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Effect of different physico-chemical factors and agricultural wastes on mycelia growth and fruit bodies production of *Volvariella bombycina* (Schaeff: Ex.Fr) Singer

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ABSTRACT

In this study, effect of physical factors (temperature and pH), simple organic and in organic compounds (carbon and nitrogen) and different agricultural wastes were optimized for mycelia biomass production and fruit body yield in *Volvariella bombycina* (Schaeff. Ex. Fr.) Singer, a Nigerian edible mushroom. This fungus produced different quantity of mycelia biomass within the temperature range of 15-40^{0}C. The best mycelia yield (300-330 mg100cm-^{3}) were observed at 26-28 ^{0}C while no mycelia growth were seen at 0,10,12,40 and 42^{0}C. Similarly, pH range of 5.6 to 7.6 supported varying degree of mycelia production. The best vegetative growth (290 mg 100cm-^{3}) was obtained at pH 6.8 while growth was inhibited at pH of 5.4 and 7.8. Among the carbon compounds, mannose supported optimal yield (340 mg100cm-^{3}) at 2.0% concentration. This was followed in order by fructose and glucose (P = 0.05). In the series of nitrogen compounds, yeast extract (0.20%) enhanced best mycelia biomass production (290 mg100cm-^{3}) followed in order by 0.10 and 0.20% of tryptophan and peptone respectively. For, the agricultural wastes, the highest mycelia extension (90.3mm) were observed in oil palm pericarp wastes followed in order by sawdust, cotton wastes and rice straw (P = 0.05). For assessment of fruit bodies (FB) production, the highest average number of fruit bodies (ANF) were 33.0 in oil palm pericarp wastes within 1-3 flushes (1-3F) while sawdust and cotton wastes produced 27.0 and 25.0 ANF respectively. Rice straw and corn cob had 21.0 and 15.0 ANF respectively. It was observed that wastes such as cassava peels, cocoa leaves, cow dung and poultry manure produced no fruit bodies. © 2011 Trade Science Inc. - INDIA

KEY-WORDS

Chemical compounds;
Mycelia biomass;
Volvariella bombycina;
Agricultural wastes.

INTRODUCTION

The genus *Volvariella bombycina* comprises of various species which are either edible or medicinal[5,13]. In the tropical countries, well known *Volvariella* species include *V. volvacea, V. esculenta, V. speciosa* and *V. bombycina.* The name '*Volvariella'* is derived from the word 'volva' which is a structure that covered the

egg of this fungus during the developmental stages. The volva opens up and very young fruit body emerges from it. This structure remains persistent throughout the life time of this organism[12].

Volvariella bombycina usually grow in a shady place on the rotting wood, leaf litter and in rich agricultural soil especially in coffee and palm plantation[22]. This edible species is recognized by its large sporophore. The pileus which is usually white in colour may range between 5-20cm in diameter. The stipe may be up to 20cm in length. The mushrooms in the genera of *Volvariella* have been reported to be rich in proteins, glycogen, lipids and mineral elements[5,12]. Up till now no indigenous mushroom from Nigeria has been cultivated on a large scale.

Volvariella bombycina has great potential as a commercial mushroom in Nigeria because, villagers in South western Nigeria usually utilize it as a substitute for meat. Therefore, this fungus serves as a good protein source for the poor and delicacy for the rich. Up till now, a lot of information abound in literatures on the cultivation of *V. volvacea, V. esculenta* and *V. speciosa*[5,13,19] but there is little or no information about the growth of *V.bombycina*. Therefore, the present study is undertaken to shed light on useful information about the requirements for the mycelia growth and fruit bodies' production in this fungus with the aim of encouraging the cultivation of *V. bombycina* as a commercial mushroom in Nigeria.

MATERIALS AND METHODS

Sample collection and culturing

Volvariella bombycina (Schaeff. Ex. Fr.) Singer, sporophores were collected from the rotten wood under shade at Imini village, Afijio local government area of Oyo State Nigeria. Mycelial culture of this fungus were obtained by tissue culture[10]. The mycelia were propagated on plates of PDA. Mycelial growth of this mushroom were assessed by mycelia dry weight method Fasidi[5]. The various compounds needed to form the sub-merged medium were dissolved in 1000ml of distilled water and pH adjusted to 6.8. The medium was dispensed into 150 ml conical flasks(100ml per conical flask) and sealed with aluminium foil. Mycelia starter culture of each mushroom was generated by tissue culture employing the method of[11,12] culture of each fungus were sub-cultured on plates of PDA supplemented with 0.5% yeast extract. Mycelial biomass production of each mushroom were determined by mycelia dry weight method described by Jayasinghe *et al*,[10]. The different compounds required to form the synthetic medium were dissolved in 1litre of de-ionised 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 autoclaved at 1.02kg cm-^{2} pressure at temperature of 121^{0}C for 15 min. On cooling .0.05g of streptomycin sulphate was added to inhibit bacterial growth. Each flask was then inoculated with vigorously growing mycelia culture (5-day old) of *V.bombycina* using 7 mm diameter cork borer. The flasks were incubated at 28±2^{0}C for 7 days after which mycelia were harvested using the procedures of Fasidi and Kadiri[4].

TABLE 1: Effect of Temperature on mycelia growth of *Volvariella bombycina*

Temperature (^{o}C)	Mycelia dry weight (mg100cm$^{3)}$
0	-
10	-
12	-
14	40j
16	70i
18	110h
20	150g
22	190f
24	240d
26	300b
28	330a
30	270c
32	200ef
34	110h
36	67i
38	30k
40	-
42	-

Values followed by the same letter(s) are not significantly different by Duncan's multiple range test (P = 0.05).

The effect of temperature and pH compounds on the mycelia growth of of *V.bombycina* was carried out using the procedure of Jayasinghe *et al*,[9] while the

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influence of carbon and nitrogen on mycelia biomass yield was done using the method of Gbolagade[16].

Effect of Agricultural wastes on mycelia growth and fruit bodies

Influence of different agricultural wastes on mycelia growth of of *V.bombycina* were carried out using these procedures. All wastes (except saw dusts, cow dung and poultry manure) were chopped into smaller particle 1-3 cm pieces sizes using a cutting machine. Each waste was soaked separately in hot water for 2hours. These were squeezed using Chinese cloth to remove excess water and dispensed into100.0mm diameter Petri dishes.. Cow dung and poultry manure were soaked with10% water before dispensing each into 100 .0mm diameter Petri dishesm[13]. These Petri dishes were sterilized at 1.02 kg cm^{-2} (pressure) at 121 ^{0}C for 15 minutes. After cooling, they were inoculated with a 7.0mm, mycelia disc of a vigorously growing (6-day old) cultures of *V.bombycina*. Each treatment was replicated three times. Incubation was carried out at 30 ± 2^{0}C for10 days, after which the diameter of mycelia extension was measured using meter rule. The mycelia density was carried out using the procedures of Jonathan[12].

For fructification, 200.0g of each prepared substrate with 8% rice bran (additive) were separately packed into 250ml jam bottles and sealed with aluminum foil. These were autoclaved at 1.02 kg cm^{-2} (pressure) at 121 ^{0}C for 15 minutes. On cooling; each bottle was inoculated with 1.0g of 15 day old spawn of *V.bombycina* which has been previously prepared using cotton wastes using the procedures of Fasidi[5]. The period of fructification was between 4 to 6 weeks and the average number of fruit bodies (ANF 1-3) harvested for 4,5 and 6th weeks were determined Fasidi[5],

Analysis of Data

The data generated from these studies were subjected to analysis of variance (ANOVA) and test of significance was determined using Duncan's multiple range test (DMRT) at 1% level of probability (P=0.05).

RESULTS AND DISCUSSION

The sub-merged culture of *Volvariella bombycina* was found to grow within the temperature range of 14 and 38^{0}C (TABLE 1). Although, the best mycelia growth (330 mg100cm-^{3}) was obtained at 28^{0}C, very good growth(240,300 and 270 mg100cm-^{3}) were also observed at 24,26 and 30^0 C respectively. This finding was similar to that obtained by Jonathan *et al*[12] for *Lepiota procera*. Conversely, the mycelia of *Volvariella esculenta* was found to grow best at 35^{0}C[13]. The variation in temperature requirements of these two genera of *Volvariella* may be attributed to their habitat, habit and substrates used for their growing.*V.esculenta* usually grow on fermenting pericarp palm wastes with high temperature regimes, while *V.bombycina* prefers growing on decaying wood or garden soil[22].

TABLE 2 : Effect of pH on mycelia growth of *Volvariella bombycina*

pH	Mycelia dry weight(mg/100ml)
5.4	-
5.6	30
5.8	70
6.0	90
6.2	120
6.4	180
6.6	260
6.8	290
7.2	230
7.4	140e
7.6	25i
7.8	-

Values followed by the same letter(s) are not significantly different by Duncan's multiple range test (P = 0.05).

TABLE 2 shows pH requirement of *V.bombycina.* The optimum pH for this fungus was 6.8 with mycelia dry weight of 290 mg 100cm^{-3}. Moderate growth (260 and 230 mg 100cm^{-3})were also observed at pH 6.6 and 7.2 respectively. This result is in agreement with that of Madunagu[20], who observed pH of 6.5 as the optimum for the mycelia growth of *Pleurotus squar-rosulus.* But on the contrary, pH of 6.0 was found to stimulate greatest mycelia biomass in *V.esculenta*[12]. The difference in pH requirements by these two *Volvariella species* may be used as a complementary tool for their systematic classification[15]. Besides, there were no growth at extreme pH (5.4 and 7.8). This may be due to the destruction inflicted on the cell membrane at these pH[15].

TABLE 3 shows the effect of different concentrations of carbon compounds on the mycelia growth of *V.bombycina*. All the tested carbohydrtes supported various degree of vegetative growth which were significant when compared with the control (P=0.05). The most stimulatory carbon compound was mannose at 2.0% concentration. This monosaccharide produced mycelia dry weight of 340 mg100cm^{-3}. This was closely followed by fructose with 240 mg100cm^{-3} at 2.0% level. Likewise, glucose also enhanced mycelia growth of 240 mg100cm^{-3} at 1.0% concentration. These results agree favourably with the report of[10] on some selected Nigerian higher fungi. Likewise, Fasidi and Olorunmaiye[6], suggested that monosaccharides generally enhanced greater mycelia production in *Pleurotus tuber-regium* than any other carbohydrate sources. Mannitol,sorbitol,sucrose,solube starch and cellulose promoted their maxima mycelia biomass yield in *V.bombycina* at 1%concentration,while glucose, sucrose, raffinose and dextrin promoted moderate growth of this fungus at 1.5% concentration. It was also observed that fructose, galactose and mannose had their best activities at 2.0% level while least growth were recorded at2.5 and 3.0% level. The poor growth of this fungus at 2.5 and 3.0 %may be due to high osmotic concentration of the culture medium which may have negative effect on the cell membrane[15]

TABLE 3: Effect of Different Concentration of Carbon compounds on mycelia growth of *Volvariella bombycina*

Mycelia dry weight (mg100 cm^{-3}) Carbon sources (Means of 3 replicates)						
Concentration in %	**0**	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**
Glucose	15.0	120f	270a	240c	180b	50bc
Fructose	15.0	80hi	170d	270b	220a	40c
Galactose	15.0	70i	120gh	190d	100d	60b
Mannose	15.0	100g	200b	340a	150c	80a
Maltose	15.0	120f	70jk	100f	50g	0
Sucrose	15.0	100g	100i	70h	40g	0
Raffinose	15.0	140de	150ef	120e	70ef	20de
Mannitol	15.0	240a	180cd	100f	60fg	25de
Sorbitol	15.0	130ef	60k	80gh	55gh	15e
Dextrin	15.0	70i	140f	70h	60fg	40c
Soluble stanch	15.0	170e	110hi	30j	25i	0
Cellulose	15.0	200b	100i	40ij	20i	0

Values followed by the same letter(s) along each vertical column are not significantly different by Duncan's multiple (P = 0.05).

The growth pattern of *V.bombycina* over different regimes of nitrogen sources is represented on TABLE 4. The most supportive nitrogen compound was 0.20% yeast extract. It stimulated mycelia growth of 290 mg100cm^{-3}. This result is similar to that obtained by Jonathan[12] for *Polyporus giganteus,Psathyrella atroumbonata* and *Lentinus subnudus.* Tryptophan,an amino acid, produced very good mycelia dry weight of 240 mg100cm^{-3} at 1.0 % concentration while peptone promoted mycelia growth of 180 mg100cm^{-3} at 0.15 % level. These nitrogen compounds have been previously reported of enhancing good growth in edible fungi[5,9,17]

TABLE 4: Effect of Different Concentration of N_2 Compounds on mycelia growth of *Volvariella bombycina*

Mycelia dry weight (mg100cm-^{3}) Nitrogen Compounds (Means of 3 replicates)						
Concentration in %	**0**	**0.05**	**0.10**	**0.15**	**0.20**	**0.25**
Asparagine	22a	40g	60h	35h	0	0
Alanine	22a	70f	80fg	50g	25fg	0
Glutamine	22a	65f	100e	50g	30fg	15g
Methionine	22a	100e	150c	70f	40ef	20g
Tryptophan	22a	150bc	240a	130c	60d	30fg
Phenyl-alanine	22a	130d	200b	100d	60d	50de
Ca $(No_3)^2$	22a	140cd	100	70f	30fg	15g
K No_3	22a	170a	130d	90e	60d	40ef
Na No_3	22a	100e	70gh	40h	20g	0
Peptone	22a	35g	55h	180a	230b	100bc
Urea	22a	40g	50h	70f	150c	90c
Yeast extract	22a	35g	80fg	150b	290a	120a

Values followed by the same letter(s) along each vertical column are not significantly different by Duncan's multiple range test (P = 0.05).

TABLE 5 shows that *V.bombycina* had the greatest mycelia extension of 90.3mm with oil pericarp wastes followed in order by sawdust and cotton wastes (P=0.05). The supportive nature of palm wastes for mycelia extension may linked to availability of necessary nutrients for metabolism of this fungus. Likewise, the preference of cotton wastes and rice straw for mycelia growth of this fungus is not a surprise. Other authors have implicated these substrates as a good materials for vegetative growth of *Pleurotus tuber-regium, Volvariella esculenta* and *Volvariella volvacea*[4,17,18]. For fruit body production, oil pericarp wastes produced highest number of fruit bodies (33.0ANF) for the 3 flushes. This could be attributed to the fact that palm

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wastes possessed necessary nutrients that supported the growth of *V.bombycina.* Similarly,this fungus may have ability to produce extra cellular enzymes such as cellulase, hemicellulase, lignase, laccase, lipase and amylase which were used to hydrolyze cellulose, hemicelluloses, lignin and other substrates[1,14,18,21]. Saw dust produced 27.0ANF, while cotton wastes produced 25.0ANF

TABLE 5: Effect of different agricultural wastes on mycelia biomass extension and fruit bodies yield in *Volvariella bombycina*

Agricultural wastes	ME (mm)	MD	ANF(1-3F)
Banana leaves	52.0e	+5	7f
Cassava peels	27.3g	+3	0
Com cob	65.3d	+5	15de
Cotton waste	81.7b	+6	25b
Oil palm pericarp waste	90.3a	+8	33a
Rice straw	70.4c	+8	21c
Andropogon straw	63.0d	+4	12e
Cocoa leaves	27.7g	+4	0
Cassava leaves	42.6f	+2	2g
Sweat potato leaves	30.3g	+2	0
Sawdust	83.3b	+7	27b
Cow dung	18.1h	+1	0
Poultry manure	13.3h	+1	0

Key:ME=Mycelial extension;MD=Mycelial density; ANF=Average no of fruitbodies;Flushes
Values followed by the same letter(s) are not significantly different by Duncan's multiple range test (P = 0.05).

Rice straw produced 21.0 ANF. Cow dung and poultry manures produced no fruit bodies. These may be due to their toxicity. Acidity or alkalinity nature of these may also contribute to their inhibitory roles[14]. Although cassava peels, cocoa leaves and sweet potato leaves encouraged appreciable mycelia growth (TABLE 5),it was observed that they produced no single fruit body. This could be due to their looseness and texture[8]. *Andropogon* straw, banana leaves and corn cob gave low yield of the fruit bodies. This may be due to low bulk density of these wastes[12].

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