the concentrations (500 and 1000) mg Kg^{-1} applied into *U. decumbens*. This could be possibly due to the insolubility of iron into the substrate and other growth factors such as pH, oxygen and temperature required for the cultivation of *Pleurotus* species, which may slow down the absorption rate of iron from substrate into *Pleurotus* species. The bioavailability of iron content is lower in aerobic environments, primarily because ferric iron reacts with oxygen to form insoluble ferric hydroxides, which are highly insoluble, and thus it is not readily available for uptake by organisms (Linda, 2011). The fungal cell wall is dynamic in structure and composition but its permeability under different growth factors and condition may alter the uptake of minerals (Philpott, 2006).

The available state of iron compound in substrate may contribute to the amount of iron assimilated by *Pleurotus* species, this need to be considered in any further studies on iron bioavailability in edible mushrooms, crops, fruits and vegetables**.** The previous statement conform to Hass (2004) who reveals that iron is not readily available in ionized states but in valence states, which therefore require some enzymatic processes during electron transfer in respiration, redox reactions by numerous oxygenase, hydrogenase and DNA-synthesis. Therefore, the unsuitable position of this metal to microorganisms could be associated to low availability of iron. The presence of other metals in the substrate could also affect the uptake level of iron in *Pleurotus* spp. Metal uptake is known to pass through specific transporters for the acquisition of essential metal and these transporters may cross react with other metals **(**Harms et al., 2011**)**, which might difficult for the easy assimilation of iron for *Pleurotus* species. Hence, fungi face some challenges in accumulating iron in the presence of other trace metals whose aqueous and redox chemistry make iron not readily bioavailable (Kosman, 2003).

Iron content recovered in cultivated *Pleurotus* species were inconsistent base on the concentration of iron added to the substrate. Hoa and Wang (2015); and Alam et al. (2008) have reported that the mineral and nutritional values of cultivated mushrooms are being affected by differences among strains, the composition of growth substrate, the method of cultivation, stage of harvesting, pH of the substrate, concentration of supplemented mineral in the enriched substrate and the specific portion of the fruiting bodies used for analysis.

The findings of Saha et al. (2012) had stated that the biosynthesis of siderophores in microorganisms is induced by intracellular iron deficiency in the environment, this statement therefore justify that at low concentration of iron; some microorganisms can still survive. So the availability of much iron concentration in the substrate might not be directly proportional to the amount of iron assimilated and further increase could be toxic to the organisms. Halliwell and Gutteridge (1992) supported the previous statement by revealing that excess amount of iron is deleterious to organisms, which allow free iron to catalyze the production of cell-damaging reactive oxygen species through Fenton reaction. Hence, some microorganisms will not be able to assimilate much quantity of iron even at optimum conditions.

Conclusively**,** iron metabolism is sometime difficult to absorb by microorganisms at higher dose therefore, several strategies still need to be adopted to make iron easily bioavailable for organisms and in food products. This will definitely combat the problem of iron deficiency in human.

Conflict of Interest

None

Acknowledgements

All Authors appreciate financial support of CAPES, CNPq and FAPEMIG. Olusola C. Ogidi is a recipient of CNPq/TWAS Sandwich Postgraduate Fellowship Award. Prof. M. C. M. Kasuya is also gratefully acknowledged for hosting OCO in the Laboratório de Associações Micorrízicas, Departmento de Microbiologia/BIOAGRO, UFV, Brazil.

References

Abbaspour, N., Hurrell, R., & Kelishadi, R. (2014). Review on iron and its importance for human health. *Journal Res Med Sci., 19*(2), 164-174.

Alam, N., Amin, R., Khan, A., Ara, I., Shim, M. J., Lee, M. W., & Lee, T. S. (2008). Nutritional Analysis of Cultivated Mushrooms in Bangladesh-*Pleurotus ostreatus*, *Pleurotus sajor-caju*, *Pleurotus florida* and *Calocybe indica*. *Mycobiology, 36*(4), 228-232. http://dx.doi.org/10.4489/MYCO.2008.36.4.228

Almeida, S. M., Umeo, S. H., Marcante, R. C., Yokota, M. E., Valle, J. S., Dragunski, D. C., … Linde, G. A. (2015). Iron bioaccumulation in mycelium of *Pleurotus ostreatus*. *Brazilian Journal of Microbiology, 46*(1), 195-200. http://dx.doi.org/10.1590/S1517-838246120130695

Da Fonseca, T. R. B., Silva, T. A., Alecrim, M. M., Da Cruz Filho, R. F., & Teixeira, M. F. S. (2015). Cultivation and nutritional studies of an edible mushroom from North Brazil. *African Journal of Microbiology Research, 9*(30), 1814-1822. http://dx.doi.org/10.5897/AJMR15.7526

Da Luz, J. M. R. Nunes, M. D. Paes, S. A. Torres, D. P., & Kasuya, M. C. M. (2014) Bio-detoxification of *Jatropha curcas* seed cake by *Pleurotus ostreatus*. *African Journal of Microbiology Research, 8*(11), 1148-1156. http://dx.doi.org/10.5897/AJMR2014.6617

Da Luz, J. M. R., Nunes, M. D., Paes, S. A. Torres, D. P. da Silva, M. C. S., & Kasuya, M. C. M. (2012) Lignocellulolytic Enzyme Production of *Pleurotus ostreatus* Growth In Agro industrial Wastes. *Brazilian Journal of Microbiology*, 1508-1515. http://dx.doi.org/10.1590/S1517-83822012000400035

De Assunção, L. S., da Luz, J. M. R., Silva, M. C. S., Vieira, P. A. F., Bazzolli, D. M. S., Vanetti, M. C. D., & Kasuya, M. C. M. (2012). Enrichment of mushrooms: An interesting strategy for the acquisition of lithium. *Food Chemistry, 134*, 1123-1127. http://dx.doi.org/10.1016/j.foodchem.2012.03.044

Falandysz, J., & Borovička, J. (2013). Macro and trace mineral constituents and radionuclides in mushrooms: health benefits and risks. *Applied Microbiology and Biotechnology, 97*, 477–501. http://dx.doi.org/10.1007/s00253-012-4552-8

Fleurat-Lessard, P., Dédaldéchamp, F., Thibault, F., Béré, E., & Roblin, G. (2011). Antifungal effects of iron sulfate on grapevine fungal pathogens. *Scientia Horticulturae, 130*(3), 517–523, http://dx.doi.org/10.1016/j.scienta.2011.07.004

Ganeshpurkar, A., Rai, G., & Jain, A. P. (2010). Medicinal mushrooms: Towards a new horizon. *Pharmacognosy Review, 4*(8), 127–135. http://dx.doi.org/10.4103/09737847.70904

Haas, H. (2004). Molecular Genetics of Iron Uptake and Homeostasis in Fungi. In R. Brambl & G. A. Marzluf (Eds.), *The Mycota III, Biochemistry and Molecular Biology* (2nd Ed.).

Halliwell, B., & Gutteridge, J. M. C. (1992). Biologically relevant metal ion-dependent hydroxyl radical generation an update. *FEBS Letters, 307*, 108–112. http://dx.doi.org/10.1016/0014-5793(92)80911-Y

Harms, H., Schlosser, D., & Wick, L. Y. (2011). Untapped potential: exploiting fungi in bioremediation of hazardous fungi, *Nature Reviews Microbiology, 9*, 177-192. http://dx.doi.org/10.1038/nrmicro2519

Hoa, H. T., & Wang, C. L. (2015) The Effects of Temperature and Nutritional Conditions on Mycelium Growth of Two Oyster Mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). *Mycobiology, 43*(1), 14-23, http://dx.doi.org/10.5941/MYCO.2015.43.1.14

Kassebaum, N. J., Jasrasaria, R., Naghavi, M., Wulf, S. K., Johns, N., Lozano, R., Christopher J. L. M. (2014): A systematic analysis of global anemia burden from 1990 to 2010. *Blood, 123*(5), 615–624. http://dx.doi.org/10.1182/blood-2013-06-508325

Kasuya, M. C. M., Rodrigues da Luz, J. M., Nunes, M. D., Silva, M. C. S., Carvalho, D. R., Assunção, L. S., ... Bento, C. B. P. (2014). Production of Selenium-Enriched Mushrooms in Coffee Husks and Use of This

Colonized Residue: In coffee in health and disease prevention. ISBN: 9780124095175

Kim, S. W., Hwang, H. J., Park, J. P., Cho, Y. J., Song, C. H., & Yun, J. W. (2002). Mycelial growth and exo-biopolymer production by submerged culture of various edible mushrooms under different media. *Letters in Applied Microbiology, 34*, 56-61. http://dx.doi.org/10.1046/j.1472-765x.2002.01041.x

Kim, Y. K., Xiao, C. L., & Rogers, J. D. (2005). Influence of culture media and environmental factors on mycelial growth and pycnidial production of *Sphaeropsis pyriputrescens*. *Mycologia, 97*(1), 25–32. http://dx.doi.org/10.3852/mycologia.97.1.25

Kosman, D. J. (2003). Molecular mechanisms of iron uptake in fungi. *Molecular Microbiology, 47*(5), 1185–1197. http://dx.doi.org/10.1046/j.1365-2958.2003.03368.x

Linda, J. (2008). Iron and siderophores in fungal–host interactions. *Mycological Research, 112*, 170-183. http://dx.doi.org/10.1016/j.mycres.2007.11.012

Nunes, M. D., Cardoso, W. L., Da Luz, J. M. R., & Kasuya, M. C. M. (2014). Lithium chloride affects mycelial growth of white rot fungi: Fungal screening for Li-enrichment. *African Journal Microbiology Research 8*(21), 2111-2123. http://dx.doi.org/10.5897/AJMR2014.6619

Papaspyridi, L. M., Aligiannis, N., Christakopoulos, P., Skaltsounis, A. L., & Fokialakis, N. (2011). Production of bioactive metabolites with pharmaceutical and nutraceutical interest by submerged fermentation of *Pleurotus ostreatus* in a batch stirred tank bioreactor, 11th International Congress on Engineering and Food (ICEFF11). *Procedia Food Science, 1*, 1746–1752. http://dx.doi.org/10.1016/j.profoo.2011.09.257

Philippoussis, A., & Diamantopoulou, P. (2011). Agro-food industry wastes and agricultural residues conversion into high value products by mushroom cultivation. *Proceedings of the 7th International Conference on Mushroom Biology and Mushroom Products* (ICMBMP7).

Philpott, C. C. (2006). Iron uptake in fungi: A system for every source. *Biochimica et Biophysica Acta, 1763*, 636-645. http://dx.doi.org/10.1016/j.bbamcr.2006.05.008

Reeslev, M., & Kjøller, A. (1995). Comparison of Biomass Dry Weights and Radial Growth Rates of Fungal Colonies on Media Solidified with Different Gelling Compounds. *Applied and Environmental Microbiology, 61*(12), 4236-4239.

Saha, R., Saha, N., Donofrio, R. S., & Bestervelt, L. L. (2012). Microbial siderophores: a mini review. *Journal of Basic Microbiology, 52*, 1-15.

Seth, E. C., & Taga, M. E. (2014). Nutrient cross-feeding in the microbial world. *Frontier Microbiology, 5*(350), 1-6. http://dx.doi.org/10.3389/fmicb.2014.00350

Silva, M. C. S., Naozuka, J., da Luz, J. M. R., Assunção, L. S., Oliveira, P. V., Vanetti, M. C. D., ... Kasuya, M. C. M. (2012). Enrichment of *Pleurotus ostreatus* mushrooms with selenium in coffee husks. *Food Chemistry 131*, 558-563. http://dx.doi.org/10.1016/j.foodchem.2011.09.023

Tedesco, M. J., Gianello, C., Bissani, C. A., Bohnen, H., & Volkweiss, S. J. (1995). Analysis of Soil, Plants and other Materials, 2ed., Porto Alegre, Department of Soil, Federal University of Rio Grande do Sul (Technical Bulletin of Soil, 5) p. 174.

Vieira, P. A. F., Gontijo, D. C., Vieira, B. C., Fontes, E. A. F., Assunção, L. S., Leite, J. P. V., … Kasuya, M. C. M. (2013). Antioxidant activities, total phenolics and metal contents in *Pleurotus ostreatus,* mushrooms enriched with iron, zinc or lithium. *Food Science and Technology, 54*, 421-425.

Zhang, X. G., Peng, Y. N., Li, X. R., Ma, G. D., & Chen, X. Q. (2015). Screening of iron-enriched fungus from natural environment and evaluation of organically bound iron bioavailability in rats. *Food Science and Technology, 35*(1), 58-65. http://dx.doi.org/10.1590/1678-457X.6454