Domestication of an indigenous Ghanaian edible mushroom-*Pleurotus sajor-caju*: variations in the proximate and mineral contents of the wild and cultivated species.

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Abstract

Growth parameters of mycelia and morphology of an indigenous *Pleurotus sajor-caju* strain (PscW) originally collected at the Wli Agumatsa Forest in Ghana was compared on four substrate agar media. These substrate media, elephant grass, rice straw, thatch and sawdust (*Triplochiton scleroxylon* and *Chlorophora excela)* were used as the basal media, and this was followed by fructification on sawdust. In addition, proximate analysis and ten mineral contents of the wild species were studied. Solid agar media made from three of the substrates rice straw, thatch and sawdust supported faster mycelial growth rate relatively equally (0.77, 0.75, 0.73 cm/day, respectively), as compared to the elephant grass based agar (0.64 cm/day). The density of the white coloured longitudinally linear mycelia was highest on elephant grass, followed by thatch and sawdust. When fruited on sawdust, it was observed that the interval between flushes did not influence the number and weights of fruit bodies obtained in successive flushes and that the highest yield in terms of both the number and weight of fruit bodies were obtained in the first flush (47 and 46 % respectively) with reduction in consequent flushes. Of the essential macro elements analysed the mushroom samples were rich in phosphorus, potassium and sodium with the trend in decreasing order being K<P<Na. Of the micro elements analyzed iron was the most dominant. *P. sajor-caju* is another species of oyster mushroom that can be cultivated in Ghana to contribute to diversity of cultivated mushrooms in Ghana for consumption and for biodiversity conservation in our forests. .

Keywords: Domestication, mushroom, substrate media, mineral contents

Introduction

Millions of tons of cultivated, medicinal and wild mushrooms are consumed each year worldwide (Royse, 2013). The total estimated value of the mushroom industry is greater than $45 billion (Chang, 2006) with cultivated, edible mushrooms comprising about 67% of the total or about $30 billion (Royse, 2013). Six different species make up about 90% of the total world production of edible mushrooms. The common cultivated mushroom *Agaricus bisporus* is the most widely cultivated edible mushroom and this mushroom is followed up by *Pleurotus spp.* accounting for 27% and *Lentinnla edodes* contributing approximately 17% (Royse, 2013; Dongmei, 2010)

Several species of edible and medicinal mushrooms are available in the forests of Ghana. In a recent survey carried out in the Wli Agumatsa waterfalls in the Volta region of Ghana, twenty species of mushrooms were collected of which eighteen were identified using phenotypic methods. Of the identified mushrooms five are edible and belong to the order Agaricales (3), Polyporales (1) and Auriculariales (1) of the genera *Pleurotus* (2), *Lentinus* (1), *Volvariella* (1) and *Auricularia* (1). Three species of mushrooms were recorded for the 1st time in Ghana namely: *Favolus brasilensis*, and oyster mushrooms *Pleurotus sajor-caju* and *P. albidus*.

Oyster mushrooms (*Pleurotus* species), the second largest commercially produced mushroom in the world, are found growing naturally on rotten woody material (Phillips, 2006). *In vivo* research has shown that consumption of oyster mushrooms lowers cholesterol levels (Rop *et al.*, 2009) because they naturally contain lovastatin (Gunde-Cimerman and Cimerman, 1995). Seventy different species of oyster mushrooms have been characterized. One of the well known species is *P. sajor-caju*. This mushroom is considered a delicacy due to its flavor and taste

(Chang *et al*., 1981). The mushroom can be grown on various plant waste materials (Gupta *et al*., 2013; Mane et al., 2007; Chang *et al*., 1981), which have either been sterilized, pasteurized or fermented and can be grown on a relatively large scale. Nutritional analysis of the mushroom using HPLC analysis indicate that its fruitbodies are rich in proteins (27.4-34.8%) with alanine, the major amino acid, soluble sugars (28.6-32.2%) with glucose, trehalose and glutamic acid being the major sugars and minerals (Gupta *et al*., 2013).

In Ghana, *P. ostreatus* is the most cultivated mushroom with its production to date standing at 300 metric tons per annum. It is grown on composted sawdust of *Triplochiton scleroxylon* locally known as 'wawa' supplemented with rice bran and lime (Obodai et al., 2007) and recently on a mixture of *Triplochiton scleroxylon* and *Chlorophora excelsa*. Due to deforestation, over grazing and bushfires mushrooms are dwindling in numbers and the fear of losing them has led to the domestication of both edible and medicinal mushrooms found in Ghana. This study therefore reports on the domestication trials of *P. sajor-caju* in Ghana with its relative changes in the nutritional and mineral values of the wild and cultivated species.

Materials and methods

Study Area

Wli Agumatsa Waterfall is located in the Agumatsa wildlife sanctuary in the Agumatsa Afadjato range in the Hohoe District of Ghana. This Waterfall is of great significance to the surrounding communities. It is said to be the tallest waterfall (800m) in the whole of West Africa and is the most popular tourist attraction in the whole of the Volta Region (www-1). The immediate valley and the surroundings extending up to 35 km² are a designated forest reserve (wildlife sanctuary)

with over 200 birds' species, bats, over 400 species of butterflies and the endangered mona monkeys and antelopes (www-2). The main source of water for the water fall is the Agumatsa river which flows through a distance of about 25 km from its source in Togo before culminating into a fall over a cliff of over 100 meters high at Wli. About 200 g of fresh wild *P. sajor-caju* mushrooms (Fig 1) were collected in the Wli Agumatsa waterfalls in May and November, 2013.

Sample collection, transportation and tissue culture preparations

The collected mushroom samples were placed in brown envelopes and identified within 4 h of picking. Its edibility was confirmed by the tourist guide who had harvested some for his household. Tissue culture preparations were made from the fresh, well-formed and firm fruit bodies, and then incubated at 28 + 2 ^{o}C. One hundred and fifty grams were then dried in a field dryer overnight and used for all chemical analysis.

***Media requirement of P. sajor-caju* strain PscW**

Four types of agar media were prepared as described by Narh Mensah and Obodai (2014) from four agricultural wastes: rice straw (RS) (*Oryza sativa*), sawdust (SD) (a mixture of *Triplochiton scleroxylon* and *Chlorophora excela*), elephant grass (EG) (*Pennisetum purpureum*) and thatch (TH) (*Imperata cylindrica*). The experiment was performed in triplicates.

Measurement of mycelia growth rates and mycelia densities

Daily readings of radial mycelia growth and mycelia densities were measured as described by Narh Mensah and Obodai (2014).

The mycelia densities were based on direct observation of the thickness (rated from 1 being least dense and 5 being highly dense) of the mycelia on the media. Mycelia were classified as least dense when they could barely be seen when observed from the cover of the plate, whereas highly

dense mycelia were very visible and were opaque such that the media could not be seen when observed from the cover of the plates.

Spawn and substrate preparation

Spawn was prepared on sorghum grains in accordance to Oei (1991). The oyster mushrooms were then grown on sawdust of a mixture of *Triplochiton scleroxylon* and *Chlorophora excelsa* using the plastic bag method (Auetragul, 1984; Obodai *et al*., 2007). Yield characteristics recorded include weights per flush, number of fruit bodies per flush and interval between flushes.

Chemical analysis

The whole mushrooms (pileus and stipe) of the first flushes harvested were dried and pulverised for chemical analysis. Proximate analysis of crude fat, protein, fibre and carbohydrate were performed according to standard methods (AOAC, 2005). The 6.25 factor was used to convert nitrogen to crude protein (Breene, 1990). Total carbohydrate was determined by subtracting the amount of ash, protein; and fat from total dry matter. All the minerals (Copper (Cu), Iron (Fe), Manganese (Mn), Magnesium (Mg), Lead (Pb), Zinc (Zn), Calcium (Ca) were analyzed by the AAS (PerkinElmer AANALYST 400) spectrophotometer (JENWAY 6300) after wet digestion of the samples (AOAC, 2005). The ascorbic acid method for colour development was used in the determination of Phosphorus (P). Flame photometer (JENWAY PFP 7) was used in the determination of Potassium (K) and Sodium (Na). Similar analysis were carried out for the wild samples.

Statistical analysis

Data obtained from triplicates were analyzed with GenStat Discovery Software (4th Edition). The correlations table was generated using GenStat Discovery Software (4th Edition) with the 12 observations from all the set-ups. Mycelia growth rates were obtained from linear trendline equations from the plot of the incubation period versus the radial mycelia extension averaged from the triplicates of each setup.

Results and Discussion

Growth of P. sajor-caju strain PscW on media

The mycelia growth rates varied slightly among RS, TH and SD (0.77, 0.75, 0.73 cm/day respectively). The slowest growth rate (0.64 cm/day) was observed when the strain was incubated on EG (Fig 2). This was apparent by the third day of incubation (Fig 3). However, although there was minimal variation in the data obtained from the triplicates of each set up for EG, SD and TH, the variation in the data obtained for PscW on RS was wide (Fig 3). There were no mycelia colour changes throughout the incubation period (8 days) and the mycelia of the mushroom showed a white coloured longitudinally linear morphology on all the agar media studied (Fig 4). Concentric rings were observed on EG (Fig 4). The observed mycelia density was highest on EG, followed by TH and SD. The least mycelia density was observed on RS.

Although the reported mycelia morphology is consistent with observations made by Atri *et al.* (2012) for *P. sajor-caju* collected from India and incubated at 25 ℃ on potatoes dextrose agar, there were some differences in the mycelia growth rates in the two studies. The highest mycelia growth rate presently reported is 0.77 cm/day on RS and was 1.20 cm/day on PDA (Atri et al., 2012). While this difference could be attributed to the probable difference in the strains used, it can better be attributed to the nutritional composition of the basal substrate in the agar media used.

Mycelia density does not correlate positively with mycelia growth rate on the various media used. for An observation made by some of the authors on *Lentinus squarrosulus* strains Lsf and Sqw grown on agar media prepared using agro-waste based media (EG, RS, SD and TH).

Yield characteristics of P. sajor-caju strain Pscw on sawdust

The yields of the mushrooms were generally not evenly distributed across the flushes. The highest yields (47 % and 46 % in terms of weight and number of fruit bodies obtained respectively) were obtained in the first flush. The yield decreased with consequent flushes, and the lowest yield both in terms of weight and number of fruit bodies (11 % and 6 % respectively) was recorded in the fourth flush. This observation has been previously reported on various *Pleurotus* species and strains including *P. sajor-caju* (Ashraf et al., 2013) cultivated on various substrates. The mushroom fruit bodies were generally greyish under the cropping conditions employed and over-matured fruit bodies had very wavy edges which curled inwards (Fig 5). The total yields of the fruit bodies of the oyster mushroom under study and the *P. ostreatus* strain EM-1 which is under cultivation had comparable biological efficiencies (results not shown).

The correlation between the number and weight of fruit bodies and the interval between successive flushes obtained per bag is presented in Table 1. The results show various degrees of positive correlations among the parameters under study. There were weak positive correlations between the intervals between flushes and the weight of fruit bodies (0.04) and number of fruit bodies (0.15) obtained, whereas a relatively strong positive correlation was recorded between the number and weight of fruit bodies (0.61) obtained from each bag during the cropping period.

These results indicate that the number and weight of *P. sajor-caju* strain PscW fruit bodies obtained have little or no dependence on the interval between flushes. Thus, the interval between flushes does not influence the number and weights of fruit bodies obtained in successive flushes.

Although this is the case, the interval between successive flushes could have economic implications for commercial producers of the mushroom especially when there is high demand for the product. Long intervals between flushes could result in periods within which little or no mushrooms will be available for sale.

On the other hand high positive correlation between the weight and number of fruit bodies show that a high number of fruit bodies will result in a correspondingly high total weight of fruit bodies obtained per flush per bag. As such, the total weight of PscW fruit bodies harvested per flush is dependent on the number of fruit bodies harvested at a time. This fact could influence sales of the mushroom as both traders and customers can approximate the weight of the mushroom sold by observing the quantity sold. This is especially useful in Ghana where traditionally, vegetable traders only approximate quantities sold without weighing.

***Proximate composition of cultivated P. sajor-caju strain PscW* mushroom**

Protein, one of the most important food factors, with its sufficiency in a diet indicating its adequacy and quality was found to be 21.65 % and 21.34 % (Figure 6) for the wild and cultivated mushrooms (Obodai et al., 2014) respectively using methods described in AOAC, 2005. These values were slightly lower than those recorded by Gupta et al., 2013 (27.4-34.8 %) but comparable with values obtained by Bano and Rajarathanam (1982) (19.4 %). The average protein content of *P. sajor-caju* studied was found to be higher than those of common food items which ranges from 7.6% in potato to 18.4% in cabbage and 12.7 % in wheat and 9.4% in corn but lower in egg and meat which contain 50.6% and 83% protein respectively (Bano, 1976; Chang, 1997). It was interesting to note that the cultivated mushroom contained a higher fat content (19.52 %) as compared to the wild (3.88 %). This value is the highest value recorded in comparison to similar work carried out for other cultivated mushrooms (Obodai and Apetorgbor, 2009). The authors recorded a relatively high fat content of 10.8 % in *Auricularia auricula* and very low fat content of 1.1-2.0 % in *Pleurotus ostreatus* and *P. oeus* stains. Çaglarlrmak *et al.* (2002) also recorded lower values of fat contents as follows: *P. ostreatus*, 0.14 % and *Volvariella volvacea*, 0.74 %.

Carbohydrate, one of the major constituents of PscW mushrooms was in the range of 18.49 % and 9.00 % for the wild and cultivated species respectively. These values are lower than those recorded for *P. ostreatus* and *P. oeus* strains, which ranged from 46.65 to 81.8% (Obodai and Apetorgbor, 2009; Bano and Rajarathanam, 1982). The percentage of crude fiber in the wild strain is slightly higher than that of the cultivated strain. Nevertheless, the similarity in all composition profiles reported in literature suggests that all parameter content may be affected quantitatively by the substrate (Paz et al., 2012).

Mineral analysis

Essential macro elements

The variations and mean concentrations of ten macro (Na, K, Ca, P and Mg), micro (Fe, Zn, Cu and Mn) elements and the heavy metal (Pb) were examined in the wild and compared with cultivated species of the mushroom. Macro element contents are presented in Table 2. Of the essential macro elements analysed the mushroom samples were rich in P, K and Na. The trend in decreasing order of macro elements content for the wild mushroom was K<P<Na<Mg<Ca as compared with values of the cultivated one which was K<Na<P<Mg<Ca (Obodai et al., 2014).

Trace elements/Heavy metals contents

Living organisms require varying amounts of "heavy metals". Iron, cobalt, copper, manganese, molybdenum, and zinc are required by humans. Excessive levels can be damaging to the organism. Other heavy metals such as mercury, plutonium, and lead are toxic metals and their accumulation over time in the bodies of animals can cause serious illness. Copper is an essential metal which serves as a constituent of some metalloenzymes, including polyphenol oxidase (Rosenzweig & Sazinsky, 2006), and is required in haemoglobin synthesis and the catalysis of metabolic growth (Silvestre et al, 2000). The concentration of Cu determined in the mushroom was 0.003 ± 0.00 mg/kg which is far below the safe limit of 40 mg/kg set by WHO (WHO, 1982). Copper levels in mushroom reported in literature are 12-181 mg/kg (Tùzen, 2003) and 13.4-50.6 mg/kg (Soylak et al., 2005). These values recorded from wild mushrooms were picked from soil samples near industrial sites.

Of all the trace elements analysed in this study, Fe content recorded the highest value of 0.34 ± 0.00 mg/kg which is lower than 15 mg/kg set by WHO. Levels of Fe reported in literature were 180-407 mg/kg (Isiloğlu et al., 2001) and 146-835 mg/kg (Tùzen, 2003). The trend in decreasing order of micro elements in the mushroom is Fe<Zn<Pb<Mn<Cu. All these values were far below the safe limits acceptable by WHO.

Fig 1: Wild *Pleurotus sajor-caju* growing on a tree in the Wli Agumatsa reserve

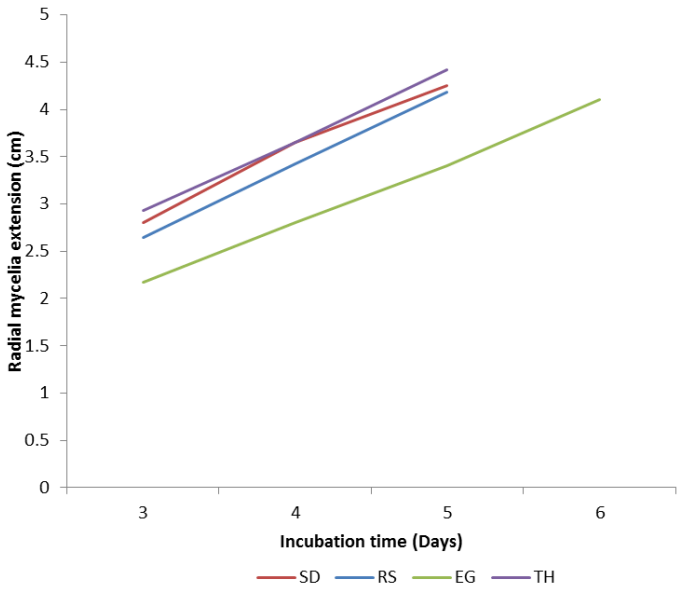

Fig 2: Mycelia growth rate of *P. sajor-caju* strain PscW on all agro-based agar media within the incubation period

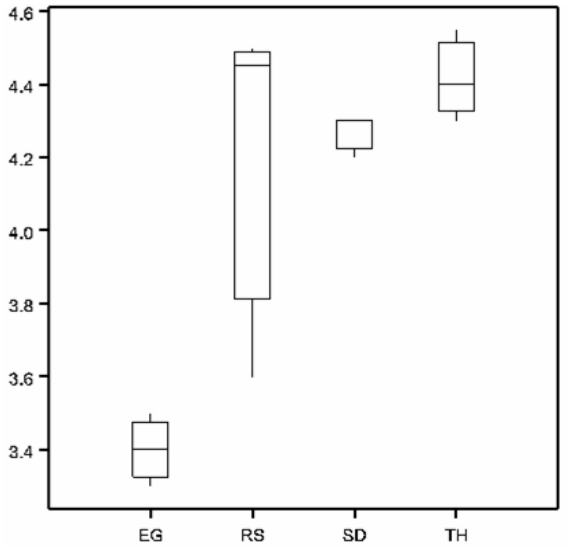

Fig 3: Box and whisker plots showing *P.sajor-caju* strain PscW radial mycelia extension by the 3rd day of incubaiton.

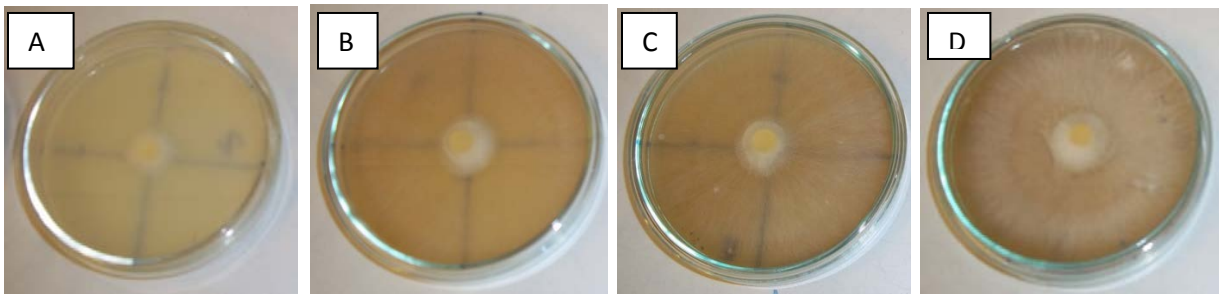

Fig **4**: *P. sajor-caju* strain PscW mycelia on the 8th day of incubation at 25 ℃. From the left sawdust agar, rice straw agar, thatch agar and elephant grass agar.

Fig 5: Cultivated *P. sajor-caju* growing on a mixture of sawdust from *Triplochiton scleroxylon* and *Chlorophora excelsa*

Table 1: Correlations between number and weight of fruit bodies and interval between successive flushes

Correlations

Interval_between_flushes			
Number_of_fruit_bodies	0.1504		
Weight_of_fruitbodies	0.0424	0.6095	
	Interval_between_flushes	Number_of_fruit_bodies	Weight_of_fruitbodies

Number of observations: 12

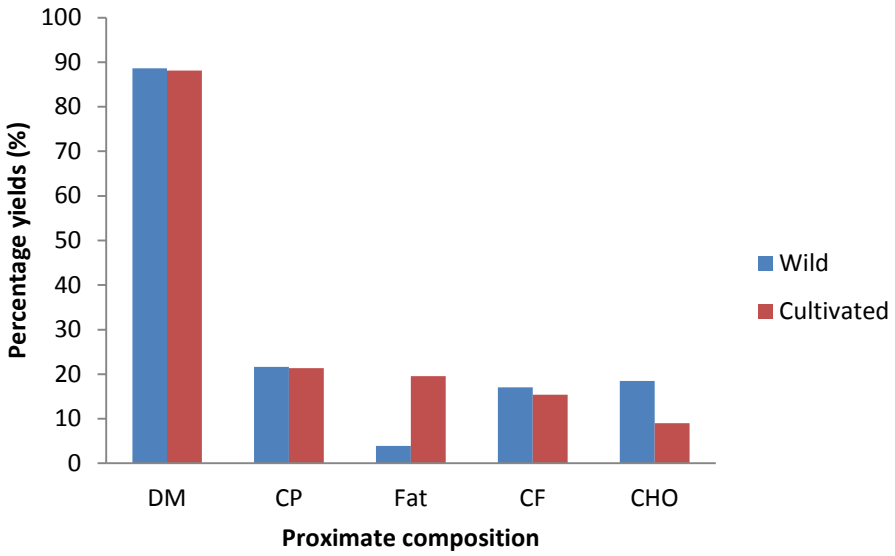

Fig 6: Comparison of proximate composition of wild and cultivated *Pleurotus sajor-caju* mushrooms. DM, CP, CF and CHO are dry matter, crude protein, crude fibre and carbohydrate respectively

Table 2: Macro and micro element composition of wild *P. sajor-caju* strain PscW

Component	Element	Composition (mg/kg dw)
Macro elements	Ca	0.586 ±0.00
	Mg	1.044 ±0.01
	K	11.3±0.05
	Na	5 ± 0.01
	P	6.055 ± 0.03
Micro elements	Fe	0.34 ± 0.00
	Zn	0.142± 0.002
	Cu	0.003 ± 0.00
	Mn	0.004 ± 0.00
Heavy metal	Pb	0.124 ± 0.001

References

AOAC 2005. Official methods of analysis of AOAC International, 18th Edition. AOAC International, Gaithersburg, Maryland, USA.

Ashraf, J., Ali, M.A., Ahmad, W., Ayyub, C.M., Shafi, J. 2013. Effect of different substrate supplements on oyster mushroom (Pleurotus spp.) production. *Food Science and Technology*, 1(3), 44-51.

Atri, N.S., Sharma, S.K., Gulati, A. 2012. Study on mycelial growth pattern of five wild Pleurotus species from North West India. *American-Eurasian Journal of Scientific Research*, **7**(1), 12-15.

Auetrugal, A. 1984. The highest aspects for cultivating oak mushroom (*Lentinula edodes*) in plastic bags. *Mushroom Newsletter for the Tropics* 5 (2): 11-15.

Bano, Z. 1976. The nutritive value of mushrooms. *Proceedings of first symposium on survey and cultivation of edible mushrooms in India, R R L*. Sringarm 2: 172.

Bano, Z., Rajarathanam, S. 1982. '*Pleurotus* mushroom as a nutritious food'. In: S.T. Chang and T. H. Quimo (Eds), *Tropical Mushrooms: Biological Nature and Cultivation Methods* The Chinese University Press, Hong Kong. pp. 363-380.

Breene, W. 1990, "Nutritional and medicinal value of speciality mushrooms", *Journal of Food Protection*, Vol. 53 pp. 883-894.

Chang, S.T. 1997. ''Nutritional and Medicinal properties of mushrooms''. In: k.E. Mshigeni,T.N. Ngwira, G.E. Kiangi, K. Mchombu, O.D. Mwandemele, M.E. Ngwira (eds). *Proceedings of the First West African Regional Workshop on Zero Emissions concept*, Benin. 15-19 November, 1997, pp. 35-37.

Chang, S.T., Lau, O.W., Cho, K.Y. 1981. The cultivation and nutritional value of Pleurotus sajor-caju. *European Journal of Applied Microbiology and Biotechnology*, **12**(1), 58-62.

Çaglarlrmak, N., Ünal, K. Ötles, S. 2002. Nutritional value of edible wild mushrooms collected from the Black Sea region of Turkey. *Micologia Applicada International* 14 (1) 1-5.

Gupta, A., Sharma, S., Saha, S., Walia, S. 2013. Yield and nutritional content of *Pleurotus sajor caju* on wheat straw supplemented with raw and detoxified mahua cake. *Food Chemistry*, **141**(4), 4231-4239.

Isıloğlu, M., Yilmaz, F., Merdivan, M. 2001. Concentrations of trace elements in wild edible mushrooms. *Food Chemistry* 73: 169-175.

Mane, V.P., Patil, S.S., Syed, A.A., Baig, M.M. 2007. Bioconversion of low quality

lignocellulosic agricultural waste into edible protein by Pleurotus sajor-caju (Fr.) Singer. *Journal of Zhejiang University, Science, B*, **8**(10), 745-51.

Narh Mensah, D.L., Obodai, M. 2014. Morphological characteristics of mycelia growth rate of two strains of the indigenous medicinal mushroom, *Lentinus squarrosuls* Mont. (Singer), on solid media. *African Journal of Agricultural Research*, **9**(23), 1753-1760.

Obodai, M., Dzomeku, M., Awotwe, B., Takli, R.K., Narh, D.L. 2007. Manual on mushroom cultivation technology in Ghana. CSIR-Food Research Institute.

Obodai, M. and Apetorgbor, M.M. (2009), "Proximate composition and nutrient content of some wild and cultivated mushrooms of Ghana", *Journal of the Ghana Science Association*, Vol.11 No.1, pp. 1-6.

Obodai, M., Ferreira, I., Fernandes, Â., Barros, L., Narh Mensah, D.L., Dzomeku, M., Urben, A., Prempeh, J., Takli, R. 2014. Evaluation of the chemical and antioxidant properties of wild and cultivated mushrooms of Ghana. *Molecules*, **19**(12), 19532-19548.

Rosenzweig, A.C., Sazinsky, M.H. 2006. Structural insights into dioxygen-activating copper enzymes. *Current opinion in structural biology*, **16**(6), 729-735.

Silvestre, M.D., Lagarda, M.J., Farra, R., Martineze-Costa, C., Brines, J. 2000. Copper, iron and zinc determination in human milk using FAAS with microwave digestion. *Food Chemistry*, 68: 95- 99.

Soylak, M., Saracoglu, S., Tùzen, M., Mendli, D. 2005. Determination of trace metals in mushroom samples from Kayseri, Turkey. Food Chem. 92: 649-652.

Tùzen M (2003). Determination of heavy metals in soil, mushroom and plant samples by atomic absorption spectrometry. Micro Chem. J. 74: 289-297.

World Health Organaiztion (1982). Evaluation of Certain Foods Additives and Contaminants (Twenty-Six Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report series, No. 683 Geneva.

(Www-1) URL (23 September 2013) www.touringghana.com/ecotourism/agumatsa.asp

(Www-2) URL (23 September 2013) www.ruford.org/rsg/projects/Patrick_k_ahumah_o