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Research Article

Growth and yield performance of oyster mushroom (*Pleurotus ostreatus*) on water hyacinth as a substrate

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Abstract: Cultivation of oyster mushroom (*Pleurotus ostreatus*) on the aquatic weed water hyacinth is an eco-friendly way of controlling and management of such a problematic weed. In the present work, water hyacinth has been used as a low-cost substrate in combinations with rice-straw of ratio 3:1 for the cultivation of *P. ostreatus*. The objective of this study was to cultivate oyster mushroom in water hyacinth compost degraded with lingo-cellulolytic fungi. The experiment was performed in control, before fungal treated compost and fungal treated compost. The data was analyzed on various aspects like completion of mycelium growth in different media like Potato dextrose agar media and water hyacinth media, the duration for spawn run, the appearance of pinheads, fruiting bodies and number of fruit bodies produced. The nutrient analysis was done in all types of fruit bodies. *Aspergillus flavus* treated compost took short duration (24 ± 1 days) and control sample compost took a long duration (27.3 ± 1.53 days) for fruiting. The highest production was recorded in *Trichoderma* sp. treated compost (68.8%) and lowest in control compost (30.7%). This study has successfully demonstrated the possibility of water hyacinth as a substrate in mushroom production and management of water hyacinth.

Key words: *Pleurotus ostreatus*; Water hyacinth; mycelium growth; NPK analysis; biological efficiency.

Introduction

Mushrooms are saprophytic fungi that grow on dead and decaying organic materials and are various shape, size, color, flavor, texture and taste. They have fleshy fruiting bodies that are considered one of the delicious fruits and are commonly produced worldwide (Madbouly and Al-Hussainy, 1996). They played an important role as a human food due to its nutritional and medicinal properties (Etich *et al.*, 2013). In nature, *Pleurotus* species live on parts of plants which are generally poor in nutrients and vitamins. Based on the characteristics of mating compatibility the genus of *Pleurotus* contains a broad family of approximately 40 known biological species (Jose and Janardhanan, 2000) and commonly referred as 'oyster mushrooms' due to its general morphological appearance like an oyster.

Among them, *P. ostreatus, P. florida, P. sajor-caju,* and *P. eryngii* are mostly cultivated in Nepal. Currently, mushroom production is dominated by Oyster mushroom occupying 86% of total national output (Raut 2017, 2019). *Pleurotus* is an efficient lignin degrading mushroom and can grow well on different types of lingo-cellulosic waste. They are able to colonize and degrade a large variety of lingo-cellulosic residues. These mushrooms are also found to be one of the most efficient lignocelluloses solid state decomposing types of white rot fungi (Baysal *et al.*, 2003).

Eichhornia crassipesis is aquatic weed which is listed as one of the '100 world's worst invasive alien species' by IUCN (Rakotoarisoa *et al.*, 2015). It is a monocotyledonous aquatic plant. It belongs to the family Pontederiaceae and order Liliales. It is low in lignin content (10%) and

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contains high amounts of cellulose (20%) and hemicellulose (33%) (Gressel, 2008). Water hyacinth (Jalakumbhi in Nepali) is an alien invasive species and now creating great environmental and economic problems covering major wetlands of the world. It causes serious harm and has adverse effect on water resources, fisheries, irrigation, drainage canals and public health. Controlling and management of this weed has become difficult because it has high capability of fast growing and spreading in aquatic ecosystems (Jha and Thapa, 2018). It shows rapid growth in neutral pH, high in macronutrients, warm temperature 28-30℃ and light intensities. It is potentially suitable substrate for mushroom cultivation (Kivaisi *et al.,* 2004). Since beginning oyster cultivation is based on rice straw in Nepal and substrate has become the major constraint in the production. Because rice straw is a major constituent of animal feed as well and it has to compete with animal husbandry. In such context introduction of water hyacinth as a substrate for Oyster production will be the best alternative.

Materials and Methods

The research was conducted in Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Fungi Isolation:

The fungi that used for decomposition of substrate were isolated from forest soil. *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger* and *Trichoderma* sp. were obtained from forest soil.

Compost preparation:

Dried water hyacinth (without roots) and rice straw was mixed in 3:1 ratio to make compost for cultivation of *P. ostreatus*. Water hyacinth and rice straw were chopped into 3-5 cm and soaked in water for 24 hours to moisten. These were autoclaved at 121℃ and 15lbs. After cooling they were placed on the steep cemented floor by making pile. They were covered by black plastic sheet for 15 days to make compost. After 15 days about 500 gm of the substrates was taken out for nutrients test and it was dried under green house. They were again autoclaved and isolated fungi were inoculated in them. The experiment was laid out in a completely randomized design (CRD) with three replications and six treatments i.e. Control, before fungal treated compost, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger* and *Trichoderma* sp. treated compost. And after 15 days, about 500 gm substrates were again separated for the nutrient analysis. Then, the substrates were again sterilized in an air tight container at 15 psi for one hour to inoculate mushroom seed.

Substrate preparation and spawning:

After cooling 3 kg wet weight of each substrate with 70-80% moisture were packed in transparent polypropylene bags (35×45 cm) for spawning. Spawns of *P. ostreatus* were prepared on wheat grains in polypropylene packets and spawned @ 5% dry weight of substrate. Grain spawn was sprinkled layers by layers in substrate filled polypropylene bag.

Cultivation:

Cultivation trials of *Pleurotus ostreatus* were conduct on six different treatments. The bag was packed well without empty spaces and then the bag opening was tied very tightly using thread. The bags were perforated using scalpel for exhaust of gases. The bags were then inoculated for spawn running under complete darkness at control temperature of 25±2℃. The temperature during cultivation was controlled by artificial light. And during fruiting the temperature was maintained between 17- 20℃. The humidity of bags were accomplished by spraying of water on them twice a day. The data were analyzed statistically on various aspects described as follows.

Data recording:

Time was recorded in days for the completion of mycelium growth on substrates, appearance of pinheads and maturation of fruiting bodies in different treatments. The data were also recorded for the total number of fruiting bodies and total yield at first, second and third flush. Nutrients like C, N, P and K of compost were analyzed before and after fungal treatments in compost. The harvested mushrooms were oven dried and used for nutritional analysis. The ash, protein, fat, carbohydrate and fiber contents of the mushroom samples were determined using standard methods (AOAC 2000, 2003). Similarly, Proximate analysis of *P. ostreatus* grown on *Trichoderma* sp. treated compost of first flush were analyzed using MS-EXCEL (version 10) and one-way analysis of variance (ANOVA).

Results and Discussion

Rate of mycelium growth in different media:

Trichoderma sp. was the fastest growth of mycelium in PDA (Potato Dextrose Agar) media and *Aspergillus niger* was the slowest growth of mycelium in PDA media. Similarly, in water hyacinth media, *Trichoderma* sp. was the fastest growth mycelium in the media and *Aspergillus fumigatus* was the slowest growth.

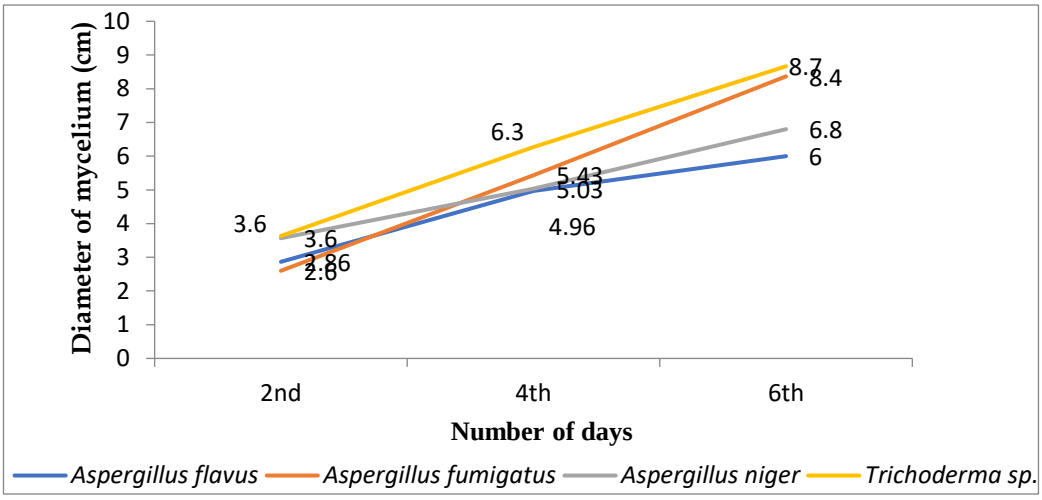


Figure 1: Rate of mycelium growth in PDA media

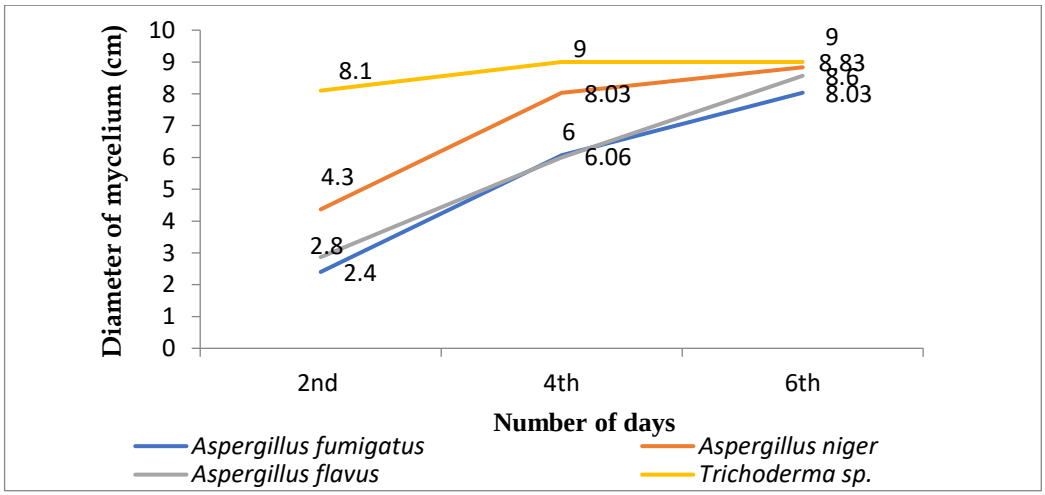


Figure 2: Rate of mycelium growth in water hyacinth media

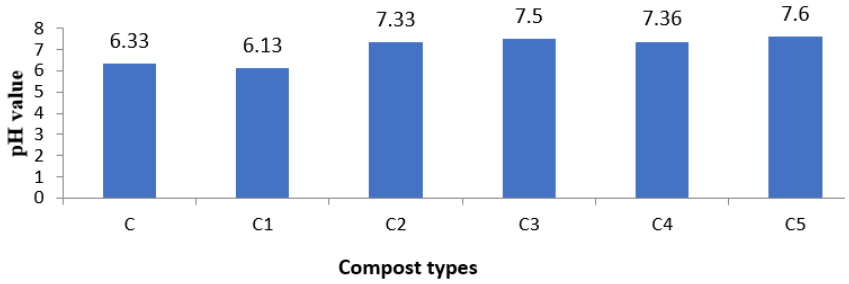


Figure 3: pH value of different types of compost.

The average pH value of before fungus treated compost and control compost are 6.13 and 6.3 respectively which is slightly acidic in nature. Similarly, the average pH value of lignocellulolytic fungi treated compost are 7.3 for *Aspergillus flavus* treated compost, 7.5 for *Aspergillus fumigatus* treated compost, 7.36 for *Aspergillus niger* treated compost and finally *Trichoderma* sp. treated compost is 7.6. A Lignocellulolytic fungus is treated with water hyacinth and rice straw compost.

Table 1: Nutrient contents in different fungal treated compost (% ± standard deviation).

Samples	Carbon (%)	Nitrogen (%)	Phosphorus (%)	Potassium (%)
C	0.93±0.15	2.5±0.15	0.39±0.15	2.01±0.15
C1	2±0.32	2.34±0.05	0.5±0	2.15±0.08
C2	2.7±0.1	2.6±0.14	0.6±0.02	2.18±0.07
C3	2.2±0.2	2.5±0.19	0.45±0.01	1.43±1.15
C4	1.73±0.15	2.34±0.06	0.39±0.01	2.1±0.04
C5	2.8±0.15	2.23±0.1	0.49±0.01	2.18±0.1

C = Control compost; C1= Before fungus treated compost; C2= *Asppergillus flavus* treated compost; C3= *Aspergillus fumigatus* treated compost; C4= *Aspergillus niger* treated compost; C5= *Trichoderma* sp. treated compost

Table 2: Duration of spawn run, pin head formation and fruiting

Sample	Spawn run (days ± std)	Pin head formation (days ± std)	Fruiting (days ± std)
C	16±1	22.67±1.52	27.3±1.53
C1	13.3±1.53	18.33±1.53	25±1
C2	12.67±0.56	18±1	24±1
C3	14±1	19.3±1.53	25.3±1.53
C4	13±1	18.67±1.15	26±1
C5	13±1	19.67±0.58	24.33±0.58

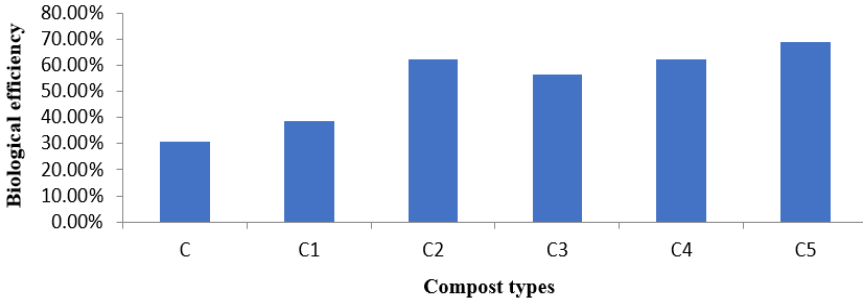
C = Control compost; C1= Before fungus treated compost; C2= *Asppergillus flavus* treated compost; C3= *Aspergillus fumigatus* treated compost; C4= *Aspergillus niger* treated compost; C5= *Trichoderma* sp. treated compost

Table 3: Number of primordial and yield in first, second and third flush.

Sample	First flush		Second flush		Third flush		Total Yield
	No. of primordia	Yield (g/bag)	No. of primordia	Yield (g/bag)	No. of primordia	Yield (g/bag)	
C	29.33±2.5	326.33±11.5	16±1	227.66±5.68	11.66±1.52	90±2	614±49.72
C1	33.66±3.5	435±13.23	23.66±1.52	249±6.56	15.66±1.52	103.3±1.53	776.6±1.15
C2	40.6±1.54	544±5.56	30±1	455±8.88	23.6±1.52	242.6±4.04	1241.6±4.93
C3	52±2	544.33±4.7	35.33±1.52	397.33±5	26.6±2.31	186.33±3.8	1128±2
C4	59.6±1.53	597.6±4.04	46.3±2.52	449±3.61	30.3±1.53	200.6±9.01	1247.3±14.7
C5	70.33±1.53	671.3±3.21	51.6±1.53	512.33±2.1	36.3±1.53	192.33±9.7	1376±9.64

C = Control compost; C1= Before fungus treated compost; C2= *Asppergillus flavus* treated compost; C3= *Aspergillus fumigatus* treated compost; C4= *Aspergillus niger* treated compost; C5= *Trichoderma* sp. treated compost

Figure 4: Efficiency of mushroom in different compost types.



C = Control compost; C1= Before fungus treated compost; C2= *Asppergillus flavus* treated compost; C3= *Aspergillus fumigatus* treated compost; C4= *Aspergillus niger* treated compost; C5= *Trichoderma* sp. treated compost

Table 4. Proximate nutritional composition of *Pleurotus ostreatus* grown on *Trichoderma* sp. treated compost of 1st flush.

Nutritional qualities	Percentage
Moisture (% of fresh weight)	80.36±0.25
Ash (% DWB)	18.06±0.15
Fiber (% DWB)	8.1±0.1
Protein (% DWB)	26.3±0.3
Fat (% DWB)	1.47±0.03
Carbohydrate (% DWB)	46.13±0.36
Energy	302.95±0.54 (kcal/100 gm)

DWB= Dry weight biomass of mushroom.

pH of compost

The pH value range between 6.13-7.6 in all compost. Rapid mycelial growth of mushroom (*Pleurotus sajorcaju*) takes place at pH 6.4-7.8 (Iqbal and Shah, 1989). Lohr *et al.,* (1984) reported an average leachate pH of 8.0 for fresh mushroom compost.

Nutrient analysis of compost

The highest percentage of carbon was found in *Trichoderma* sp. (2.8%) and the least percent of carbon was found in control compost (0.9 %), which is not treated by any fungus.

The highest percentage of nitrogen was found in *Aspergillus flavus* (2.6%) and the least percent of nitrogen was found in *Trichoderma* sp. treated compost (2.23%).

The highest percentage of phosphorous was found in *Aspergillus flavus* (0.6%) and the least percentage of phosphorous were found in both *Aspergillus niger* treated compost and control compost (0.39%) which is not treated by any fungus.

The highest percentage of potassium was found in *Aspergillus flavus* and *Trichoderma* sp. with (2.18%) and the least percent of potassium was found in *Aspergillus fumigatus* treated compost (1.43%). Wheat straw is a basic component for fermented *Agaricus* compost and contains 1% protein, 13% lignin, 39% hemicellulose and 40% cellulose (Mamiro, 2003) while Heltay *et al.,* (1960) documented that it contained 48% cellulose, 20% lignin, 0.5% total nitrogen, 0.04% phosphate (P_20_5), 0.1% potash (K_20), 4.1% silica, carbon/nitrogen ratio of 104 and a pH of 6.9. Dry banana leaves contain 1.45% nitrogen and it is very productive for the oyster mushroom cultivation (Chan-Ho *et al.,* 1979).

Days required for spawn running

In different compost types, spawn run duration took place from 12.67 days to 16 days. Maximum duration of mycelium run was in control sample (16 days) and less days was taken in *Aspergillus flavus* treated compost (12.67 days). These results agree with the findings of Khan *et al.,* 2013 which reported the spawn running took two to three weeks after inoculation. Anakalo *et al.,* (2008) observed spawn run on the water hyacinth substrate after 12 days of incubation. The poor mycelia growth of the control compost might be due to the higher nitrogen content (2.5%) of the substrates.

Excess nitrogen may cause stratum degradation when nitrogen is increased (Rajarathan and Bano, 1989).

Days required for pin head formation

Duration of pin head formation, maximum days was taken in control sample (22.67) days and less duration for mycelium run was taken in *Aspergillus flavus* treated compost (18 days). Khan *et al.,* (2001) described the cultivation of oyster mushroom using different ligno cellulosic substrates and found that pinhead formations take place after 7-8 days while sporocarps formation take place after 10-12 days of spawn running. Shah *et al.,* (2004) investigated oyster mushroom cultivation and observed that pin heads like structure are formed after 6-7 days of spawn running. Khan *et al.,* (2004) studied the cultivation of *Pleurotus ostreatus* on different lingo cellulosic substrates and the results showed that pinhead formed after 17-29 days of spawning. Murugesan *et al.,* (1994) worked on the production of oyster mushrooms using water hyacinth and paddy straw as substrates. The time taken for pinhead appearance using only water hyacinth was 17 days and in case of paddy straw it was 10 days. Patra & Pani (1995) revealed that mushroom (*Pleurotus* sp.*)* took 20-24 days but Jiskani (1999) stated 25-50 days for pinhead formation, whereas Jiskani *et al.,* (1999) concluded that pinhead formation took 51.6 days after spawning in case of using wheat straw.

Days required for fruiting bodies formation

Similarly, maximum duration for fruiting was 27.3 days in control compost and less time taken in *Aspergillus flavus* treated compost (24 days). These findings are in conformity with Quimio (1976, 1978) who reported that fruiting bodies appeared 3-4 weeks after inoculation of spawn.

Nageswaran *et al.,* (2003) concluded that the first harvest stage at 13 days for water hyacinth, 17 days for paddy straw and 16 days for the water hyacinth and paddy straw mixed in ratio of 3:1. Kim *et al.,* (2008) studied mycelial growth rate of *Pleurotus* species. The results indicated that addition of bacterial culture strain P-7014 and its supernatant to the mushroom growing media resulted in fast mycelial growth of mushroom. Mycelial growth rate of *Pleurotus eryngii* was increased up to 1.6-fold and primordial formation take place one day earlier than the normal. Heltay (1987) observed that fruit bodies appeared after 49 days of spawning of *P. florida* using different lingo cellulosic substrates like rye, barley, and wheat bran.

Yield of *Pleurotus ostreatus*

The crop of *Pleurotus ostreatus* was harvested in three flushes. The maximum yield was obtained in first flush than the second and third flush. Maximum average yield 688 gm was estimated from *Trichoderma* sp. treated compost. So water hyacinth and rice straw compost formed with treatment of *Trichoderma* sp. is recommended as a best substrate for the cultivation of Oyster mushroom which is in agreement with the findings of Hami (1990) who studied the Oyster mushroom cultivation on sawdust of different woods and found that *Pleurotus ostreatus* gave the maximum yield.

Number of fruiting bodies

The caps of *Pleurotus ostreatus* was counted in three flushes, average 11.66-70.33 were formed in three flushes. *Trichoderma* sp. treated compost gives more number of fruiting bodies than other treated compost as a substrate.

Biological efficiency

Biological efficiency is the percentage production of fresh mushroom in different substrate composition. Among all the six treatment, *Trichoderma* sp. treated compost found the most biological efficient for the growth and the total yield of the *P. ostreatus*. It

gives 688 gm/kg with B.E. 68.8%. The production was high due to proper amount of nitrogen and carbon present in the compost. The lowest biological efficiency was recorded in control compost with 30.7% and its yield was 307 gm/kg dry substrate used. The total yield was collected from three flushes of harvested mushroom. Biological efficiency differ the various substrates were due to different substrate compositions. The obtained results agreed with the results from Nageswaran *et al.,* (2003). 52% biological efficiency was recorded when *Pleurotus sajor-caju* was cultivated in substrate of water hyacinth and rice straw in ratio of 3:1. These variations are mainly related to spawn rate, fungal species used and supplement added to the substrate (Mane *et al.,* 2007).

Chemical analysis of *Pleurotus ostreatus*

Beluham & Ranogajec (2011) reported that mushroom are a potential source of total carbohydrate in range of 42.62-66.78 gm/100 gm, very low in fat content 1.34-6.45 gm/100 gm and also rich in protein 27.95-38.99 gm/100 gm depending on the type of species. Ahmed *et al.,* (2009) determined the effect of agro waste on moisture content, crude protein, fat, crude fiber and ash content. Soybean straw showed maximum crude protein (23.50%) and ash (8%). Maximum moisture (92.45%) and crude fiber content (8.10%) in the fruiting bodies were recorded on Paddy straw cultivation. Bandopadhyay 2013 revealed that the mushroom grown on water hyacinth and rice straw comprises moisture 82.7%, ash 18.4%, fiber 9.4% and 22.2% protein.

Conclusion

This study demonstrated that composted water hyacinth inoculated with different fungal strain act as a good substrate for oyster mushroom (*Pleurotus ostreatus*) production. From the study it was unequivocally proved that the mixture of water hyacinth and rice straw in the ratio of 3:1 is a good substrate for cultivation of oyster mushroom. Among the isolated fungi from soil, *Trichoderma* sp. shows the fastest growth of the mushroom in water hyacinth media. *Trichoderma* sp. treated compost gives higher production than other fungal strain treated compost. Short duration for the harvesting was in *Aspergillus flavus* treated compost and *Trichoderma* sp. treated compost. The nutritional composition of the *P. ostreatus* obtained on first flush that was treated with *Trichoderma* sp compost is comparable to the mushroom which is grown on common substrates.

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Photo 1: Isolating fungi

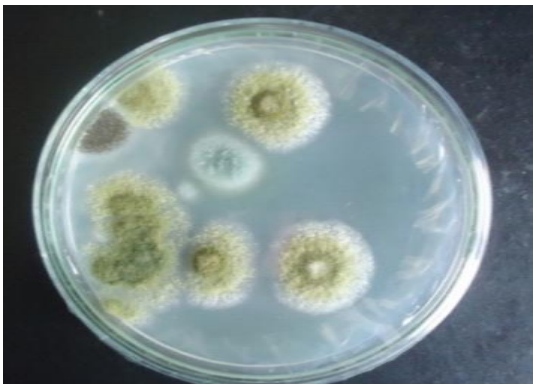

Photo 2: Fungal colonies obtained from upper soil layer soil (*A. flavus, A. fumigatus, A. niger & Trichoderma* sp.)

Fungi grown in PDA (Potato Dextrose Agar) media

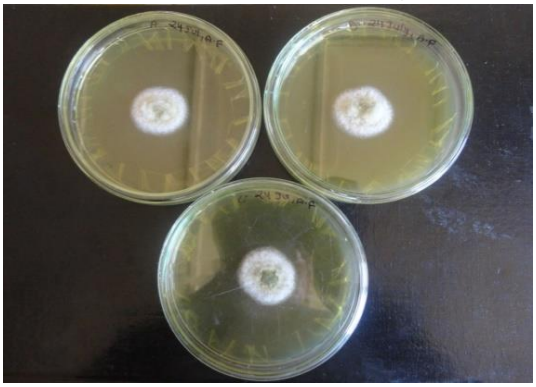

Photo 3: *Aspergillus flavus*

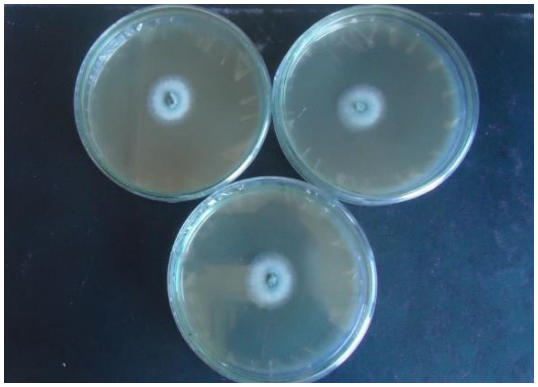

Photo 4: *Aspergillus fumigatus*

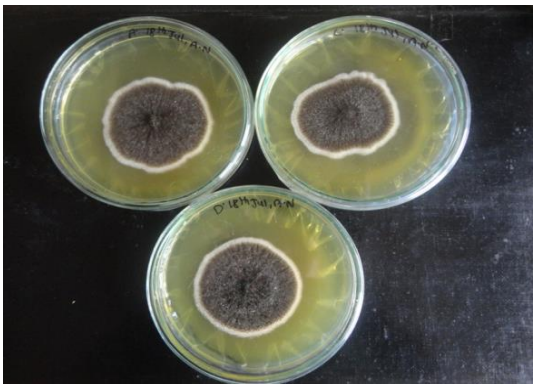

Photo 5: *Aspergillus niger*

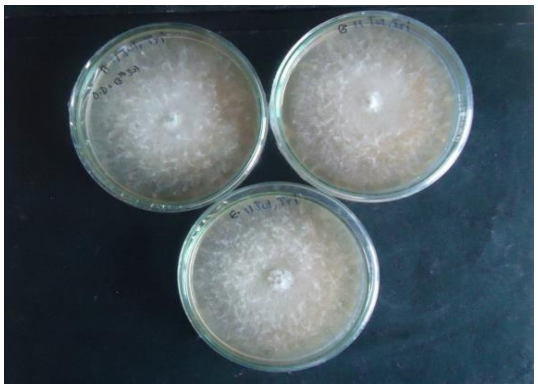

Photo 6: *Trichoderma* sp.

Fungi grown in Water hyacinth media

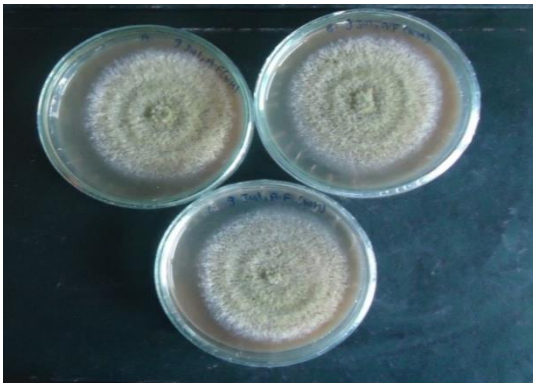

Photo 7: *Aspergillus flavus*

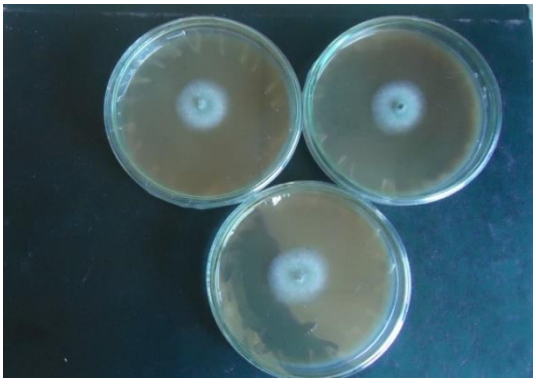

Photo 8: *Aspergillus fumigatus*

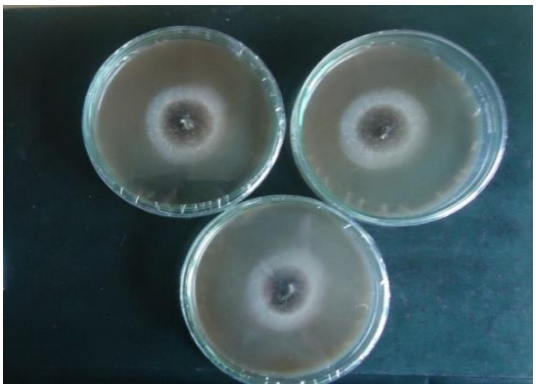

Photo 9: *Aspergillus niger*

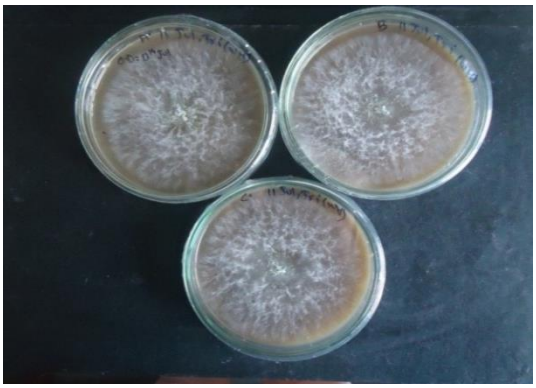

Photo 10: *Trichoderma* sp.

Microscopic view of isolated fungi

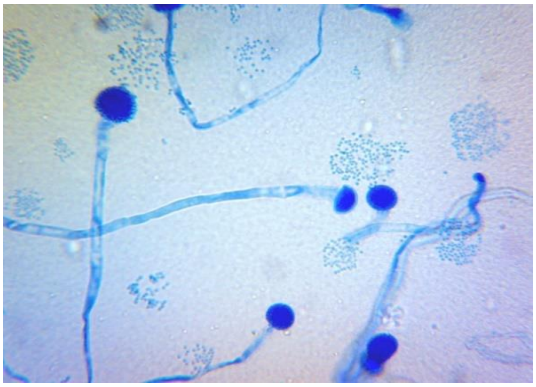

Photo 11: *Aspergillus flavus* at 40x

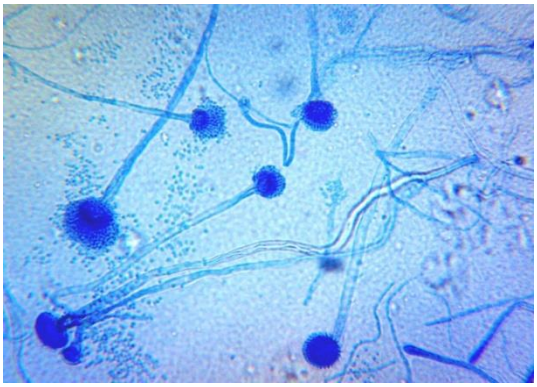

Photo 12: *Aspergillus fumigatus* at 40x

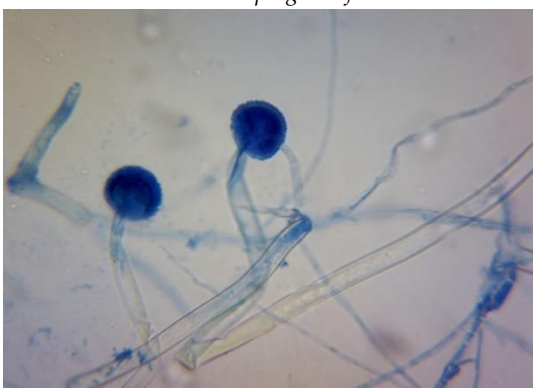

Photo 13: *Aspergillus niger* at 40x

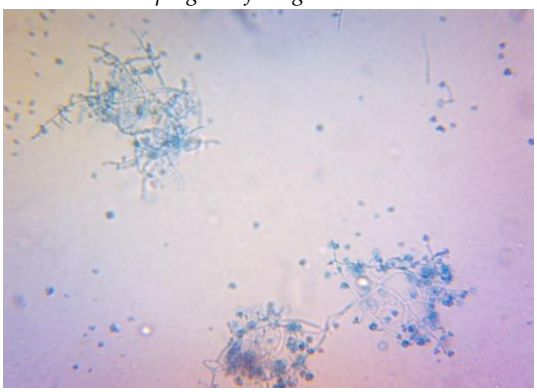

Photo 14: *Trichoderma* sp. at 40x

Photo 15: Water hyacinth at collection site

Photo 16: Flower of water hyacinth

Photo 17: Drying of water hyacinth

Photo 18: Chopping dried water hyacinth.

Photo 19: Pre-wetting of water hyacinth substrate.

Photo 20: Inoculation of fungi.

Photo 21: Spawning

Photo 22: Mushroom bags for cultivation.

Photos 23: Formation of mycelium & Pin head formation of *Pleurotus ostreatus*

Photo 24: Mature fruiting body of *Pleurotus ostreatus.*

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Conflict of interest: Nil.