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Yield Response of Oyster Mushroom (Pleurotus ostreatus) Cultivated on Creeping Bent Grass (Agrostis sp) Biomass Supplemented with Wheat Bran, Cotton Seed and Waste Paper

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Abstract
Nowadays more attention has been given to mushroom production as new sources of nutrients, medicinal uses; especially for degenerative diseases and environmental sustainability through solid organic waste recycling. The yield of oyster mushroom (*Pleurotus ostreatus*) grown on the substrate composed from creeping bent grass (*Agrostis* sp.) waste paper, wheat bran supplemented with cotton seed waste indicated the positive response of this plant. The experimental design constitutes ten treatments (T1-T10) in three replicates from the middle of March 2018 to the end of May 2018. Fastest mycelia colonization was observed in the treatments T10, T9, T8 and T7, 11 days each from inoculation, while the slowest mycelia colonization was observed in treatment T1, T2, T3 and T4, 15 days each from inoculation. Relatively, longest production cycle was observed for treatment T1, T2 and T3: 53 days each, while the shortest production cycle was recorded for treatment T10, T9, T8 and T7: 45days. Highest fresh weight 2050g per 800g dry substrate; highest number of fruits, 85 and largest cap diameter 12cm were recorded for treatment T10. The lowest total fresh weight 1590g per 800 g dry weight of the substrate was recorded for the treatments T1, T2, T3, T4 and T5 respectively. Lowest number of fruits(58)was recorded for T1, and smaller cap diameter, 8cm was recorded for T5. The highest number of aborts were observed for T4 and T9, while the least number with T1. No significant difference was observed for the stipe length and number of bunches of the different treatments. Highest biological efficiency, 250 % was recorded for T10 and the lowest 197 % each for T1, T2, T3, T4 and T5. The study reveals that less proportion of creeping bent grass (*Agrostis* sp.) biomass together with high proportion of cotton seed waste and equal amount of wheat bran and waste paper as substratum is ideal for growing the oyster mushroom.
Keywords/phrases: Creeping bent grass, cotton seed waste, oyster mushroom, waste paper, yield

Introduction

Mushrooms are fleshy, spore-bearing reproductive structures of fungi most of them are highly protenaceous. The edible, medicinal and poisonous species are nature's gift with large potentialities useful to mankind. For a long time, wild edible mushrooms have played an important role as a human food, once considered as the "food of the gods" and still treated as a garnish or delicacy or being treated as healthy food or as functional food (Chang and Miles, 2004). Bio waste control is one of the major challenges in the world today. Various ways and methods have been developed in controlling waste from various sources, such as chemical, biological and other wastes (Ezeonu *et al*. 2012). Cultivation of edible mushrooms is the cheap and prospective biotechnology for lignocellulosic organic waste recycling to combat environmental pollution (Beteez and Kustudia, 2004; Sánchez, 2009). Besides, mushroom production indirectly provides materials that are used to improve the soil structure for production of other crops (FAO, 2009) and as animal feed (Williams *et al.,* 2001). Moreover, successful cultivation and trade of mushrooms can strengthen livelihood assets, enhance an individual's and a community's capacity to access other economic opportunities (Elaine and Nair, 2009). In mushroom production, so many factors affect the nutritional composition, growth and yields of mushroom. These include difference in strains, methods of cultivation, stage of harvesting, composition of growth substrates and environmental factors such as temperature and relative humidity (Benjamin, 1995).

The genus *Pleurotus* is well-known for conversion of substrates into biomass and supporting profitable agribusiness and hence gaining rapid popularity amongst the entrepreneurs (Naraian *et al*. 2011), and the production cost of this mushroom is low and easy to adopt by the marginal farmers as it is less expensive(Banik and Nandi 2004; Pant *et al*. 2006). The chemical composition of the fresh fruiting bodies of oyster mushroom, *Pleurotus ostreatus* indicates a large quantity of moisture (90.8%), rich in proteins (30.4%), fat (2.2%), carbohydrates (57.6%), fiber (8.7%) and ash (9.8%) with 345 K (cal) energy value on100 g dry weight basis. It contains vitamins such as thiamin (4.8 mg), riboflavin (4.7 mg) and niacin (108.7 mg), minerals like calcium (98 mg), phosphorus (476 mg), ferrous (8.5 mg) and sodium (61 mg) on 100 g dry weight basis (Akyuz and Kirbag, 2010; Daba *et al*., 2008).

A number of byproducts of agriculture and ago-forestry system have been evaluated for their usability as a mushroom substrate and found remarkable differences in yield and yield related parameters (Gume *et al*., 2013; Asefa Keneni 2018; Asefa and Lakew 2016). The bio-conversion of *Gravilaea robusta* dry leaves together with

different proportion of cotton seed waste yielded high oyster mushroom biomass and biological efficiency (Asefa Keneni 2018). There are a number of agro-processing and forest by products which have no immediate uses in certain environments need to be evaluated for their usability as mushroom substrates. The Creeping bent grass (*Agrostis* sp.) is a vigorously growing perennial with vegetative spread by stolen and tiny seeds growing across a wide range of agro-ecological zones in Ethiopia. Conversion of this little used grass to valuable mushroom fruit body will be paramount importance in supplying the community with additional nutritional resources. In line with this, the usability of creeping bent grass (*Agrostis* sp) for oyster mushroom has been attempted and its performance is reported here.

MATERIALS AND METHODS

Organism and culture conditions

The oyster mushroom, *(Pleurotus ostreatus)* strain obtained from Mycology Laboratory, Addis Ababa University, Addis Ababa, Ethiopia was grown on Potato dextrose agar slant at 4°C in the refrigerator. The pure culture of *Pleurotus ostreatus* was transferred on plate containing Potato Dextrose Agar (PDA) under aseptic condition in laminar flow chamber and inoculated by 1 cm×1 cm agar block and incubated at 28°C. The growth of the culture and presence of contamination were visually inspected at three days interval. The spawn (mushroom seed) of *Pleurotus ostreatus* was developed on yellow colored sorghum grain, wheat bran and calcium sulfate (gypsum) in the ratio of 88:10:2 respectively (Dawit, 1998).

Substrate collection

Creeping bent grass (*Agrostis* sp.) was collected from the main campus of Ambo University premises and wheat bran from the local processing mill in Ambo town.

Treatments

Ten treatments (T1–T10) comprising different proportions of creeping bent grass (*Agrostis* sp.) biomass, cotton seed, waste paper and wheat bran along with lime stone (Calcium Carbonate 1%) on dry weight basis were used in this investigation as shown in Table 1.

Table1: The composition of different treatments

Treatments	Agrostis sp(biomass)	Cotton seed waste (g)	Waste Paper(g)	Wheat bran	Total (g)
T1	500	-	150	150	800
T2	450	50	150	150	800
T3	400	100	150	150	800
T4	350	150	150	150	800
T5	300	200	150	150	800
T6	250	250	150	150	800
T7	200	300	150	150	800
T8	150	350	150	150	800
T9	100	400	150	150	800
T10	50	450	150	150	800

Preparation of the substrate

Creeping bent grass (*Agrostis* sp.) collected were in to 3-4cm long small pieces length. Both the grass biomass and cotton seed waste were weighed and soaked in sufficient amount of water over night in separate container. The waste paper was cut into small pieces approximately (3-5 cm), weighed and soaked in sufficient amount of water immediately before use. Excess water present in the substrates was drained thoroughly and mixed with required amount of wheat bran and one percent calcium carbonate and filled in sterilizable polyethylene bags (Kurtu pestal). The substrates were autoclaved at 15Psi pressure and 121°C temperatures for 1h. Each substrate (800 g) with 70% moisture was mixed with 10% spawn (dry weight/wet weight basis) and the inoculated polythene bags were then tightly tied with string made from polyester/cotton cloth. Pin holes were made through the bags (1/100 cm2) for drainage and aeration. It was kept in a spawn running room at room temperature in the dark until primordial were formed. After primordial formation, large holes were made on the polythene bag to allow normal development of fruiting bodies. Bags were transferred to mushroom house under normal environmental conditions and relative humidity of the room was maintained at 85-90% by keeping water in open containers at different corners of the room and when the temperature become raised the floor of the production room was watered in sufficient amount in order to maintain the temperature to the optimum mycelia and fruiting body formation of the oyster mushroom. The cultivation bags were irrigated using tap water every morning and evening until all flushes of *Pleurotus ostreatus* fruiting bodies were harvested. Adequate ventilation was provided to prevent increased CO_2 concentration in the room by opening the door and windows of the room for

half an hour in the morning and in the evening. The mushrooms were manually harvested at maturity which was indicated by upward curving of the edges of the cap.

Biological efficiency of the different substrate mix was calculated as the ratio of weight (g) of fresh mushrooms harvested to dry weight (g) of the substrate (Chang *et al.,* 1981).

$$\text{Biological Efficiency} = \frac{\text{Weight of fresh fruiting bodies (g)}}{\text{Weight of dry substrate (g)}} \times 100$$

Data analysis

The data on mean weights and percent biological efficiency through one way ANOVA. The data groups were analyzed using Statistical Package for Social Sciences (SPSS) for windows 20.0. Treatment mean were compared using LSD.

Results and Discussion

Mycelia colonization of the substrate

The production bags that received different substrates mixture showed significantly ($p \leq 0.05$) different mycelia colonization rate. Treatments 7,8,9,and 10 took 11 days for complete mycelia colonization, while treatments 1, 2, 3 and 4 took relatively longer days(15days). Treatment 5 and 6 took mycelia colonization between the longer and the shorter days (Table 2). This observation was by far faster than the observation reported by Mondal *et al*. (2010) ranged from 21 to 24.75 days grown on substrate composed from banana leaves and rice straw. Even longer duration, 2-3 weeks after inoculation was reported (Pathania et *al*.2017). The shorter mycelia colonization in this investigation may be due to the presence of sufficient nutritional supplements for the oyster mushroom. Unlike the mycelia colonization the first primordial formation of the different production bags showed least ($p \geq 0.05$) variation ranging from 4 - 6 days (Table 2). This observation was in line with the reports of Mondal *et al.* (2010) who observed lowest duration (5.50 days) for primordial initiation on saw dust, banana leaves and rice substratum in different proportions than in rice straw alone. The present study results are comparatively shorter than the observation on wheat straw and apple pomace (Pathania *et al*. 2017).The total days required for complete production cycle of the oyster mushroom using substrate composed from creeping bent grass (*Agrostis* sp) biomass as major substrate showed significant ($p \leq 0.05$) differences. The fastest production cycle was 45 days and slowest 53 days (Table 2). This observation was similar to the report of Ogundele *et al*. (2017) who indicated that the average total days for oyster mushroom grown on softwood (*Daniella oliveri*) sawdust and hardwood (*Anogeissus leiocarpus*) sawdust was 45 days. There were three harvest in the present experiment whereas only two harvests with *Daniella oliveri* and *Anogeissus leiocarpus* saw dust (Ogundale, *et al*.,2017). Long production cycle was reported by Asefa Keneni (2018) 111-100days each, with five harvesting cycle was observed when *Gravilae robusta* dry leaf and cotton seed waste as substratum. In general, fastest mycelia run and shortest time taken for complete colonization of the different mixes of the substrates satisfy the required amount of assailable nutrients for the mycelia growth in this investigation.

Table2: Duration of the different phases of growth on different substrate mix

Treatment	Days taken for complete mycelia colonization	Days taken for first primordial formation after colonization	Total days required for complete production cycle
T1	15^{b}	6^{b}	53^{c}
T2	15^{b}	6^{b}	53^{c}
T3	15^{b}	5^{ab}	53^{c}
T4	15^{b}	5^{ab}	47^{ab}
T5	14^{b}	5^{ab}	49^{b}
T6	14^{b}	5^{ab}	49^{b}
T7	11a	4^{a}	49^{b}
T8	11^{a}	4^{a}	46^{a}
T9	11^{a}	4^{a}	46^{a}
T10	11^{a}	4^{a}	45^{a}

Mushroom yields per cycle of harvest

The yields of mushroom biomass during the first harvest showed significant ($p \leq 0.05$) variations. During this harvest treatment T10 gave the highest yield and the treatments 3, 4, and 8 gave the lowest yield. All the remaining treatments showed fresh weight of mushroom between the highest and lowest (Table 3). During the second harvest, treatment T5 gave the lowest yield, while treatment T10 gave the highest yield and, all the remaining treatments gave intermediate yields between the lowest and the highest. In the third harvest, all the treatments were showed significantly different ($p \leq 0.05$) mushroom fresh weight. At this time of harvest T9 showed the highest mushroom fresh weight while T10 showed the least mushroom fresh weight (Table 3). All

the rest of the treatments showed intermediate yields between the highest and lowest. In general the yields of the mushroom biomass decreases step wise from the 1st harvest to the 3rd harvest. This observation was similar to the report of Ogundele *et al*.(2017) who indicated that the average fresh weight of oyster mushroom grown on softwood (*Daniella oliveri*) sawdust and hardwood (*Anogeissus leiocarpus*) subsequently decreased from the first harvest to the second harvest and explained these from the point of view of the deceasing nutrient concentration in the substrate. Similarly Yang *et al*. (2013) reported that the oyster mushroom fresh weight varied based on the substrate composition and highest mushroom yield obtained from 80% cotton seed hull and wheat 20% bran as compared to other substrate composition used. Asefa and Lakew (2016) reported that highest biomass was found in the first flush than the rest of the harvest. Pathania *et al.* (2017) suggested that oyster mushroom was harvested in three flushes and maximum average yield was estimated from the sawdust and the biomass harvested declined progressively from the third to the fifth harvest invariably with all treatments.

Table3: Mushroom biomass (fresh weight) in different harvesting cycle

Treatments	1st	2nd	3rd
T1	720^{d}	520^{e}	360^{e}
T2	660^{h}	480^{g}	450^{b}
T3	650^{i}	500^{f}	440^{bc}
T4	650^{i}	510^{e}	430^{c}
T5	750^{g}	450^{h}	400^{d}
T6	670^{f}	680^{b}	400^{d}
T7	700^{e}	600^{d}	450^{b}
T8	800^{b}	500^{f}	450^{b}
T9	650^{i}	620^{c}	550^{a}
T10	1000^{a}	700^{a}	350^{ef}

Pinning to maturation during different harvesting time

The duration between pinning to maturation were not significantly ($p \geq 0.05$) varied. But treatments 8, 7, and 9 showed relatively shorter days from pinning to maturation which was less by half a day from the rest of the treatments in the first and second harvest while it was equal for the all the treatments in the third harvest (Table 4). These results were found to be shorter than the pinning to maturation period reported by Asefa Keneni (2018) ranging from 9 days in the longest and 6 days in the shortest harvest. The results of present investigation was in line with the observation of Gume *et al*.(2013) where 4 days for maturation of oyster mushroom grown on sawdust and coffee waste. Kinge *et al*. (2016) observed the time from primordial initiation to harvest ranged from 3.25-5.50days while longer pinning to maturation 10 days after pinhead formation was reported by Pathania *et al*. (2017).

Table 4: Pinning to maturation duration (Mean) under different harvesting cycle

Treatments / Flushes	1st	2nd	3rd
T1	6	5.5	5
T2	6	5.5	5
T3	6	5.5	5
T4	6	5.5	5
T5	6	5.5	5
T6	6	5.5	5
T7	5.5	5	5
T8	5.5	5	5
T9	5.5	5	5
T10	5.5	5	5

Yield related parameters

a. Number of aborts, bunches and fruits

The average number of fruits of the treatments bags showed significant ($p \leq 0.05$) differences. Treatments 8, 9 and 10 showed the highest number (average) of fruits (80-85) while treatment T1 gave the least number of fruits 58 (Fig 1). This observation is similar to Mondale *et al*. (2010) who reported that, the number of effective fruiting body ranged from 8.5 to 37.25 and the maximum number on sawdust. Highest number (130) of fruits were recorded for T4, a substrate composed from *Gravilae robusta* dry leaves and cotton seed waste (Asefa Keneni, 2018).The average number of aborts also showed significant variations ($p \leq 0.05$). The treatment 4, 8 and 9 showed higher number of aborts (105-100), while treatment 1 and 2 showed lower number of aborts 85-87. The rest of the treatments showed number of aborts between the highest and lowest (Fig 1).

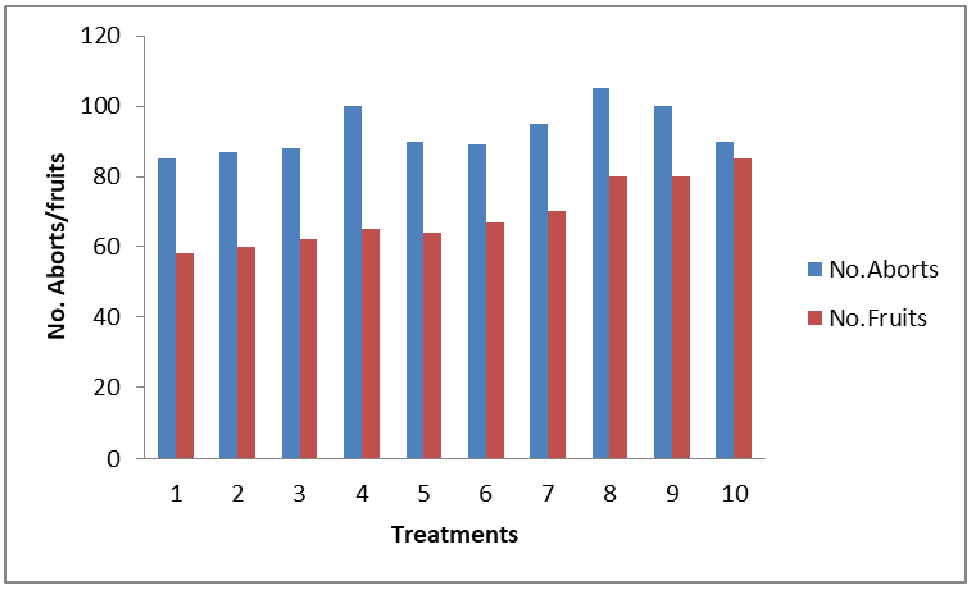

Fig 1: Number of aborts and mature fruits bodies on the different substrata

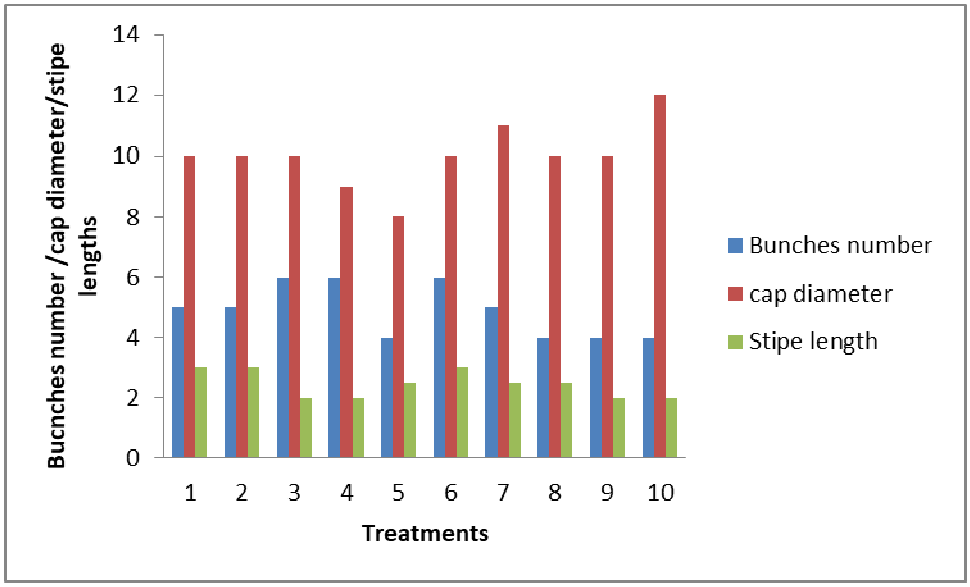

Fig 2: Bunch number, Cap diameter and stipe length of the oyster mushroom

Average number of bunches in the various treatment mix did not showed significant variations ($p \leq 0.05$) (Fig 2) and n all the treatments average bunches ranged from 4-6 (Fig 2).

b. Cap diameter and stipe length

Cap diameter: The average cap diameter of the fruit bodies grown on treatment bags did not showed significant ($p \geq 0.05$) variations. Treatment T5 showed smaller average cap diameter while treatment T10 showed largest cap diameter. The rest of the treatments showed intermediate cap diameter between the larger and smaller. This observation was in line with the reports of Yang *et al*., (2013) who observed that substrate composed from cotton seed hull 80% and wheat bran 20% gave the highest cap diameter. Largest cap diameter (8cm) was recorded for treatment T6 mushroom grown on *Gravilae robusta*, cotton seed (Asefa keneni, 2018). In case of 1st flush pileus diameter was found highest (7.798 cm) on sawdust and the lowest (4.13 cm) diameter was recorded on banana leaves and rice straw (1:3) (Mondal *et al*. 2010)

Stipe length: The average stipe length of the treatments showed no significant ($p \leq 0.05$) differences. Treatment 3, 4, 9 and 10 gave relatively shorter (2.00 cm) stipe length while treatment 1, 2 and 6 gave relatively longer stripe length (3.0 cm) each. And the rest of the treatments showed intermediate stipe length between the longer and shorter (Fig 2). Similar stipe length variations were reported Asefa Keneni (2018) on substrates composed from *Gravilae robusta* dry leaf and cotton seed. Short stipe length was reported by Yang et al. (2013)for mushroom grown on substrate composed from cotton seed hull (20%) rice straw (60%) and wheat bran (10%).

Total biomass and biological efficiency

The highest total biomass (2050g / 800g) was recorded on treatment T10, while the lowest biomass 1590 g of fresh mushroom per 800 g of dry substrate on treatments T1T2,T3, T4, T5, (Fig 3). Mondal *et al*. (2010) obtained from rice straw maximum biological yield (159.3 g) and the minimum biological yield (36.35 g) from banana leaves and rice straw (1:1) in the first harvest. Highest fresh weight 1246.5 g was recorded for T 9 and T1 showed the lowest fresh weight, 538g per 600 g dry weight of the substrate (Asefa Keneni, 2018).

In the present experiment the highest biological efficiency was recorded from treatment T10, (250%) and the lowest from treatment T1-5(197%) (Fig4). This result is higher than the reports in the literature. Yang *et al*. (2013) reported the highest biological efficiency 125.6% from the substrate composed of cotton seed hull (80%) and wheat bran (20%) as compared to other substrates. Pathania *et al.* (2017) observed biological efficiency 54.23 % on a substrate composed of 0.50apple pomace + 1.50 wheat straw whereas highest biological efficiency 208% for oyster mushroom from a substrate composed from *Gravilae robusta* dry leaf and cotton seed waste (Asefa Keneni ,2018).

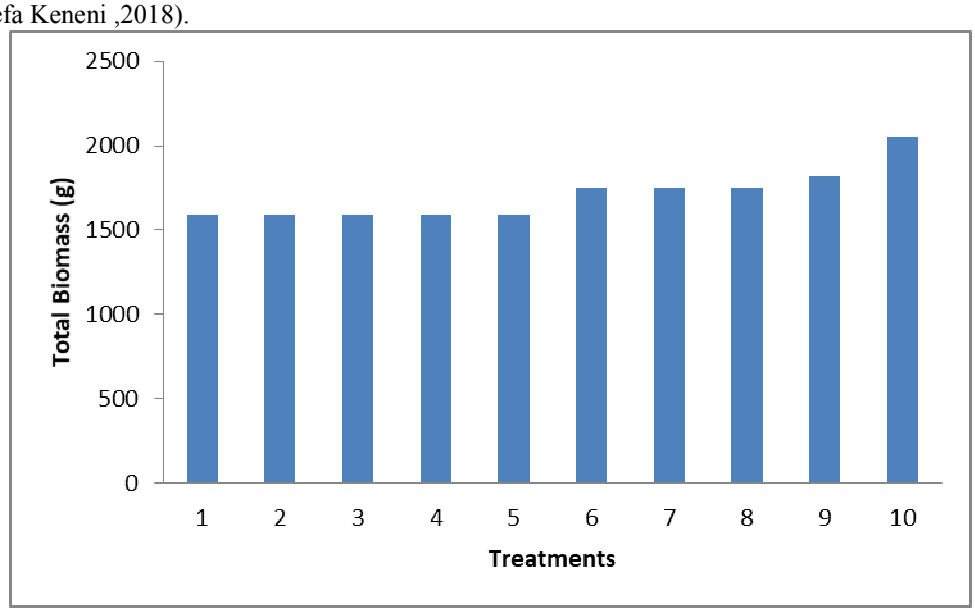

Fig 3. Total biomass of oyster mushroom grown on the different substrate mix

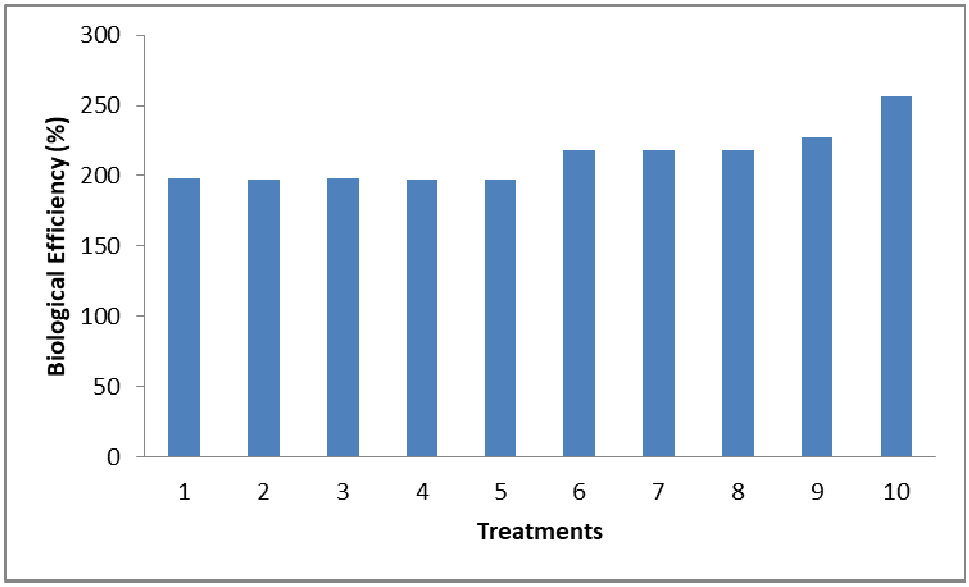

Fig 4: The Biological efficiency of the oyster mushroom grown on the different substrate mix

Fig 5: (A) *Agrostis sp. in the field (*B) *Agrostis sp biomass*, (C) waste paper (D) cotton seed waste: (E) wheat bran (F) oyster mushroom culture on dextrose agar surface, (G) spawn prepared on yellow colored sorghum grain ready to use, (H) Fully colonized substrate (I) Primordial formed on the production bag, (J) Mature fruit bodies of oyster mushroom.

CONCLUSION

The production of mushroom depends on several factors of which availability of nutrient rich substrates which are comparatively cheap is crucial for success of this enterprise. In this study the usability of creeping bent grass (*Agrostis* sp) biomass along with cotton seed waste, waste paper and wheat bran of different proportions were evaluated as substratum for oyster mushroom production. The total yield as well as the biological efficiency of oyster mushroom grown on different substrate composition showed significant differences which are found to be high and more promising than the earlier reports. As a result it is possible to conclude that creeping bent grass (*Agrostis* sp) biomass alone or together with other substrates can be utilized as a mushroom substratum for farm scale and /or large scale production of oyster mushrooms.

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