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Effect of Different Levels of *Plectranthus glandulosus* on Mycelial Growth and Production Efficiency of Oyster Mushroom (*Pleurotus* Spp.)

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***Abstract* – The cultivation of mushrooms for food and medicine is increasingly popular across the world, including Africa. However, the production is small relative to the demand because of proper production techniques. Ethno botanic studies reveal the use of *Plectranthus glandulosus* leaves in fungal control. The objective of this study was, therefore, to assess the ability of *P. glandulosus* leaf powder to influence mycelia growth of oyster mushroom *(P. ostreatus)* specie. Malt extract and agar containing *P. glandulosus* at different concentrations (1, 2 and 5%) were used as growth media while the substrate for spawn production was prepared on maize powder thoroughly mixed with sawdust and rice husk in a ratio of 1:3:3. Results showed that *P. ostreatus* grows in different media but this growth was reduced when the concentration of P. *glandulosus* increases. At the highest concentration of *P. glandulosus* leaf powder (5%), the growth was completely inhibited after 10 days. Comparing the different concentrations of *P. glandulosus* and the control (agar) there was no significant difference between 1, 2 and 5% concentrations 4 days after inoculation (DAI) while the control had a better growth of 1.88cm. However, from 6 DAI to 10 DAI, there was no significant difference between the control, 2% and 1% concentrations of *P. glandulosus.* Results also showed that *P. glandulosus* incorporated as 1% in agar medium, resulted in mycelia producing 21 pinheads and 13 fruiting bodies which are not significantly different from what is produced by the control (20 and 14 respectively).However, at the 2% concentration of *P. glandulosus* more pinheads (23) and fruiting bodies (16) were formed which are significantly different from all other treatments.**

***Keywords* – *P. glandulosus,* Agar, Malt Extract, *P. ostreatus,* Mushroom.**

I. Introduction

Africa constitutes at least 25% of the total mushroom biodiversity worldwide but contributes barely 0.4% of total mushroom sales and new mushroom products on the global market. Yet mushrooms are well known in most indigenous African recipes (Mpeketula, 2008) and, at the onset of the rainy seasons, it is customary to find rural people across Cameroon going out to search mushrooms from decaying wood and palm trees (Yongabi et al.,2004).

The cultivation of mushrooms for food and medicine is increasingly popular across the world, including Africa. However, the pace of progress in Africa is slow. Never before in contemporary times has the potential of mushrooms been so widely known and advocated. Mushroom production capacity of sub Saharan Africa is, proportionally, minimal compared to that of Latin America, China and Europe (Martinez-Carrera, 2002; Sanchez et al.,2002). Chioza and Ohga (2014) reported 240 kg per grower in Malawi, with a sale price of about two USD per kg. These values are not significantly different across sub Saharan africa. The mushroom sector in Africa is characterized by a lack of infrastructure, inadequate technical support, a scarcity of mushroom scientists and poor knowledge of mushroom diversity (Okhuoya et al., 2010).

Plectranthus glandulosus, Lamiaceae is one of 18 species of genus *Plectranthus* known around the world (Abdel-Mogib et al. 2002). This plant is found in the flora of West Africa (Ngassoum et al. 2001) and also in the Cameroonian flora (Amvam et al. 1998; Ngassoum et al. 2001; Tatsadjieu et al. 2008). It is an herbaceous plant and carries violet flowers during the flowering period. Ethno botanic studies reveal the use of its leaves in the conservation of stored maize and beans against insect and fungal damages (Tatsadjieu et al., 2008). Nukenine et al. (2007) reported that the powder of *P. glandulosus* leaves causes significant mortality, a reduction in damage, and inhibition of progeny production and a suppression of the population growth of *S. zeamais*.

The objective of this study was, therefore, to assess the ability of *P. glandulosus* leaf powder to influence mycelia growth of oyster mushroom specie.

II. Materials and Methods

MATERIALS

The sclerotia of the fungus *(Pleurotus ostreatus*) used for the study were obtained from the Laboratory of Crop protection of the Institute of Agricultural Research Bambui. The leaves of *P. glandulosus* were collected in July 2012 from Ngaoundere (latitude 7°22' North and longitude 13°34' East, altitude 1.100 m.a.s.l.) located in

the Vina Division of the Adamawa region of Cameroon. The plants were less than one-year old and only the green leaves were collected. The identity of the plant was confirmed at the Cameroon National Herbarium in Yaounde. The leaves were dried at room temperature for seven days, and then crushed. The crushed leaves were ground until the powder passed through a 0.20 mm sieve and then stored in a freezer at –20°C for use .

METHODS

Preparation of culture media

10g, 4g and 2g of powdered *P.glandulosus* were measured and put into separate glass jars containing agar or Malt extract dissolved in 250ml of water, to make dilutions of 5%, 2% and 1% respectively. This was then autoclaved for 15 minutes at *121* °C and 15psi.

Treatments:

- Pure Agar
- Pure Malt extract
- Agar + 5% *P.glandulosus*
- Agar + 2% *P.glandulosus*
- Agar + 1% *P.glandulosus*
- Malt extract + 5 % *P.glandulosus*
- Malt extract + 2 % *P.glandulosus*
- Malt extract + 1 % *P.glandulosus*

Inoculation in Petri dishes

Inoculation of the Petri dishes was done by agar tissue culture transfer under sterile conditions .The sterilized substrates were each poured in a set of four Petri dishes and allowed to coagulate. mother culture of *P.ostreatus* collected using a heated and hot cork borer of 4.0mm circumference, was used in inoculating the petri dishes. They were then sealed with paraffin, labelled (date and composition) and stored in the incubator at 20^0 C.

Spawn preparation

The spawn of Oyster mushroom (Pleurotus spp.) was prepared on maize powder thoroughly mixed with sawdust and rice husk in a ratio of 1:3:3. This mixture was filled in polypropylene bags after the adjustment of its moisture contents to about 30%. Substrate weight of each bag was 500g.The substrate was pasteurized for three hours by using a metal drum designed for pasteurization. spawning of substrate was done at 10% of substrate on wet weight basis. The spawned bags were incubated in dark for impregnation with the mushroom mycelia. After incubation, the bags were transferred in to the cropping room for fruiting at 30 to 35°C temperatures and 70 to 90% humidity. (Khan et al., 2009). Sufficient light and controlled ventilation was allowed during the cropping period. Water was also sprayed regularly to keep the surface of the substrate moist. Mature fruiting bodies were harvested from two flushes by twisting them slightly near the base. No remnants of harvested sporophores were allowed to remain in the substrate.

Data collection

The growth of mycelia in Petri dishes and glass jars were inspected for growth and contamination, and data collected. Data collection from Petri dishes was taken from 4days after inoculation date, by measuring the diameter of mycelia spread on the culture media from the centre, using a meter ruler. The collection was done 4, 6, 8 and 10 days after. Results were recorded for further analysis.

From substrates in bags, number of pinheads formed and total number of fruiting bodies was recorded and the experiment was laid out according to the completely randomized design with three replications.

Data analysis

JMP (SAS) statistical software was used in conducting ANOVA for the different treatments .

III. RESULTS

Effect of Plectranthus glandulosus leaf powder on Pleurotus ostreatus mycelia growth in petri dishes.

The growth of *P. ostreatus* varied according to media composition incubation period ($F_{(3;12)}$ = 81.10-366.16, P<0.0001 for all the media except Agar+5%*P. glandulosus* where P>0.05).

Table 1: Growth of *Pleurotus ostreatus* in Agar and in combinations with *Plectranthus glandulosus* leaf powder

Period	Mean ±Standard error (Mean±SE)				$F_{(3; 12)}$
	Agar	Agar+5%*P. glandulosus*	Agar+2%*P. glandulosus*	Agar+1%*P. glandulosus*	
0	0.00 ± 0.00^{dA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{eA}	0.00 ± 0.00^{eA}	—
4	1.88 ± 0.23^{cA}	0.60 ± 0.15^{aB}	1.28 ± 0.08^{dB}	1.25 ± 0.13^{dBC}	11.18**
6	3.00 ± 0.22^{bA}	0.90 ± 0.35^{aB}	2.28 ± 0.07^{cA}	2.28 ± 0.07^{cA}	14.41***
8	3.88 ± 0.35^{bA}	1.13 ± 0.45^{aB}	3.25 ± 0.12^{bA}	3.85 ± 0.38^{bA}	18.54***
10	5.28 ± 0.16^{aA}	1.13 ± 0.68^{aB}	5.43 ± 0.18^{aA}	5.40 ± 0.04^{aA}	29.83***
$F_{(4;15)}$	81.10***	1.82^{ns}	366.16***	120.03***	

^{ns} P>0.05; *** P<0.0001.

Means ± S.E. followed by the same capital letter in rows and the same lower letter in the column do not differ significantly at $P < 0.05$ (Tukey's test)

^{ns} P > 0.05, * P < 0.05, ** P < 0.001, *** P < 0.0001

Table 1 showed that the *P. ostreatus* spawn grows in different media but this growth was reduced when the concentration of *P. glandulosus* increases. At the highest concentration of *P. glandulosus* leaf powder (5%), the growth was completely inhibited after 10 days.

As days increased from 4-10 days after inoculation (DAI), there was no significant difference mycelia growth for 5% *P. glandulosus* concentration in agar. While agar

alone showed significant difference in mycelia growth from 4-6 DAI and 8-10 DAI, there was no significant change from 6-8 DAI. With 2% and 1% *P. glandulosus* concentration in agar, there was significant difference every 2DAI from 4-10 DAI.

Comparing the different concentrations of *P. glandulosus* and the control (agar) there was no significant difference between 1,2 and 5% concentrations 4DAI while the control had a better growth of 1.88cm. However, from 6 DAI to 10 DAI, there was no significant difference between the control, 2% and 1% concentrations of P. *glandulosus.*

Table 2: Growth of *Pleurotus ostreatus* in Malt extract and in combinations with *Plectranthus glandulosus* leaf powder.

Period	Mean ±Standard error (Mean±SE)				$F_{(3; 8)}$
	Malt Extract	Malt Extract+5%*P. glandulosus*	Malt Extract+2%*P. glandulosus*	Malt Extract+1%*P. glandulosus*	
0	0.00 ± 0.00^{eA}	0.00 ± 0.00^{eA}	0.00 ± 0.00^{eA}	0.00 ± 0.00^{eA}	—
4	1.52 ± 0.06^{dA}	0.40 ± 0.04^{dC}	1.32 ± 0.01^{dB}	1.37 ± 0.02^{dAB}	189.40***
6	2.40 ± 0.06^{cB}	0.70 ± 0.01^{cC}	2.35 ± 0.01^{cB}	2.66 ± 0.01^{cA}	854.91***
8	3.15 ± 0.09^{aC}	0.95 ± 0.02^{bD}	3.43 ± 0.02^{bB}	3.66 ± 0.01^{bA}	745.66***
10	3.88 ± 0.02^{aC}	3.88 ± 0.01^{aC}	6.35 ± 0.03^{aB}	6.83 ± 0.03^{aA}	4229***
$F_{(4; 10)}$	764.43***	7040***	17270***	19260***	

^{ns} P>0.05; *** P<0.0001.

Means ± S.E. followed by the same capital letter in lines and the same lower letter in the column do not differ significantly at $P < 0.05$ (Tukey's test)

^{ns} P > 0.05, * P < 0.05, ** P < 0.001, *** P < 0.0001

Table 2 shows that the growth of *P. ostreatus* varied according to media composition incubation period ($F_{(3;8)}$ = 764.43-19260, P<0.0001 for all the media).

Malt extract showed a significant difference in mycelia growth from 4-6 and 6-8 DAI but no difference from 8-10 DAI. For the other treatments, there was a significant change in mycelia growth every 2DAI from 4-10 DAI. Comparing the different treatments 4 DAI, there was no significant difference between the control (malt extract) and 1% *P. glandulosus* concentration but there was a significant difference between the control and the other two concentrations. From 6 DAI to 10 DAI, 1% *P. glandulosus* concentration had a significant better growth rate of mycelia than the other treatments. While the control and 5% *P. glandulosus* concentration had no significant difference in terms of mycelia growth 10 DAI, 2% *P. glandulosus* had a significant better growth than the control and 5% *P. glandulosus.*

Primordia and fruiting bodies formed from mycelia of Plectranthus glandulosus in agar

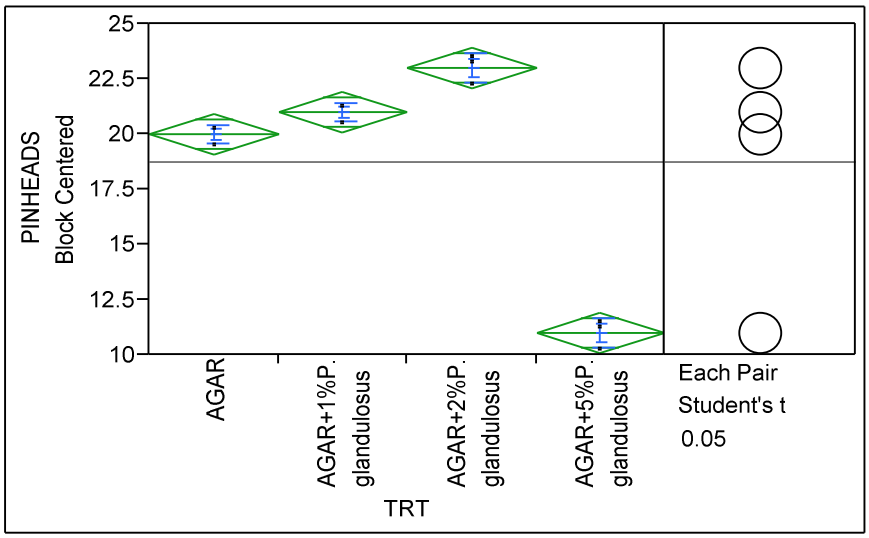

Fig.1. Oneway Analysis of Pinheads By Treatment of *Plectranthus glandulosus* in agar

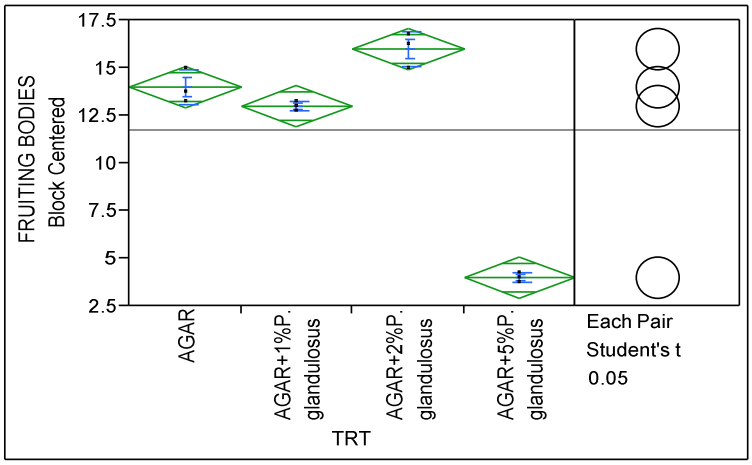

Fig 2. Oneway Analysis of Fruiting bodies By Treatment of *Plectranthus glandulosus* in agar

Table 3. Effect of *Plectranthus glandulosus* in agar on number of primordial and fruiting bodies

TREATMENTS	NO. PINHEADS (PRIMORDIA)	NO. FRUITING BODIES
AGAR	20^{B}	14^{B}
AGAR + 1% *P. glandulosus*	21^{B}	13^{B}
AGAR + 2% *P. glandulosus*	23^{A}	16^{A}
AGAR + 5% *P. glandulosus*	11^{C}	4^{C}

Levels not connected by same letter in same column are significantly different.

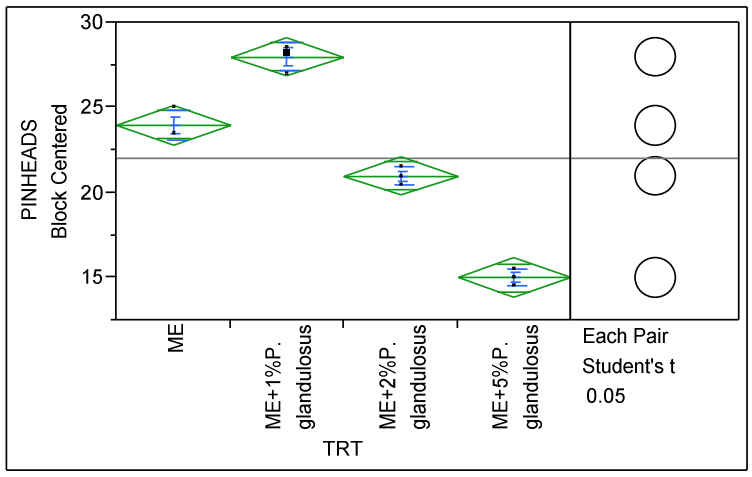

Fig.3. Oneway Analysis of Pinheads By treatment of *Plectranthus glandulosus* in Malt extract

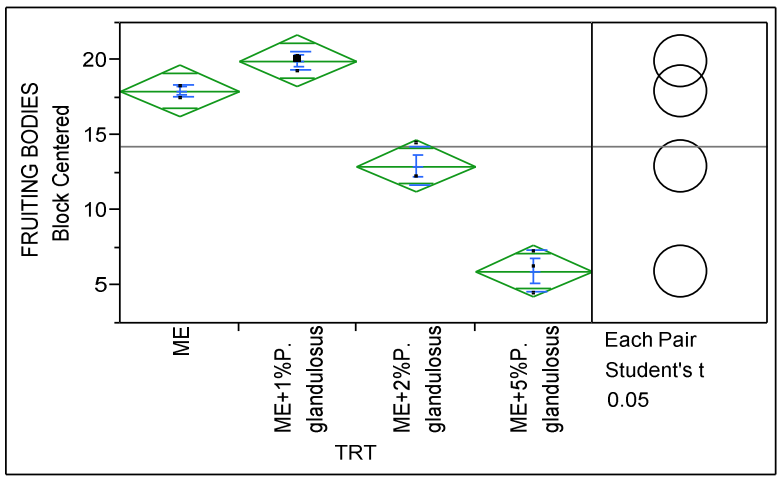

Fig.4. Oneway Analysis of Fruiting bodies by treatment of *Plectranthus glandulosus* in Malt extract

Table 4. Effect of *Plectranthus glandulosus* in malt extract on number of primordial and fruiting bodies

Treatments	No. Pinheads (Primordia)	No. Fruiting Bodies
Malt extract	24^{B}	18^{A}
Malt extract + 1% *P. glandulosus*	28^{A}	20^{A}
Malt extract+ 2% *P. glandulosus*	21^{C}	13^{B}
Malt extract + 5% *P. glandulosus*	15^{D}	6^{D}

Levels not connected by same letter in same column are significantly different.

Table 3, fig 1 and fig 2 show that, *P. glandulosus* incorporated as 1% in agar medium, resulted in mycelia producing 21 pinheads and 13 fruiting bodies which are not significantly different from what is produced by the control (20 and 14 respectively).However, at the 2% concentration of *P. glandulosus* more pinheads (23) and fruiting bodies (16) were formed which are significantly different from all other treatments.

Table 4 , fig 3 and fig 4 describes the statistical data about number of primordia (pinheads) and fruiting bodies formed from mycelia of malt extract.

When *P. glandulosus* is incorporated into malt extract, there is a significant difference between the treatments in terms of number of pinheads formed. At 1% concentration of *P. glandulosus* in malt extract, 28 pinheads were formed against 24 for the control(malt extract) while at higher concentrations of 2% and 5%, the number of pinheads (21 and 15 respectively) were significantly lower than that of the control. With regards to fruiting bodies formed, there was no significant difference between the control and 1% concentration of *P. glandulosus and* they had 18 and 20 fruiting bodies respectively. On the other hand, there was a significant difference between the control (18) and 2% and 5% (13 and 6 respectively) concentration of *P. glandulo*

IV. Discussion

Incorporation of *P. glandulosus* in agar and malt extracts had a beneficial effect on mycelia growth and mushroom development when the concentration was lower than 5%. While malt extract proved to be a better medium than agar for mycelia development and mushroom development (pinheads and fruiting bodies), it is also interesting to know that incorporating *P. glandulosus* into agar at 2% concentration, greatly improved on the number of pinheads and fruiting bodies formed (23 and 16 respectively). *P. glandulosus* powder contains several monoterpenes (Ngassoum et al. 2001; Nukenine et al. 2007) which could contribute in altering the composition of the medium or substrate necessary for mycelia growth and mushroom development. Yield of mushroom in these substrates could be related to other factors like aeration, water holding capacity etc. Proper gaseous exchange in the substrate is essential for mycelia development. The presence of *P. glandulosus* may play in role in gaseous exchange and thereby increasing mushroom production.

Supplementation of substrates with various sources of organic nitrogen, improves growth and development of various species of basidiomycetes (Loss et al. 2009, Moonmoon, et al. 2011). Organic sources of nitrogen can be easily used by fungi since the absorption of these molecules is more efficient and allows the fungi to obtain more energy for mycelia growth and mushroom formation. Özçelik & Peksen, 2007 reported that addition of organic matter decreases the granulometry of substrate and improves the moisture retention . For mushroom formation, the fungus requires a considerable amount of water, due to the high content of water in mushrooms (Tewari, 1986). Probably, P. glandulosus assists in the water retention capacity of substrates.

V. Conclusion

From results obtained, it is proven that *P. glandulosus* positively influences the growth and development of *P. ostreatus* at lower concentrations below 5% while at high concentrations of 5%, they retard growth and development. However, this study was conducted on agar and malt extract with just 3 concentrations of 1,2 and 5%. Therefore, more research is encouraged on other media and more varying concentrations.

References

[1] Abdel-Mogib M, Albar HA, Batterjee SM. 2002. Chemistry of genus *Plectranthus*. Molecules.7:271–301.
[2] Amvan ZPH, Biyiti L, Tchoumbougnant F, Menut C, Lamaty G, Bouchet P. 1998.
[3] Chemical composition and antifungal activity of thirteen essential oils from aromatic plants of
[4] Cameroon. flavour fragrance Journal 13: 107–114.
[5] Chioza A, Ohga S. 2014. Cultivated mushrooms in Malawi: A look at the present situation. Advances in Microbiology. 4(1)6-11
[6] Khan F., Gaikwad S.M., Khan M.I., 2009. Entropy driven binding of *O*-glycan and glycoproteins to *Artocarpus hirsuta* Lectin: An SPR study. Asian J. Biochem., 4: 106-116.
[7] Loss, E., Royer, A. R., Barreto-Rodrigues, M., & Barana, A. C. 2009. Use of maize wastewater for the cultivation of the Pleurotus spp. mushroom and optimization of its biological efficiency. Journal of Hazardous Materials, 166, 1522-1525.
[8] Martinez-Carrera D .2002. Current development of mushroom biotechnology in Latin America. Micologia Aplicada International. 14 (2):61- 71.
[9] Moonmoon, M., Shelly, N. J., Khan, M. A., Uddin, M. N., Hossain, K., Tania, M., & Ahmed, S. 2011. 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 Sciences, 18, 323-328 .
[10] Mpeketula PMG .2008.Indigenous mushroom species cultivation, processing and utilization for food security and conservation. National Research Council of Malawi, Conference Proceedings. 95-105

[11] Ngassoum MB, Jirovetz L, Buchbauer G, Fleischhacker W. 2001. Investigation of essential oils of *Plectranthus glandulosus* Hook f. (Lamiaceae) from Cameroon. *J Essent Oil Res 13*: 73-75.

[12] Nukenine EN, Adler C, Reichmuth Ch. 2007. Efficacy evaluation of plant powders fromCameroon as post-harvest grain protectants against the infestation of *Sitophilus zeamais* Motchulsky (Coleoptera:Curculionidae). Journal of Plant Disease and Protection, 114(1): 30-36.

[13] Özçelik, E., & Peksen, A. (2007). Hazelnut husk as a substrate for the cultivation of shiitake mushroom (Lentinula edodes). Bioresource Technology, 98, 2652-2658.

[14] Sanchez JE, Huerta G, Montiel E (Eds.) .2002. Mushroom Biology and Mushroom Products, Proceedings of the 1V International Conference. University of Morelos, Cuernavaca, Mexico. 468 pp

[15] Tatsadjieu NL, Etoa F-X, Mbofung CMF, Ngassoum MB. 2008. Effet of *Plectrantus glandulosus* and *Ocimum gratissimum*. Essential oils on growth *Apergillus flavus* and Aflatoxin B_1 production. Tropicultura 26(2): 78–83.

[16] Tewari, R. P. 1986. Mushroom cultivation. Extension Bulletin. Indian Institute of Horticulture Research, 8, 36.

[17] Yongabi K, Martinez-Carrera D, Agho M (2004) Ethnomycological studies on wild mushrooms in Cameroon, Central Africa. Micologia Aplicada International, 16(2):34-36.

[18] Yongabi KA (2004) Studies on the use of medicinal plants and macrofungi (Lower Plants) in water and wastewater purification. Proceedings of an International E-conference organized by the International Organization for Biotechnology and Bioengineering (IOBB), Sweden, June 14-25.