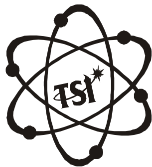
ISSN : 0974 - 7508 Volume 7 Issue 4



Trade Science Inc.

Natural Products
An Indian Journal

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NPAIJ, 7(4), 2011 [211-216]

Effect of physical and chemical factors on mycelial growth of ten wild Nigerian mushrooms during cellulase and amylase production

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Received: 16th July, 2011 ; Accepted: 16th August, 2011

ABSTRACT

In the previous studies, selected wild mushrooms from Nigeria were assessed for amylase and cellulase production. Within the same submerged liquid medium used for these enzyme analyses, the test higher fungi also produced some quantities of mycelial biomass under different growing conditions. In the present studies, influence of environmental factors and nutrient sources on mycelial biomass production of ten wild Nigerian mushrooms (Pogonomyces hydnoides, Termitomyces clypeatus, Nothopanus hygrophanus, Podoscypha bolleana,Corilopsis occidentalis, Agaricus blazei, Termitomyces globulus, Coriolus versicolor, Pleurotus tuber-regium and Agaricus sp.) during cellulase and amylase production were investigated. All the test fungi were able to produced mycelial biomass between temperature range of 25 and 40 ^{0}C. The optimal temperature that supported the best vegetative growth (113 mg/30ml) during amylase production in Agaricus sp was 25 ^{0}C. Agaricus blazei, Coriolus versicolor and Termitomyces globulus also produced mycelial biomass of 106,104 and 103 mg/30ml respectively at 25^{0}C. Likewise, all the fungi were able to produce mycelia at pH range of 3.8 and 7.8. The best mycelial dry weight (113 mg/30ml) were obtained during cellulase production in Termitomyes globulus at pH of 6.8. Termitomyces clypeatus had the highest significant mycelial yield of 117 mg/30ml when the medium was supplemented with carboxymethylcellulose (CMC). Similarly, high mycelial yield of 119, 116, and 114 mg/30ml were obtained for Pleurotus tuber-regium, Coriolus versicolor and Agaricus sp. respectively when the medium were supplemented with yeast extract at 28^{0}C and pH of 6.8.
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KEYWORDS

Mushroom culture;
Environmental factors;
Nutrients;
Mycelial biomass;
Enzymes.

INTRODUCTION

Macro fungi or higher fungi could be classified into two major groups. These are toadstools and mushrooms. The former are made up poisonous species while the latter comprises of members that are usually edible[1,2]. Macro fungi are lower plants that lacked flowers and fruits of the higher orders. The word 'mushroom' is a general term without any taxonomic significance. This is because mushrooms comprise of several groups that are not systematically related[2,3]. Generally, mushrooms are fungi that develop

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macroscopic fruiting bodies. They include morels, polypores, gilled fungi, jelly fungi, coral fungi, stinkhorns, bracket fungi, puffballs, and bird's nest fungi[4,5]. The fruitbodies of a typical mushroom represents the carpophores or reproductive structures of a microscopic fungi in which the actual organism is made up of hyphae or mycelia which are buried in the rich substrate used for growing[1,2]

Mycelial biomass of a fungus consists of fine, thread-like hyphae, which grow extensively through the organic rich substrate of the ecosystem. This normally takes place under adequate growing requirements such as temperature, pH, relative humidity and moisture in addition with nutrients to support the reproductive capacity in fungi[6-8].

The life cycle of a typical mushroom starts from spore to primary and secondary mycelium and then to tertiary mycelium which is known as the basidiocarp or the fruit bodies. This is then followed by series of cell division which results into formation of spores which could escape to the atmosphere. On settling on a suitable substratum, the spore will germinate again to produce primary mycelia and the cycle continues[2,8].

Cultivation of edible fungi usually involves three major steps. The first stage deals with the production of high starter inoculum known as mycelium, while the second step is the preparation of spawn. The last step is the formulation of compost and spawning to produce fruitbodies. Out of these three stages, the mycelial generation stage is the most important in the process of mushroom production because, if there are problem with the initial mycelial propagation, it will directly affect the fruitbodies yield[9-11]. Mycelia of mushrooms could be propagated under adequate culturing condition. The role of nutrients, environmental factors and enzymes are very important in the production of mycelial starter culture of a typical edible fungus[12,13].

In the earlier studies carried out by Jonathan and Adeoyo[14], ten wild Nigerian mushrooms (Pogonomyces hydnoides, Termitomyces clypeatus, Nothopanus hygrophanus, Podoscypha bolleana,Corilopsis occidentalis, Agaricus blazei, Termitomyces globulus, Coriolus versicolor, Pleurotus tuber-regium and Agaricus sp.) produced different significant quantities (P=0.05) of amylase and cellulase in the submerged liquid media. In the present studies, influence of environmental factors and nutrient sources on mycelial biomass yield of these fungi during cellulase and amylase production was investigated. This was necessary because little or no attention have been given to mycelial biomass production of Nigerian mushrooms under the influence of enzyme action, nutrients and environmental factors. Therefore, this present study was focused on providing useful information that could help in improving the mycelial biomass yield of selected wild macro fungi from Nigeria for better mushroom production.

MATERIALS AND METHODS

Mushroom samples used

The test mushrooms used were Termitomyces clypeatus, Termitomyces globulus, Pleurotus tuber-regium, Coriolus versicolor Agaricus sp, Podoscypha bolleana, Agaricus blazei, Pogonomyces hydnoides and Nothopanus hygrophanus, Corilopsis occidentalis. They were collected from different farmlands and forests within Akoko land in Ondo State, Nigeria. The collection period was between May and September, 2009.

Preparation of mycelial starter culture

Mycelial starter culture of each mushroom was generated by tissue culture employing the method of Jonathan and Fasidi[14]. The mycelial culture of each fungus were sub-cultured on plates of PDA supplemented with 0.5% yeast extract. Mycelial biomass production of each mushroom was determined by mycelial dry weight method described by Jonathan and Fasidi[15]. The different compounds required to form the synthetic medium were dissolved in 1litre of deionised water and pH adjusted to 6.3. The medium was dispensed into 250 ml jam bottles (30ml per bottle) and the mouth was covered with aluminium foil. They were sterilised in the autoclave at 121^0C for 15 min. After cooling 0.05g of streptomycin sulphate was added to suppress bacterial growth. Each bottle was then inoculated with actively growing mycelial culture of a specific mushroom using 7 mm diameter cork borer. They were incubated at 28 ± 2^0C for 7 days after which mycelia were harvested[15].

Effect of pH

The mycelia of each test sample was cultured separately on plates of potato dextrose agar (supplemented with 0.5% yeast extract1. The basal medium used has the following compositions: yeast extract, 2.5g; KH_2PO_4 0.05g ; $MgSO_4 . 7H_20$,0.05g; $FeSO_4$;KNO_3 1.55g and 1000ml of de-ionised water. The vitamins calcium pantothenate (500µg)was added following the procedures of Camacho-Rhuiz (16). These were dispensed into 150 ml conical flasks 30 ml per flask and supplemented with different nutrients The pH of the liquid medium was adjusted by using 0.1N HCl and 0.1N NaOH to 3.8, 4.8, 5.8, 6.8, 7.8 and sterilized. This was followed by inoculation with 7mm mycelial disc plug of the macrofungus using sterile cork borer. The inoculated flasks were incubated at $30^0 \pm 2^0$C for 7 days. Each treatment was replicated three times[17].

Effect of temperature

Similar basal medium used in the previous experiment was employed. After sterilization and inoculation with the appropriate mushroom. The flasks were incubated at 25^0C, 30^0C, 35^0C, and 40^0C for 7 days. Each treatment was replicated three times Harvesting was done using the procedures of Gbolagade[11]

Effect of carbon source

The mycelia of each test sample was cultured separately on plates of potato dextrose agar (supplemented with 0.5% yeast extract1. The basal medium used was similar to that of pH experiment[15]. The carbon sources used include carboxyl methyl-cellulose (CMC), glucose, maltose, and sucrose. After cooling, each bottle was inoculated with 7mm alga disc plug of the macrofungus and incubated at $30^0 \pm 2^0$C for 7 days. Each experiment was replicated thrice. The mycelial biomass was determined by mycelial dry weight method (Jonathan and Fasidi[15].

Effect of nitrogen sources

Similar synthetic liquid medium used in the carbon experiment was employed. The nitrogen sources used include urea, yeast extract, peptone, and $NaNO_3$. 30ml of each medium were dispensed into 100ml Erlenmeyer flask and sterilized; this was followed by inoculation with one 7mm alga disc plug of the macrofungus and incubated at $30^0 \pm 2^0$C for 7 days. Each treatment was replicated three times. The mycelial biomass was determined as outlined in the previous experiment[17]

Analysis of data

All results were subjected to analysis of variance (ANOVA) using general linear model option SAS. Test of significance was determined by Duncan´s multiple range test at 0.5% level of probability.

RESULTS AND DISCUSSION

TABLE 1 shows the pattern of mycelial biomass production by different wild Nigerian higher fungi during enzyme activities in different regime of temperature treatment. The most supportive temperature for the best mycelial biomass yield (113mg/100ml) was 25^0C for Termitomyces globulus during cellulase production and 30^0C for Agaricus sp during amylase production. The second best mycelial yield (108 mg/100ml) was obtained in Pleurotus tuber-regium during cellulase assay at 30^0C. Coriolus versicolor had the mycelial biomass yield of 107 mg/100ml (at 25^0C) in the sub-merged medium used for cellulase assay, closely followed by Agaricus blazei (106 mg/100ml) at the same temperature. Likewise, all other test fungi had their maximum mycelial biomass yield within the temperature range of 25 and 30^0C.

Mycelial growth of different edible fungi in relation to the effect of temperature has been widely reported[16-19]. It has been observed that at optimal temperature, a particular fungus will produce excellent mycelial biomass provided other factors are not limiting. [16,20] Although, all the test mushrooms in this study had their best mycelial growth at the temperature range of 25-30^0C, it was reported that Volvariella esculenta produced the best biomass at 35^0C[19]. This may be as a result of species specific requirements for environmental factors. At higher temperatures, the level of biomass production in all the test mushrooms significantly reduced ($P=0.05$). This may be as a result of denaturation of important enzymes such as amylase and cellulase which catalyse some important fungal processes[22,23].

The results obtained when different wild Nigerian mushrooms were cultured under varying pH conditions were presented on TABLE 2. The highest mycelial dry

TABLE 1 : Effect of temperature on mycelial biomass production of some wild mushrooms from Nigeria during amylase and cellulase production.

Temperature (°C)	25		30		35		40	
	(Mg/30ml)							
Mushrooms	**AML**	**CEL**	**AML**	**CEL**	**AML**	**CEL**	**AML**	**CEL**
Pogonomyces hydnoides	64^{bc}	73^{de}	60^{f}	62^{ef}	66^{bc}	58^{d}	33^{c}	28^{d}
Termitomyces clypeatus	78^{abc}	96^{abc}	92^{bc}	89^{ef}	82^{abc}	82^{bc}	59^{bc}	42^{b}
Nothopanus hygrophanus	61^{c}	60^{c}	57^{f}	59^{cd}	61^{c}	68^{d}	38^{de}	33^{c}
Podoscyha bolleana	79^{abc}	87^{bcd}	75^{e}	80^{f}	84abc	42^{c}	42^{de}	28^{d}
Corilopsis oceidentalis	85^{abc}	98^{abc}	78^{de}	57de	86^{abc}	65^{d}	60^{bc}	44^{b}
Agaricus blazei	106^{a}	82^{cd}	83^{cde}	75^{bc}	93^{a}	85^{bc}	68^{ab}	67^{a}
Termitomyces globules	85^{abc}	113^{a}	103^{ab}	94^{ab}	80^{abc}	65^{bc}	79^{a}	67^{a}
Coriolus versicolor	92^{abc}	107^{ab}	104^{ab}	102^{ab}	89^{ab}	92^{ab}	50^{cd}	69^{a}
Pleurotus tuber-regium	96^{ab}	105^{ab}	90^{bcd}	108^{a}	92^{a}	98^{a}	79^{a}	58^{a}
Agaricus sp.	95^{ab}	108^{ab}	113^{a}	88^{bcd}	96^{a}	80^{c}	80^{a}	46^{b}

Values followed by the same letter (s) along each vertical column are not significantly different by Duncan´s multiple range test (P≥0.05).). Each value is an average of three replicates.
KEY : AML=amylase, CEL=cellulase

TABLE 2 : Effect of pH on mycelial biomass production of some wild mushrooms from Nigeria during amylase and cellulase production

pH	3.8		4.8		5.8		6.8		7.8	
	AML	CEL	AML	CEL	AML	CEL	AML	CEL	AML	CEL
P.hydnoides	41^{b}	40^{b}	41^{b}	40^{b}	59^{bc}	74^{cd}	64^{bc}	73^{dc}	62^{bc}	64^{dc}
T.clypeatus	67^{ab}	65^{ab}	67^{ab}	65^{ab}	75^{abc}	95^{abc}	78^{abc}	96^{abc}	84^{c}	90^{bc}
N.hyrophanuus	59^{ab}	34^{b}	59^{ab}	34^{b}	43^{c}	67^{d}	61^{c}	60^{c}	58^{c}	80^{cc}
P.bolleana	63^{ab}	44^{b}	63^{ab}	44^{b}	70^{abc}	77^{bcd}	79^{abc}	87^{bcd}	63^{bc}	57^{c}
C.occidentalis	49^{ab}	69^{ab}	49^{ab}	69^{ab}	86^{ab}	100^{abc}	85^{abc}	98^{abc}	73^{abc}	80^{cc}
A.blazei	75^{a}	62^{ab}	75^{a}	62^{ab}	107^{a}	86^{abcd}	106^{a}	82^{cd}	60^{bc}	73^{cd}
T.globules	76^{a}	92^{a}	76^{a}	92^{a}	89^{ab}	99^{abc}	85^{abc}	113^{a}	74^{ab}	82^{cc}
C.versicolor	77^{a}	88^{a}	77^{a}	88^{a}	96^{ab}	103^{ab}	92^{abc}	107^{ab}	94^{a}	110^{a}
P.tuber-regium	78^{a}	72^{ab}	78^{a}	72^{ab}	90^{ab}	111^{a}	96^{ab}	105^{ab}	97^{a}	107^{a}
Agaricus sp.	83^{a}	65^{ab}	83^{a}	65^{ab}	98^{a}	106^{a}	95^{ab}	108^{ab}	89^{a}	103^{a}

Values followed by the same letter(s) along each vertical column are not significantly different by Duncan´s multiple range test ((P≥0.05).). Each value is an average of three replicates.
KEY : AML=amylase, CEL=cellulase

weight (113mg/100ml) in Termitomyces globulus was produced at pH6.8 during cellulase production while 111amylaseand 110 mg/100ml of biomass were obtained in Pleurotus.tuber-regium and Coriolus vesicolor at pH of 6.8 and 7.8 respectively. These results indicated that each mushroom have a definite pH value in which the maximal mycelial biomass would be produced under specific enzyme action. Similar effect of pH on mycelia production in Psathyrella atroumbonata was reported by Jonathan and Fasidi[17]. This author observed the greatest vegetative growth of L.procera at pH of 6.5[2]. Convesely,Gbolagade et al[20], obtained the best mycelial yield of Lentinus subnudus at pH of 5.5. The variations obtained in different macrofungi may be due to specific pH requirements by each fungus.

Apart from Agaricus blazei that has its best mycelial dry weight of 107mg/100ml during amylase production at pH5.8, all other test mushrooms had their best mycelial biomass production in the submerged liquid medium used for cellulase assay (TABLE 2). Pogonomyces hydnoides,Podoscyha bolleana,Corilopsis occidentalis and Pleurotus tuber-regium produced their best mycelial biomass at pH 5.8 while Termitomyces clypeatus, T.globulus and Agaricus sp has their highest mycelial dry weight at pH 6.8.Nothopanus hygrophanus and Coriolus versicolor thrived excellently at pH7.8. The different pH requirements by various wild mushrooms used in this study are in accordance with earlier observations[5,18,19,20].

TABLE 3 shows that all the carbohydrate sources used supported different significant levels of mycelial yield. The most supportive carbon source was glucose

TABLE 3 : Effect of carbon sources on mycelial biomass production of some wild mushrooms from Nigeria during amylase and cellulase production

Carbon compounds	CMC		Glucose		Maltose		Sucrose	
	(mg/30ml)							
Mushrooms	**AML**	**CEL**	**AML**	**CEL**	**AML**	**CEL**	**AML**	**CEL**
Pogonomyces hydnoides	63^{bc}	74^{de}	61^{cd}	72^{cd}	47^{b}	68^{ab}	43^{b}	36^{bc}
Termitomyces clypeatus	78^{abc}	99^{abc}	72^{cd}	107^{a}	67^{b}	86^{ab}	64^{ab}	64^{ab}
Nothopanus hygrophanus	60^{c}	58^{e}	51^{d}	78^{bcd}	52^{b}	81^{ab}	35^{b}	41^{bc}
Podoscyha bolleana	81^{abc}	88^{bcd}	64^{dc}	100^{abc}	56^{b}	62^{b}	56^{ab}	68^{ab}
Corilopsis oceidentalis	85^{abc}	98^{abc}	71^{cd}	111^{a}	63^{ab}	84^{ab}	54^{ab}	78^{a}
Agaricus blazei	107^{a}	82^{cd}	96^{abc}	92^{abcd}	73^{ab}	77^{ab}	66^{ab}	31^{c}
Termitomyces globulus	85^{abc}	107^{a}	71^{cb}	71^{d}	80^{ab}	84^{ab}	85^{a}	78^{a}
Coriolus versicolor	91^{abc}	107^{ab}	76^{bcd}	102^{ab}	80^{ab}	108^{a}	85^{a}	88^{a}
Pleurotus tuber-regium	98^{ab}	106^{ab}	108^{ab}	108^{a}	94^{a}	108^{a}	74^{ab}	83^{a}
Agaricus sp.	97^{ab}	107^{ab}	112^{a}	97^{abcd}	80^{ab}	94^{ab}	57^{ab}	72^{ab}

Values followed by the same letter(s) along each vertical column are not significantly different by Duncan´s multiple range test. Each va(P≥0.05). lue is an average of three replicates.
KEY: AML=amylase, CEL=cellulase

followed in order by CMC and maltose while sucrose was the least carbon compound (P=0.05). The highest mycelial yield (112mg/30ml) was supported by glucose for Agaricus sp.during amylase production followed closely by Corilopsis occidentalis with mycelial dry

weight value of 111mg/30ml during cellulase assay. Likewise, Pleurotus tuber-regium produced mycelial biomass of 108mg/30ml in dextrose and maltose supplemented medium. Coriolus vesicoloror also has biomass of 108 mg/100ml in maltose medium followed by Agaricus blazei and Termitomyces globulus with mycelial dry weight value of 107mg/30ml when the medium was supplemented with CMC while the least value (31mg/30ml) was obtained for Agaricus blazei with sucrose.

Monosaccharides have been reported as the most stimulatory carbon compound for the vegetative growth of several other mushrooms. Gbolagade[11], obtained best mycelial production of Lepiota procera with mannose,Jonathan and Fasidi[15], obtained highest mycelial yield of Tricholoma lobayensis with mannitol. However, some authors also observed glucose as the most supportive carbon compound for the mycelial production in mushrooms[18,19,21,22,23]. The results obtained in this study is similar to that observed for Psathyerella atroumbonata and Volvariella esculenta by Jonathan. The preference o[2] f this hexose to other carbon compounds in this study may be due to the ease by which glucose is being metabolise to produce cellular energy. Maltose stimulated significant mycelial production (108mg/100ml) in Coriolus versicolor and Pleurotus tuber-regium. The enhancement of growth by this disaccharide may be due to the ability of the two fungi to hydrolyse maltose in the presence of enzyme[2]. Sucrose, another disaccharide did not support the luxuriant growth compared with glucose and maltose (TABLE 3). The reason for the poor growth may be due to the fact that these molecules are too large to be transported across the fungal membrane[24,25]. Poor mycelial growth may also be linked to the inability of these mushrooms to produce necessary enzymes which could catalyse the hydrolysis of this oligosaccharides into simple sugars[25]. Jonathan[2] reported that sucrose in submerged liquid medium has tendency of raising solute concentration of the medium to toxic level which may inhibit mycelial growth. This may be the major reason why sucrose is not supportive to mycelial growth in the wild mushrooms used in this study.

TABLE 4 showed that all the nitrogen sources used for supplementing the media supported different levels of mycelial biomass yield. In the basal medium used for cellulase assay, yeast extract supported the greatest mycelial yield of 119mg/100ml in P.tuber-regium followed by urea with value of 118 mg100ml in Agaricus sp. Yeast extract also enhanced the biomass yield. The stimulation of mycelial growth by yeast extract in Volvariella esculenta was also reported by Fasidi[26]. Likewise, Gbolagade et al[23] (obtained highest mycelial yield (200mg100cm-$^{3)}$ with yeast extract in Lentinus subnudus. The promotion of growth with this complex organic nitrogen source may be due to its complex nature. Kadiri[25] suggested that yeast extract has both carbon source, amino acids and vitamins. This may be the reason why this compound enhanced excellent growth in these wild mushrooms. Yeast extract also stimulated very good growth in P.tuber-regium,T.clypeatus and P.hydnoides (TABLE 4).

Among the four nitrogen sources used in C.occidentalis, peptone sustained the best growth (92mg/100ml) in the liquid medium used for amylase assay. This was followed in order by urea, yeast extract and sodium nitrate (P=0.05). Among all other nitrogen compounds used by Jonathan et al[8] for Auricularia polytricha, peptone was reported of promoting greatest biomass yield (320mgcm-^{3}). The support of growth by peptone may also be due to its complex nature in containing both carbon sources and amino acids. Sodium nitrate, an Inorganic nitrogen compound generally inhibited good mycelial biomass yield in the

TABLE 4 : Effect of nitrogen sourceson mycelial biomass production of some wild mushrooms from Nigeria during amylase and cellulase production

Nitrogen Sources	Urea (Mg/30ml)		Peptone		Yeast extract		$NaNO_3$	
Mushrooms	**AML**	**CEL**	**AML**	**CEL**	**AML**	**CEL**	**AML**	**CEL**
Pogonomyces hydnoides	57^{cd}	68^{d}	68^{cd}	55^{c}	36^{b}	73^{bc}	37^{dc}	38^{b}
Termitomyces clypeatus	67^{cd}	104^{ab}	60de	103^{ab}	70^{b}	107^{ab}	52^{cd}	50^{b}
Nothopanus hygrophanus	49^{d}	77^{cd}	59de	71^{bc}	54^{c}	70^{bc}	24^{c}	41^{b}
Podoscyha bolleana	69^{bcd}	76^{cd}	46^{e}	43^{c}	67^{b}	59^{c}	34de	54^{b}
Corilopsis oceidentalis	84^{abc}	79^{cd}	92^{ab}	51^{c}	72^{b}	81^{abc}	70^{bc}	40^{b}
Agaricus blazei	80^{abc}	92^{bc}	84^{abc}	74^{abc}	71^{b}	63^{c}	72^{bc}	53^{b}
Termitomyces globules	80^{abc}	103^{ab}	78^{be}	80^{abc}	82^{ab}	94^{abc}	93^{a}	92^{a}
Coriolus versicolor	85^{abc}	114^{a}	78^{bc}	112^{a}	95^{a}	116^{a}	79^{ab}	105^{a}
Pleurotus tuber-regium	100^{a}	110^{ab}	91^{ab}	110^{ab}	101^{a}	119^{a}	69^{bc}	113^{a}
Agaricus Sp.	95^{ab}	118^{a}	99^{a}	110^{ab}	103^{a}	114^{a}	61b^{c}	98^{a}

Values followed by the same letter(s) along each vertical column are not significantly different by Duncan´s multiple range test (P≥0.05). Each value is an average of three replicates.
KEY: AML=amylase, CEL=cellulase

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entire wild mushroom tested. This result is in line with the earlier observation that in mushroom propagation, inorganic nitrogen sources are poor mycelial growth stimulators[26,27].

CONCLUSION

The mycelial growth of Nigerian mushrooms collected from the wild as obtained in this study were generally favoured between temperature range of 25 and 30 ^{0}C. Likewise,all the fungal samples thrives well within the pH range of 5.8 and 7.8. Carbohydrate sources such as carboxymethylcellulose (CMC), glucose and maltose were found to significantly enhanced mycelial biomass production of these mushrooms under different enzyme action. Nitrogen compounds such as yeast extract, peptone and urea also stimulated different degrees of mycelial biomass yield. These information could be a useful tool in producing mycelial starter cultures for the emerging mushroom industries in Nigeria.

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