IJBPAS, August, 2018, 7(8): 1489-1499

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

MINERAL COMPOSITION AND YIELD OF *Pleurotus ostreatus* ON RICE STRAW-BASED SUBSTRATE ENRICHED WITH NATURAL CALCIUM SOURCES

ARIANNE V. JULIAN[1*], MICHAEL R. UMAGAT[2] AND RENATO G. REYES[1, 2, 3, 4]

[1]Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines

[2]Laboratory of Industrial Biotechnology and Bio-Industry, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines

[3]Flora and Fauna Analytical and Diagnostic Laboratory, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines

[4]Center for Tropical Mushroom Research and Development, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines

***Corresponding Author: ariannejulian@gmail.com**

Received 22nd April 2018; Revised 30th May. 2018; Accepted 20th June 2018; Available online 1st August 2018

https://doi.org/10.31032/IJBPAS/2018/7.8.4501

ABSTRACT

Pleurotus ostreatus, one of the most widely cultivated mushrooms worldwide, is an excellent source of protein and other minerals but inherently contains low calcium (Ca) level. This study aimed to evaluate the influence of varying concentrations of Ca supplementation derived from natural sources including agricultural lime, eggshell and oyster shell in rice straw-based formulation on the nutritional composition and yield of *P. ostreatus*. Rice straw-based substrates were supplemented with different concentrations of the Ca sources. Each sterilized bag was aseptically inoculated with grain spawn of *P. ostreatus*. The fruiting bodies of *P. ostreatus* were harvested, weighed and air-dried until the 3rd flush. Yield and biological efficiency (BE) were recorded. Dried fruiting bodies were ground using a blender and analyzed for elemental composition. The use of these Ca sources as substrate supplements generally increased yield, biological efficiency and Ca content of *P. ostreatus*. Mushrooms grown in substrate with 8-10% agricultural lime and 6% eggshell powder produced the highest yields while using oyster shell powder did not vary with the control. Meanwhile, substrate supplementation using agricultural lime and eggshell powder in all concentrations have increased Ca in fruiting bodies. However, Ca was not absorbed in oyster shell powder-supplemented substrate. Noticeably, supplementation of all sources reduced K and P contents. However, the levels of Mg, Na, Si, Cl and S were generally increased. These findings imply the potential of agricultural lime and eggshell powder in the production of Ca-enriched mushrooms resulting to higher yield.

Keywords: *Pleurotus ostreatus*; Calcium absorption; Mushroom enrichment; Biological efficiency; Natural sources; Waste management

INTRODUCTION

Food fortification is a strategy with the goal of increasing the consumption of essential nutrients and minerals to improve health [1]. Of these minerals, Ca is one of the most abundant and plays a vital role in maintaining optimum health particularly the sustenance of bones and teeth; hence, adequate daily intake is necessary [2]. Most individuals can acquire Ca by eating a variety of food such as milk and other dairy products; green, leafy vegetables; seafood, nuts, and dried beans [1].

Cultivation of *Pleurotus* species is widespread in different parts of the world which can be attributed to its low cost production and its nutritional and medicinal properties [3]. Reportedly, these mushrooms contain various nutrients including protein and minerals such as phosphorus, iron, potassium and sodium, and vitamin C, B complex such as thiamine, riboflavin, folic acid and niacin [4]. However, Ca level remains lesser compared to vegetables [5].

In this study, inedible materials including agricultural lime, eggshells and oyster shells were used as Ca supplements. These natural sources are known to contain calcium carbonate ($CaCO_3$) as their main component [6]. Accordingly, bioconversion of these waste materials provides an excellent method to utilize renewable resources. In relation with this, several studies demonstrated the ability of mushrooms for nutrient uptake from substrate [7]. Hence, substrate supplementation is a common practice not only to improve the nutritional value but also to enhance the yield [8]. The present study evaluated the ability of *P. ostreatus* to absorb Ca from agricultural lime, eggshell and oyster shell powder; the effect on other mineral contents present in its fruiting bodies; and the total yield and biological efficiency.

MATERIALS AND METHODS

Source and Revival of Pure Culture

Pure culture of *P. ostreatus* was obtained from the Center for Tropical

Mushroom Research and Development (CTMRD), Central Luzon State University, Science City of Munoz, Nueva Ecija. Approximately, 8 mm mycelial discs from the pure culture of *P. ostreatus* were aseptically transferred using an inoculating needle into the sterilized potato dextrose agar (PDA) culture plates. These culture plates were incubated for seven days at room temperature to allow mycelial growth.

Collection of Ca Sources and Preparation of Powder

Agricultural lime and eggshells were obtained from the public market of Science City of Muñoz, Nueva Ecija. On the other hand, oyster shells were collected from a market in Anda, Pangasinan. Eggshells were washed with purified water and cut into small pieces (10 mm × 10 mm), then sun-dried for 24 hours and were placed in an oven for 30 minutes to completely dry the samples. Oyster shells were cut into pieces (10 mm × 10 mm) and soaked in distilled water for 24 hours and left to dry naturally. The materials were rinsed several times with purified water in order to eliminate impurities. The samples were sun-dried for 48 hours and placed in an oven for 30 minutes. Dried eggshells and oyster shells were pulverized using a blender and sifted using a metal sieve.

Preparation of Grain Spawn

Unmilled rice seeds were boiled until tender in 1:0.5 ratio (rice seed: water) and allowed to cool down for 15 minutes. These seeds were drained, maintained at 65% moisture content, and dispensed into small polypropylene bags. Each bag contained 40 g of boiled unmilled rice seeds and the opening was plugged with cotton and covered with paper. These bags were sterilized in an autoclave at 15 psi, 121°C for 30 minutes and were allowed to cool for 24 hours. Then, the bags were inoculated aseptically in the laminar airflow cabinet using actively growing mycelium of the pure culture of *P. ostreatus*. A portion of pure culture was placed aseptically into the opening of each bag using sterile inoculating needle. After inoculation, the bags were incubated at room temperature to allow the full mycelial ramification of the grains. After about one week, the grains were dislodged to spread the mycelium evenly through the bags.

Substrate Formulation, Preparation and Enrichment

Rice straw was soaked in a water tank overnight and then washed with running water to remove any undesirable odor. The washed rice straw was allowed to decompose for five days then cut to approximately one inch long. Seven parts of composted rice straw were combined with three parts of sawdust and one part of rice bran. Afterwards, the mixture was divided and supplemented with different concentrations (2%, 4%, 6%, 8% and 10%) of the Ca sources and the mixture without any Ca source served as control. Supplementation was followed by addition of tap water to give moisture content of 65%. Each of the enriched substrate (500 g) was compressed in a 7x14 inch polypropylene bag with 10 replicates for each treatment. These bags were individually provided with opening using cut PVC pipe to facilitate inoculation, plugged with cotton and sterilized at 15 psi, 121 °C for 1 hour. Next, the bags were allowed to cool down and each bag was aseptically inoculated with grain spawn. Initial weights of the bags were obtained and were incubated at 30°C temperature with 80-90% relative humidity until mushroom formation.

Determination of Mineral Contents in Fruiting Bodies

Dried fruiting bodies of *P. ostreatus* were ground using a blender. Two hundred mg of ground mushroom of each treatment was analyzed for elemental composition using Thermo EDX system (no ran system 6, Ultra Dry 10 mm2 SDD crystal, 129 eV resolution, NORVAR window, LN2-Free Type detector) installed in Hitachi SU1510 scanning electron microscope. Other minerals present in the fruiting bodies were also determined.

RESULTS AND DISCUSSION

Total yield and biological efficiency of *P. ostreatus*

Different substrate supplementation may influence mushroom yield and biological efficiency (BE) [8, 9]. Several nutrient additives are recommended to improve growth of oyster mushroom [10]. In this study, natural sources were used as substrate supplement and its effect on the total yield and BE of *P. ostreatus* are presented in Table 1.

Addition of different concentrations of the sources has generally increased the yield. Mushroom grown in substrate with 8% and 10% agricultural lime (AGL) produced the highest total yields of 191.80 g and 191.20 g, respectively; which are significantly higher than the control (150.60 g). Accordingly, these concentrations have higher BE of 35.57% (8% AGL) and 35.63% (10% AGL) as compared with the control (28.69%). Meanwhile, in eggshell powder (ESP)-supplemented mushroom, the yield was recorded highest in 6% (197.60 g) with 37.43% BE, while the lowest was 10% with 143.20 g. Oyster shell powder (OSP)-enriched mushroom generally increased its yield and BE but not statistically different from the control.

These results are congruent with the findings of [11] that the addition of 0.6% precipitated $CaCO_3$ resulted to higher yields and BE of shiitake (*Lentinula edodes*) mushrooms than substrate containing no additional Ca. From the data presented, it can be concluded that substrate enrichment using 8-10% AGL and 6% ESP can significantly improve the yield and BE of *P. ostreatus* in rice straw-based formulation.

Elemental composition of Ca sources

Agricultural lime, with primary active component of $CaCO_3$, is a soil additive made from pulverized limestone used for neutralizing soil acidity [12]. Similarly, eggshell is also a good soil amendment material that can be an excellent natural source of Ca which is about 90% absorbable [13]. Another good Ca source is oyster shell which can be easily obtained as waste material [14]. Table 2 presents the elemental composition of the Ca sources used for supplementation in this study.

Among the sources, AGL contained the highest Ca content of 68.74% followed by OSP with 63.95% and ESP with 57.02%. Aside from Ca as their main component, these materials also contained trace amount of other elements. Silicon and magnesium were detected in all sources. Meanwhile, ESP contained sulfur and potassium which were not identified in other sources. OSP, on the other hand, contained sodium and chlorine which were not present in AGL and ESP. These results revealed that these sources can be used as Ca additive on mushroom substrate.

Average Ca content of *P. ostreatus* in three flushes

Mushrooms possess an effective mechanism allowing them to absorb some trace elements from the substrate [15]. Therefore, supplementation of substrate is an effective technique used in *Pleurotus* species production which aims to improve yield and nutritional value of the mushroom [16]. In this study, the calcium absorption efficacy of *P. ostreatus* using three different Ca sources was evaluated. Fig. 1 illustrates the average amount of Ca in *P. ostreatus* cultivated in substrates with different levels of Ca sources in three flushes.

Table 1: Influence of varying concentrations of Ca sources on the total yield and biological efficiency of *P. ostreatus* in three flushes

Calcium sources	Concentration (%)	Total Yield (g)	Biological efficiency (%)
Agricultural lime	0	150.60±4.22^{b}	28.69±0.93^{b}
	2	179.20±14.55^{ab}	33.52±2.98^{ab}
	4	177.40±26.58^{ab}	33.70±5.18^{ab}
	6	165.80±19.66^{ab}	31.27±3.50^{ab}
	8	191.80±19.10^{a}	35.57±2.99^{a}
	10	191.20±18.14^{a}	35.63±3.75^{a}
Eggshell powder	0	150.60±4.22^{b}	28.69±0.93^{b}
	2	170.80±28.66^{ab}	31.88±5.28^{ab}
	4	169.20±19.07^{ab}	31.51±3.43^{ab}
	6	197.60±19.05^{a}	37.43±5.54^{a}
	8	154.60±17.39^{b}	29.89±3.52^{b}
	10	143.20±10.20^{b}	27.26±2.51^{b}
Oyster shell powder	0	150.60±4.22^{a}	28.69±0.93^{a}
	2	172.40±18.23^{a}	32.82±3.58^{a}
	4	173.20±25.17^{a}	33.04±4.42^{a}
	6	168.00±8.22^{a}	32.07±1.50^{a}
	8	153.60±20.38^{a}	29.18±3.86^{a}
	10	168.40±37.96^{a}	31.98±7.43^{a}

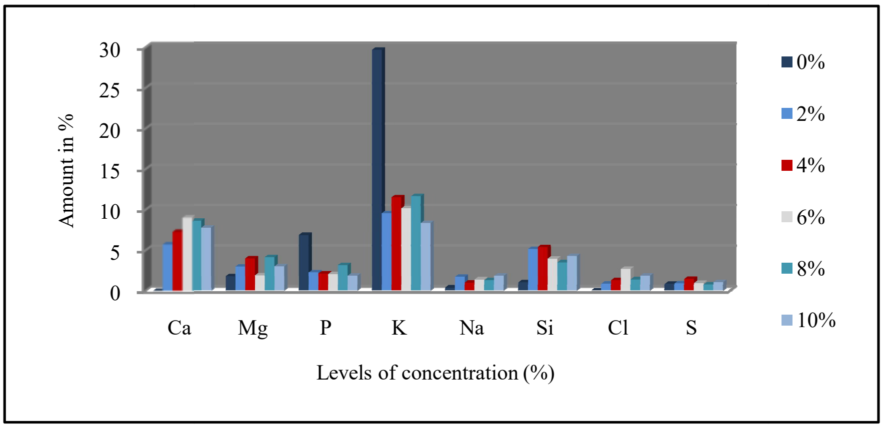
Values presented are means and SD

Treatment means in each column with the same letter of superscript are not significantly different from each other at 5% level of significance using LSD

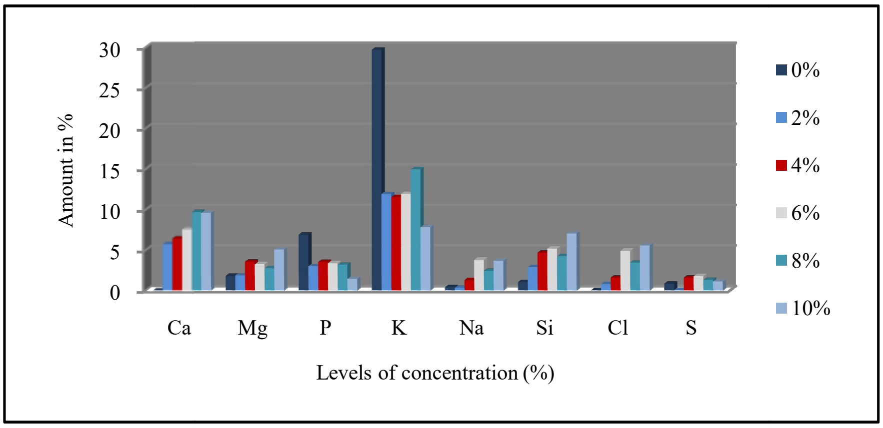
Table 2: Elemental composition of Ca sources

Minerals	AGL (%)	ESP (%)	OSP (%)
Ca	68.74	57.02	63.95
Si	0.51	0.22	0.20
Mg	0.44	0.53	0.39
S	ND	0.68	ND
K	ND	0.34	ND
Na	ND	ND	0.74
Cl	ND	ND	0.35

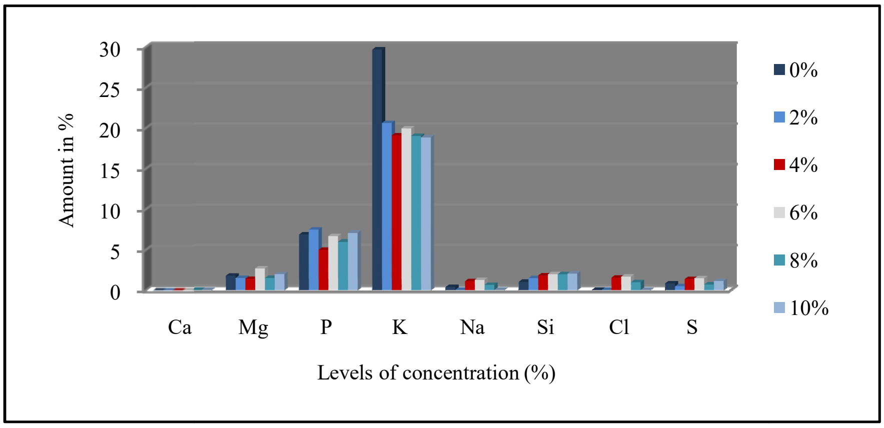
ND: Not detected



(a)



(b)



(c)

Fig. 1: Average mineral composition of *P. ostreatus* grown in rice straw-based substrate enriched with (a) agricultural lime; (b) eggshell powder; (c) oyster shell powder in three flushes

Noticeably, no Ca content was detected in mushrooms grown on non-supplemented substrate (control). Results revealed that AGL and ESP evidently improved Ca level in *P. ostreatus* while OSP supplementation at all levels showed no Ca absorption efficacy. The highest Ca contents were recorded in mushroom cultivated in rice straw-based substrate with 6% AGL, 8% and 10% ESP with 9.01%, 9.66% and 9.51% average Ca amount, respectively. The lowest absorption was noted in 2% AGL (5.71%) and 2% ESP (5.72%). Meanwhile, AGL with concentrations higher than 6% have decreased Ca content in *P. ostreatus*. Conversely, in mushrooms enriched with ESP, it can be observed that as the level of ESP increased, Ca content of fruiting body also increased and reached a saturation point in 8% and 10%. This outcome conforms to the conclusion of [17] that sawdust media supplemented with 1% Ca salts increased the Ca content of *H. marmoreus.* In addition, the ability of Ca uptake of *P. ostreatus* and *P. nameko* in sawdust media has also been documented [18]. Furthermore, *P. eryngii* cultivated in sawdust medium with Ca salts and calcinated starfish powder resulted in higher Ca content in their fruiting bodies [19, 20].

Influence on other minerals in *P. ostreatus*

Enrichment using AGL, ESP and OSP in mushroom substrate potentially impacts the elemental composition and amount of other minerals. Using different substrate combination and supplements can change the nutritional contents of *P. ostreatus* [21, 22]. This can be explained by the two kinds of interactions between nutrients. First is synergism in which a positive relationship between nutrients occurs and the second is antagonism wherein a negative effect happens between nutrients [23].

In the present study, the influence of Ca supplementation on other minerals present in *P. ostreatus* was also evaluated (Figure 1). Upon analysis, the minerals in non-supplemented mushrooms include potassium, phosphorus, magnesium, silicon, sulfur and sodium. Noticeably, all the Ca sources used have reduced the amount of K and P while AGL and ESP have generally increased other minerals such as Mg, Na, Si, Cl and S.

[24] reported that Ca, Cu, Fe, K, Mg, Mn, P and Zn were present in *P. ostreatus* cultivated in sawdust, sugarcane bagasse and corncob. Among these minerals, K and P were highest in amounts similar to the result in the present study. Similarly, Ogundele et al. (2014) reported that *P. ostreatus* grown in sawdust-based substrate had high concentration of K, and smaller quantity of Ca, Mg, Fe and Zn.

CONCLUSION

Varying concentrations of Ca from different sources affected the yield and mineral absorption efficacy of *P. ostreatus* mushroom. Based on the findings of the present study, it can be concluded that agricultural lime and eggshell powder (up to 10%), but not oyster shell powder, generally increased the yield and BE. This growth improvement may signify an association with increased Ca content (less 10%) recorded in the fruiting bodies. Concentrations of other minerals such as Mg, Na, Si, Cl and S were generally increased but K and P levels in the supplemented mushrooms were lower than control. This study suggests that using Ca-rich biological wastes may improve the concentration of Ca and yield of *P. ostreatus*, but may also induce diverse effect on the absorption of other essential minerals.

ACKNOWLEDGEMENTS

The authors would like to extend their earnest gratitude to the following institutions for their financial and technical assistance: Department of Science and Technology, Science Education Institute, Accelerated Science and Technology Human Resource Development Program (DOST-SEI ASTHRDP) and the Center for Tropical Mushroom Research and Development (CTMRD), Central Luzon State University.

REFERENCES

[1] Pravina P, Sayaji D, Avinash M, Calcium and its role in human body, International Journal of Research in Pharmaceutical and Biomedical Sciences, 4(2),2013, 659-668.

[2] Allen L, De Benoist B, Dary, Hurrell R, Guidelines on food fortification with micronutrients, 2006, WHO Library Cataloguing-in-Publication Data. http://www.who.int/nutrition/publications/guide_food_fortification_micronutrients.pdf.Accessed 6 September 2015.

[3] Kortei J, Growing oyster mushrooms (*Pleurotus ostreatus*) on composted agrowastes: An efficient way of utilizing lignocellulosic materials, Lambert Academic Publishing, Saarbrücken, Germany, 2011.

[4] Çağlarirmak N, The nutrients of exotic mushrooms (*Lentinula edodes* and *Pleurotus* species) and an estimated approach to the volatile compounds, Food Chemistry, 105, 2007, 1188–1194. https://doi.org/10.1016/j.foodchem.2007.02.021

[5] Kawai H, Sugahara T, Fujishiro S et al, Mineral contents of edible mushrooms growing on wood: Comparison with mineral contents of mushrooms growing in soil, Journal of the Japanese Society for Food Science and Technology, 37, 1990, 468-473.

[6] Choi UK, Lee OH, Kim YC, Effect of calcinated oyster shell powder on growth, yield, spawn run, and primordial formation of king oyster mushroom (*Pleurotus eryngii*), Molecules, 16(3), 2011, 2313–2322. https://doi.org/10.3390/molecules16032313

[7] Ogundele GF, Abdulazeez RO, Bamidele OP, Effect of pure and mixed substrate on oyster mushroom (*Pleurotus ostreatus*) cultivation, Journal of Experimental Biology and Agricultural Sciences, 2, 2014, 215-219.

[8] Moda E, Horii J, Spoto M, Edible mushroom *Pleurotus sajor-caju* production on washed and supplemented sugarcane bagasse, Scientia Agricola, 62,2005, 127-32. http://dx.doi.org/10.1590/S0103-90162005000200006

[9] Moonmoon M, Shelly NJ, Khan A, Uddin N, Hossain K, Tania M, Ahmed S, Effects of different levels of wheat bran, rice bran and maize powder supplementation with saw dust on the production of shiitake mushroom (*Lentinus edodes* (Berk.) Singer), Saudi Journal of Biological Science, 18(4), 2010, 323-324. https://doi.org/10.1016/j.sjbs.2010.12.008.

[10] Curvetto NR, Figlas D, Devalis R, Delmastro S, Growth and productivity of different *Pleurotus ostreatus* strains on sunflower seed hulls supplemented with N-NH4+ and/or Mn(II), Bioresource Technology, 84(2),2002, 171–176. https://doi.org/10.1016/S0960-8524(02)00013-5.

[11] Royse DJ, Sanchez-Vazquez JE, Influence of precipitated calcium carbonate ($CaCO_3$) on shiitake (*Lentinula edodes*) yield and mushroom size, Bioresource Technology, 90, 2003, 225–228. https://doi.org/10.1016/S0960-8524(03)00119-6.

[12] Mullins GL, Sources of lime for acid soils in Virginia. Virginia Cooperative Extension, 2009, http://pubs.ext.vt.edu/452/452-510/452-510.html. Accessed 10 September 2015.

[13] Amu OO, Fajobi AB, Oke BO, Effect of eggshell powder on the stabilization potential of lime on an expansive clay soil, Journal of Applied Sciences, 1(1), 2005, 80-84. https://doi.org/10.3923/jas.2005.1474.1478.

[14] Lee CH, Lee DK, Ali MA, Kim PJ, Effects of oyster shell on soil chemical and biological properties and cabbage productivity as a liming materials, Waste Management, 28(12),2008, 2702–2708. https://doi.org/10.1016/j.wasman.2007.12.005.

[15] Kalač P, Svoboda L, A review of trace element concentrations in edible mushrooms, Food Chemistry, 69, 2000, 273-281. https://doi.org/10.1016/S0308-8146(99)00264-2.

[16] Carvalho C, Sales-Campos C, De Andrade M, Mushrooms of the *Pleurotus* Genus: A review of cultivation techniques, Interciencia, 35(3), 2010, 177−182.

[17] Tabata T, Ogura T, Absorption of calcium and magnesium by the fruiting body of the cultivated mushroom *Hypsizygus marmoreus* (peck) bigelow from sawdust culture media, Journal of Food Science, 68,2003, 76-79.

[18] Tabata T, Shinohara H, Absorption of calcium salts added culture media by Hiratake (*Pleurotus ostreatus* (Fr.) Quel.) and Nameko (*Pholiota nameko* J. Ito), Journal of the Japanese Society for Food Science and Technology, 42,1995, 682–6860.

[19] Lee NH, Im MH, Choi UK, Calcium absorption by the fruit body of *Saesongi* mushroom, Food Science Biotechnology, 15, 2006, 308-311.

[20] Choi UK, Bajpai VK, Lee NH, Influence of calcinated starfish powder on growth, yield, spawn run and primordial germination of king oyster shell mushroom (*Pleurotu seryngii*), Food and Chemical Toxicology, 47,2009, 2830-2833. https://doi.org/10.1016/j.fct.2009.08.019

[21] Amuneke E, Dike K, Ogbulie J, Cultivation of *Pleurotus ostreatus*: An edible mushroom from agro base waste products, Journal of Microbiology and Biotechnology Research, 1(3), 2011, 1-14.

[22] Ashraf J, Ali MA, Ahmad W, Ayyub CM, Shafi J, Effect of different substrate supplements on oyster mushroom (*Pleurotus* spp.) production, Food Science and Technology, 1(3), 2013, 44-51. https://doi.org/10.13189/fst.2013.010302

[23] Malvi U, Interaction of micronutrients with major nutrients with special reference to potassium, Karnataka Journal of Agricultural Sciences, 24(1), 2011, 106-109.

[24] Hoa HT, Wang CL, Wang CH, The effects of different substrates on the growth, yield, and nutritional composition of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*), Mycobiology, 43(4), 2015, 423-434. https://doi.org/10.5941/MYCO.2015.43.4.423.