



# Evaluation of *Grevillae Robusta* Dry Leaves Supplemented with Cotton Seed Waste for the Cultivation of Oyster mushroom (*Pleurotostreatus*)

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## ABSTRACT

At present more emphasis has been given to mushroom production for the nutrition and medicinal uses and waste recycling technology. The main aim of the present study was to report the usability of *Grevillaerobusta* dry foliage as a major substrate for production of oyster (*Pleurotostreatus*) mushroom with supplement of different proportion of cotton seed waste. The culture of the oyster mushroom was maintained on potato dextrose agar, and the spawn was prepared on yellow colored sorghum and sterilized substrate was inoculated with 5% of the spawn wet basis on dry basis of the substrate. The experimental design constitutes nine treatments (T1-T9) in three replicates from the middle of November 2017 to the end of February 2018. Fastest mycelia run was observed in the treatments T9, T7 and T8: 10 days each from inoculation, while the slowest mycelia run was observed in treatment T5 and T6: 19 days each from inoculation. Longest production cycle was observed for treatment T3, T5 and T6: 111 days each, while the shortest production cycle was recorded for treatment 8: 100 days. Highest fresh weight 1246.5 g /600g dry substrate was recorded for T 9; highest number (130) of fruits recorded for T4 while largest cap diameter (8cm) was recorded for treatment T6. The T1 showed the lowest fresh weight, 538g/600 g/dry weight of the substrate, with 70 fruits and 6.5cm cap diameter. No significant difference was observed for the stipe length of the different treatments. Highest biological efficiency was recorded for T9: 208; and 89.5 for T1. Even though the cotton seed waste proportion supplementation varied for the treatments all except T1 gave more than 100% biological efficiency which makes *Grevillae* dry leaves, a good substratum for mass production of oyster mushroom which can produce good quality mushroom fruit bodies.

## INTRODUCTION

Mushrooms are basically fungi which have a flashy and spore bearing fruiting body growing in fields, forests; on manure heaps, on water channels, on hilly areas, on damp rotten log of wood trunks of trees, on decomposing organic matter, in damp soil rich in organic substances, mostly during and just after rainy season. They comprise a large heterogeneous group having various shapes, sizes and color, all quite different in character, appurtenance and edibility. Based on standard morphology the word "mushroom" was mostly used to describe those fungi that have stem (stipe), a cap (pileus), and gills (lamellae) or the pores on the under sides of the cap. Only a fruiting body of the mushroom can be seen whereas the rest of the mushroom remains underground as mycelium (Chang and Miles 1992).

Mushroom has been recognized as alternative sources of good quality protein. They are capable of producing the highest quality of protein per unit area and time, from the worthless agro waste which are available in huge amount all-round the year and they, represents as one of greatest untapped resources of nutrition and palatable food of the future. The nutritional value of the edible mushroom can be compared with eggs, milk, and meat (Daba *et al.*, 2008). And the mushroom contents of essential amino acids are high and close to the need of the human body. It is easily digestible and it has no cholesterol (Chang and Buswell, 1996).

Mushroom with protein content ranging from 3-7% when fresh to 25-40% when dry can play an important role in enriching human diet when meat sources are limited. The mushroom protein content is almost equal to that of corn, milk, and legumes, although still lower than meat, fish and egg (Sparks, 2006). Mushrooms are considered as a functional food, which can provide health benefits beyond the traditional nutrients they contain (Miles and Chang, 2004). Without doubt, edible mushroom in fresh, cooked or processed forms are nutritionally sound, tasteful food source for most people and can be a significant dietary component for vegetarians (Daba *et al.*, 2008). From a number of cultivated mushrooms, oyster mushroom (*Pleurotostreatus*) is the most preferred species due to its ability to utilize different organic waste due the presence of efficient enzymes such as cellulose, hemicelluloses, and lignin, for fast growth.

Several species of the genus *Pleurotus* have great commercial value in the global market of edible cultivated mushrooms, which reportedly grew from 2.13 million tons in 1996 to 3.43 million tons in 2007 (Okuda *et al.*, 2009). Nutritional value of oyster mushroom is also appreciable. 3.5 oz. of oyster mushroom provides 38 Calories and contain, 15%-25% of Protein, 2.2g of Fat, 6.5g of Carbohydrate, 2.8g of Fiber, and also contains Vitamin B1 (Thiamine) 0.56 mg, Vitamin B2 (Riboflavin) 0.55 mg, Vitamin B3 (Niacin) 12.2 mg, Phosphorus 140

mg, Calcium 28 mg, Iron 1.7 mg<sup>3</sup>. Comparatively it is high in fiber and low in carbohydrate and fat which make it more potential for both normal people and patients (Akyuz and Kirbag 2010; Daba *et al.*, 2008). Oyster mushrooms are cultivated widely as their temperature requirement of 20-30°C prevails in most of the areas. From 1997 to 2010, *Pleurotus* spp. production increased from 876 t to 6,288 t (618%). China was responsible for most of the production increase and accounted for over 85% of the world's total output in 2010. (Royse, 2014)

Edible mushrooms are grown on waste materials from farms, plantations agro factories recycled to produce value-added mushrooms. Currently, millions of tons of agricultural wastes are discarded, burned and neglected thus reducing environmental pollution (Bhawna and Thomas, 2003). These agro wastes can be used alone or in combination to create mushroom growing substrates (Chang, 1991 in an inexpensive way.

*Grevillaerobusta* (Proteaceae), commonly known as "Silky Oak" is native to Australia (Richie *et al.*, 1965; Cannon *et al.*, 1973). *G. robusta* is popular among farmers in Kenya, due its fast growth, ability to tolerate heavy pollarding and pruning of branches and because it mixes well with other crops (Tyndall, 1996; Muchinet *et al.* 2002). The species has important uses including construction material, fuel wood, shade, fodder soil erosion control, and soil fertility improvement (FAO, 2001; Holding *et al.*, 2005). So far, alkyl resorcinols, macrocyclic phenols and cinnamic acid derivatives have been reported to be constituents of this plant. In Ethiopia, *Grevillaerobusta* is a dominant tree planted as a road side shade throughout the country, in different institutions including the capital city, Addis Ababa. The use of this tree for the various purposes is not yet promoted for the scale it required. This article discusses about the usability of *Grevillaerobusta* dry leaves together with the different proportion of cotton seed waste for the cultivation of oyster (*Pleurotostreatus*) mushroom.

## MATERIALS AND METHODS

### Organism and culture conditions

The fungal strain, *Pleurotostreatus* (Oyster mushroom) was obtained from Mycology Laboratory, Department of Biology, Addis Ababa University, Ethiopia. The pure culture of *Pleurotostreatus* was transferred on to Potato Dextrose Agar (PDA) on the Petri dishes and incubated at 25°C. The growth of the culture and presence of contamination were visually inspected at three days interval.

### Grain Spawn production

The spawn (mushroom seed) of *Pleurotostreatus* was produced on yellow colored sorghum grain, wheat bran and calcium sulfate (gypsum) in the ratio of 88:10:2

respectively (Dawit, 1998). The required amount of sorghum grain was weighed and soaked over night in sufficient amount of water. The grains were washed and drained to remove the dead and floating seeds with excess of water. After removing the excess water from the grain, the required amount of wheat bran and gypsum ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) were added and transferred to 1000 ml glass bottles (75% level) leaving a head space over the grain and autoclaved at 121°C temperature for 45 minutes. After cooling, each bottle was inoculated with 20 agar blocks (1 cm × 1 cm) of 15day old mushroom culture from the Petri dish and incubated for

21 days at  $28 \pm 2^\circ\text{C}$  until the substrate were fully colonized and the mycelia invasion and contamination were inspected at five days interval.

### Treatments

Nine treatments (T1–T9) comprising different proportions of *Grevillaerobusta* dry leaves, waste paper and cotton seed waste (600 g) with 1% lime stone (Calcium Carbonate) on dry weight basis were used as shown in Table 1.

**Table1: The composition of different treatments used in this study**

Treatments	Grevillae dry leaves (g)	Cotton seed waste (g)	Waste Paper(g)	Total (g)
T1	450	50	100	600
T2	400	100	100	600
T3	350	150	100	600
T4	300	200	100	600
T5	250	250	100	600
T6	200	300	100	600
T7	150	350	100	600
T8	100	400	100	600
T9	50	450	100	600

### Preparation of the substrate

The *Grevillae* dry leaves were soaked in sufficient amount of water over night. On the next day excess water was squeezed from the leaves. 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 of calcium carbonate and filled in sterilizable yellow color polyethylene bags (Kurtupestal). The substrates were autoclaved at 15Psi pressure at 121°C temperatures for 1hour. After sterilization the substrates were transferred to transparent polyethylene cultivation bags for easy supervision of the growth of the mycelia and presence of contamination. Each substrate (600 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 cm<sup>2</sup>) for drainage and aeration. It was kept in a spawn running room at room temperature. After primordial formation, the bags were transferred to mushroom house under normal environmental conditions and relative humidity (the room maintained at 85–90%), irrigated using tap water every morning and evening with optimum ventilation. The mushrooms were manually harvested at maturity which was indicated by up ward curving of the edges of the cap. Biological efficiency was calculated and defined as the ratio of

weight (g) of fresh mushrooms harvested to dry weight (g) of the substrate (Chang *et al.*, 1981)

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

### Data analysis

The data were analyzed by comparing the mean weights and percent biological efficiency through one way ANOVA. The data groups were analyzed using Statistical Package for Social Sciences (SPSS) for windows version 20. Treatment mean were compared using LSD.

## RESULTS AND DISCUSSION

### Mycelia colonization of the substrate

The production bags that received different substrates mixture showed significantly different mycelia colonization rate ( $p \leq 0.05$ ). Treatment 9,8 and 7 showed relatively short period (10 days) for mycelia colonization than treatments 5 and 6 which took 19 days (Table.2). Sharma *et al.*, (2016) reported complete of mycelia colonization from 22.40-26.00 days. The time required for first primordial formation in the different treatment showed no significant variation ( $p \geq 0.05$ ) (Table 2). In this study, the pin head formation after complete mycelia colonization took longer time than the duration reported by Sharma *et al.*, (2016) that is 6 days in fastest and 8

days in slowest treatments. Kumari and Achal (2008) stated that colonization of the substrate was completed within 20 days of inoculation. Quimio *et al.* (1999) reported that good harvest of *P. ostreatus* was 3-4 weeks after incubation. Shah *et al.* (2004) reported that primordial formation of *P. ostreatus* appears 27-34 days of inoculation which is longer than the results of this study. The total days required for complete production cycle of the oyster mushroom using *Grevillae* dry leaves as major substrate showed significant ( $p \leq 0.05$ )

differences. Zinabue *et al.*, (2015) reported that sugarcane bagasse supported the fast mycelial growth during cultivation of *Pleurotus* species. In general, fastest mycelia run and shortest time taken for complete colonization of the substrate, the different mixes of the substrates satisfy the required amount of assimilable nutrients for the mycelia growth.

Treatment 8 relatively took shorter total days for complete production while treatment 3, 5, 6, took more days for completing the production cycle.

**Table2: Duration of the different phases of growth of the mushroom**

Treatment	Complete mycelia colonization	First primordial formation after colonization	Completion of production(days)
T1	16 <sup>b</sup>	7 <sup>a</sup>	110
T2	16 <sup>b</sup>	7 <sup>a</sup>	104
T3	16 <sup>b</sup>	7 <sup>a</sup>	111
T4	16 <sup>b</sup>	7 <sup>a</sup>	110
T5	19 <sup>c</sup>	8 <sup>a</sup>	111
T6	19 <sup>c</sup>	8 <sup>a</sup>	111
T7	10 <sup>a</sup>	6 <sup>a</sup>	110
T8	10 <sup>a</sup>	6 <sup>a</sup>	100
T9	10 <sup>a</sup>	6 <sup>a</sup>	106

#### **Yield (fresh weight) of mushroom in different harvesting cycle**

The yields of mushroom biomass during the first harvest showed significant ( $p \leq 0.05$ ) variation in the different treatments. During this harvest treatment 9 with less amount of *Grevillae* dry leaves gave the highest yield (570g) and treatment 1 gave the lowest yield (275g) (Table 3). All the remaining treatments showed fresh weight of mushroom between the highest and

lowest. During the second cycle harvest, treatments 7, 8 and 9 showed high rate of biomass, 346, 310 and 320g respectively. The biomass harvested declined progressively from the third to the fifth harvest invariably with all treatments. However, relatively good harvest was observed with treatments 4, 8 and 9. This observation is in line with reports of Asefa and Lakew (2016) and Sharma *et al.*, (2016) that highest biomass was found in the first flush than the rest of the harvest.

**Table 3: Yield (fresh weight) of mushroom in different harvesting cycle**

Treatments	1 <sup>st</sup> flush	2 <sup>nd</sup> Flush	3 <sup>rd</sup> flush	4 <sup>th</sup> flush	5 <sup>th</sup> flush
T1	275 <sup>i</sup>	140 <sup>h</sup>	73 <sup>h</sup>	50 <sup>f</sup>	-
T2	350 <sup>g</sup>	207 <sup>f</sup>	190 <sup>a</sup>	84 <sup>e</sup>	88 <sup>b</sup>
T3	300 <sup>h</sup>	238 <sup>e</sup>	109 <sup>g</sup>	94 <sup>d</sup>	73 <sup>c</sup>
T4	415 <sup>e</sup>	278 <sup>d</sup>	174 <sup>b</sup>	125 <sup>b</sup>	105 <sup>a</sup>
T5	440 <sup>d</sup>	213 <sup>f</sup>	117 <sup>f</sup>	105 <sup>c</sup>	60 <sup>d</sup>
T6	400 <sup>f</sup>	210 <sup>f</sup>	151 <sup>d</sup>	130 <sup>b</sup>	88 <sup>b</sup>
T7	500 <sup>b</sup>	346 <sup>a</sup>	140 <sup>e</sup>	108 <sup>c</sup>	75 <sup>c</sup>
T8	450 <sup>c</sup>	310 <sup>c</sup>	165 <sup>c</sup>	145 <sup>a</sup>	75 <sup>c</sup>
T9	570 <sup>a</sup>	320 <sup>b</sup>	178 <sup>b</sup>	107 <sup>c</sup>	73 <sup>c</sup>

Relatively shorter pinning to maturation were observed on treatments 8,7, and 9. While more days from pinning to maturation were taken for the treatments of 5 and 6 (Table 4). In all rest of harvesting cycle (2-5) there were no significant ( $p \geq 0.05$ ) variation on the days taken from pinning to maturation. According to Mekonnen and Semira, (2014) oyster mushroom grown on cotton hull substrates took 4 and 7 days for first and second harvesting time respectively than the saw dust substrate. The combination of substrate with less amount cotton seed waste showed shortest mean periods from pinning to maturation from first to fourth flushing in the order 10, 9, 8, and 7 days respectively as compared to other treatments. Similarly short pinning to maturation period

of 4 days was reported by Gumeet *al.*(2013) for oyster mushroom grown on sawdust and coffee waste.

The average number of fruits of the treatments bags that received different substrate mix showed significant differences ( $p \leq 0.05$ ). Treatment 4 and 7 showed the highest number (average) of fruits 130 each while treatment 1 gave the lower number of fruits 70 (Fig 1). And the rest of the treatments gave number of fruits between the highest and the lowest. The result recorded in this study was in line with Sharma *et al.* (2016) who indicated that the number of fruit bodies produced in the first harvest was higher than the subsequent flushes.

**Table 4: Duration from pinning to maturation(days) during harvesting cycle**

Treatments	1 <sup>st</sup> flush	2 <sup>nd</sup> Flush	3 <sup>rd</sup> flush	4 <sup>th</sup> flush	5 <sup>th</sup> flush
T1	8	7	7	7	-
T2	8	7	7	7	6
T3	8	7	7	7	6
T4	8	7	7	7	6
T5	9	8	7	7	6
T6	9	8	7	7	6
T7	7	6	6	6	6
T8	7	6	6	6	6
T9	7	6	6	6	6

The average number of aborts of the treatments bags that received different substrate mix showed significant

variations ( $p \leq 0.05$ ). The treatment 4 and 9 showed lower number of aborts 60 each, while treatment

8 showed highest number of abortions 91. And the rest of the treatments showed number of abortions between the highest and lowest (Fig 1).

Average number of bunches in the various treatment mix showed significant variations ( $p \leq 0.05$ ). The treatments 9 (15), 6 (14) and 4 (14) showed relatively higher number of bunches while treatment 1 (7) had fewer number of bunches (Fig 1). The average cap diameter with treatment 6 was greater than treatment 1 (Fig 2). This observation was in line with the observation of Sharma *et al.*, 2016), who indicated that, the size of

fruiting bodies was higher in case of rice straw than in all other substrates.

Stipe length: the average stipe length of the treatments bags that received different substrate mix showed significantly different ( $p \leq 0.05$ ). Treatment 3, 8, 5 and 9 gave (relatively shorter stipe length which were ranged from 2.6-2.8 cm while, treatment 1, 2, and 7 gave relatively longer stipe length 3.5 cm each. And treatment 4 and 6 showed intermediate stipe length between the longer and shorter length 3.0 each (Fig 2).

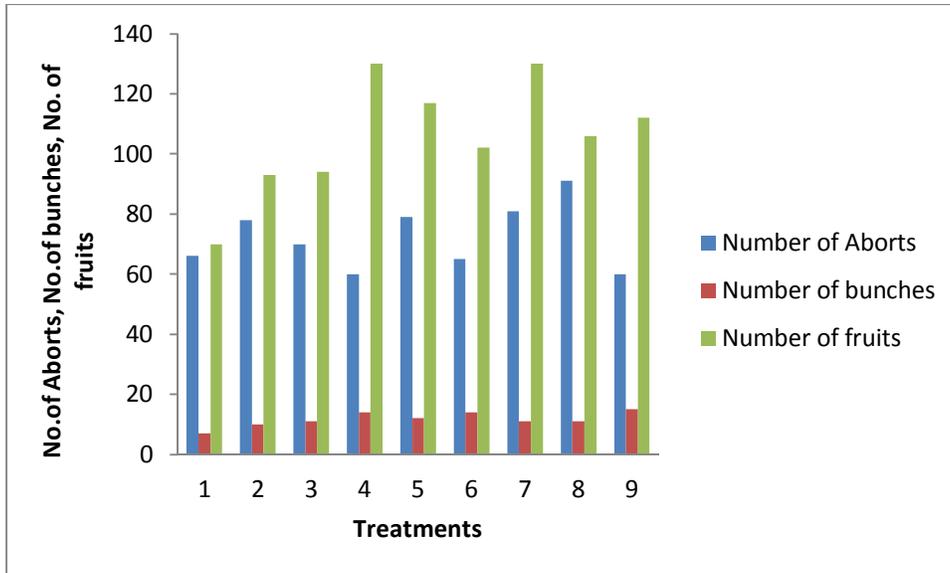


Fig 1: Number of abortions, bunches and fruits

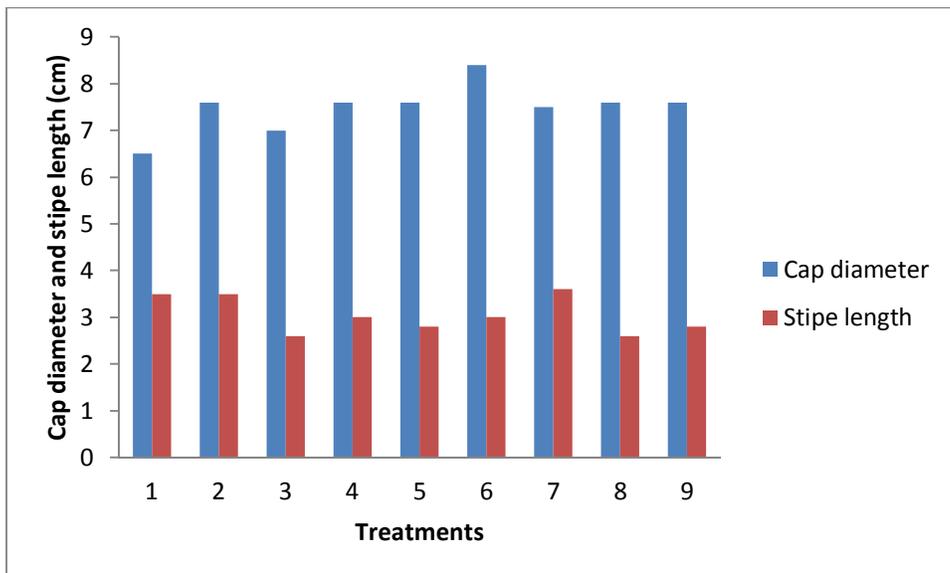


Fig 2: Cap diameter and stipe length of the different treatments

**Total biomass and biological efficiency**

The highest total biomass was recorded on treatment 9 (1246.5g fresh weight /600g of the dry substrate) while the

lowest biomass was recorded on treatment 1, (538 g of fresh mushroom /600 g of dry substrate) (Fig 3). The highest biological efficiency was also recorded from treatment 9, (208) and the lowest from treatment 1 (

89.5%)(Fig 4). Zinabuet *et al.*, (2015) reported high values of biological efficiency for *Pleurotusostreatus* grown on Sugarcane bagasse (70.5%) and *Pleurotusostreatus* grown on waste paper alone (68.9%) followed by *Pleurotusflorida* grown on sugarcane bagasse (67.6%).Patra and Pani (1995) reported biological efficiency (50-75%) of *Pleurotus* species grown on agro-industrial residues, namely; corncobs, various grasses and reed stems, vine shoots, cottonseed hulls and sugarcane bagasse. In another report Sharma *et al.*, (2016) observed thehighest yield (381.85gm) and

biological efficiency (95.46%) using rice straw , wheat straw, paper and sugarcane bagasse while lowest yield 247.87g and biological efficiency 61.96 % was obtained from sawdust.Royse *et al*(2004)stated that, yields for cottonseed hull/wheat straw substrate were three fold higher than treatments containing switch grass. The present study results are in agreement with their observation that as the proportion of cotton seed waste increased in the substratum the total biomass yield and biological efficiency also increased.

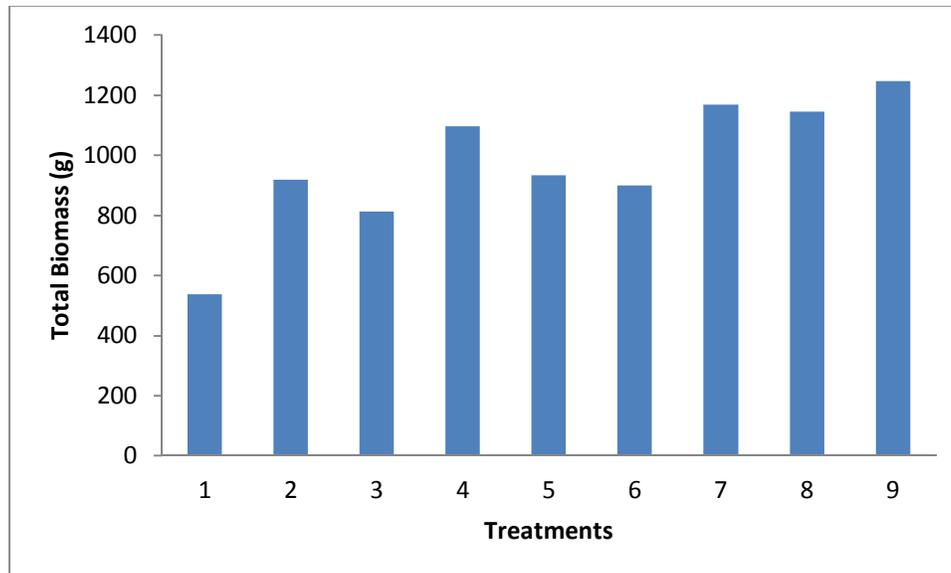


Fig 3:Totalbiomass of the different treatments

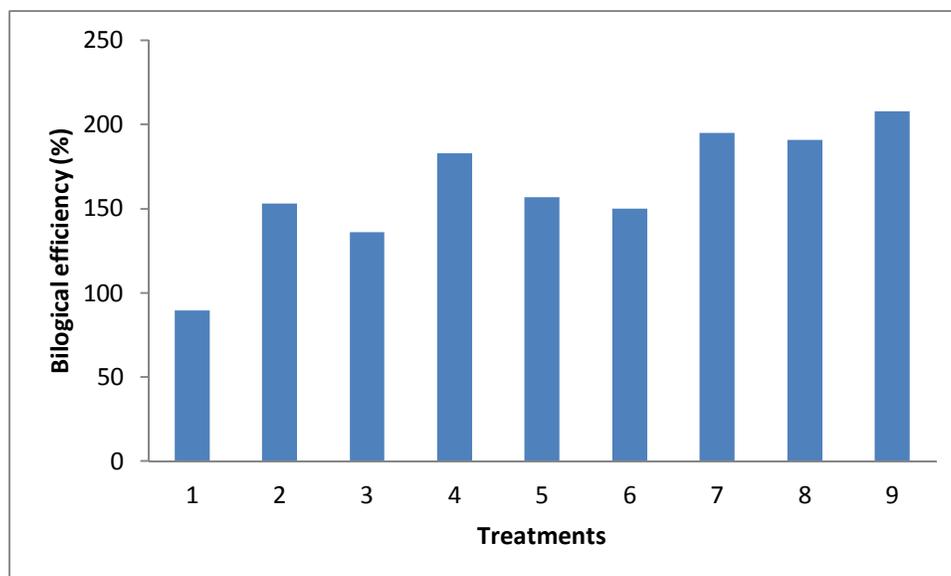


Fig 4: The Biological efficiency of the different treatments



**Fig 5: A) *Grevillaerobusta* B) *Grevillae* dry leaves, C) cotton seed D) waste paper: E) oyster mushroom culture on dextrose agar medium, F) spawn prepared on yellow colored sorghum grain ready to use, G) Fully colonized substrate in the production bag, H) Primordial formed on the production bag, I) Mature fruit bodies of oyster mushroom**

## CONCLUSION

Mushroom production technology has been considered as appropriate technology for satisfying nutritional needs and medicinal requirements and for the sustainability of the environment through recycling of organic waste which otherwise negatively affect the environment. Large bodies of information have been available on the usability of different agricultural and agro processing by products for the cultivation of mushrooms. But very few reports were available on processing dry foliage for the production of mushroom. In this study the usability of *Grevillaerobusta* dry leaves along with cotton seed waste, and waste paper were used as substratum at different proportions. The total yield of the mushroom biomass, as well as the biological efficiency showed significant difference between the treatments. However, it is evident that *Grevillaerobustae* dry leaves can be utilized as a mushroom substratum along with other agricultural by-products for production of mushrooms.

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