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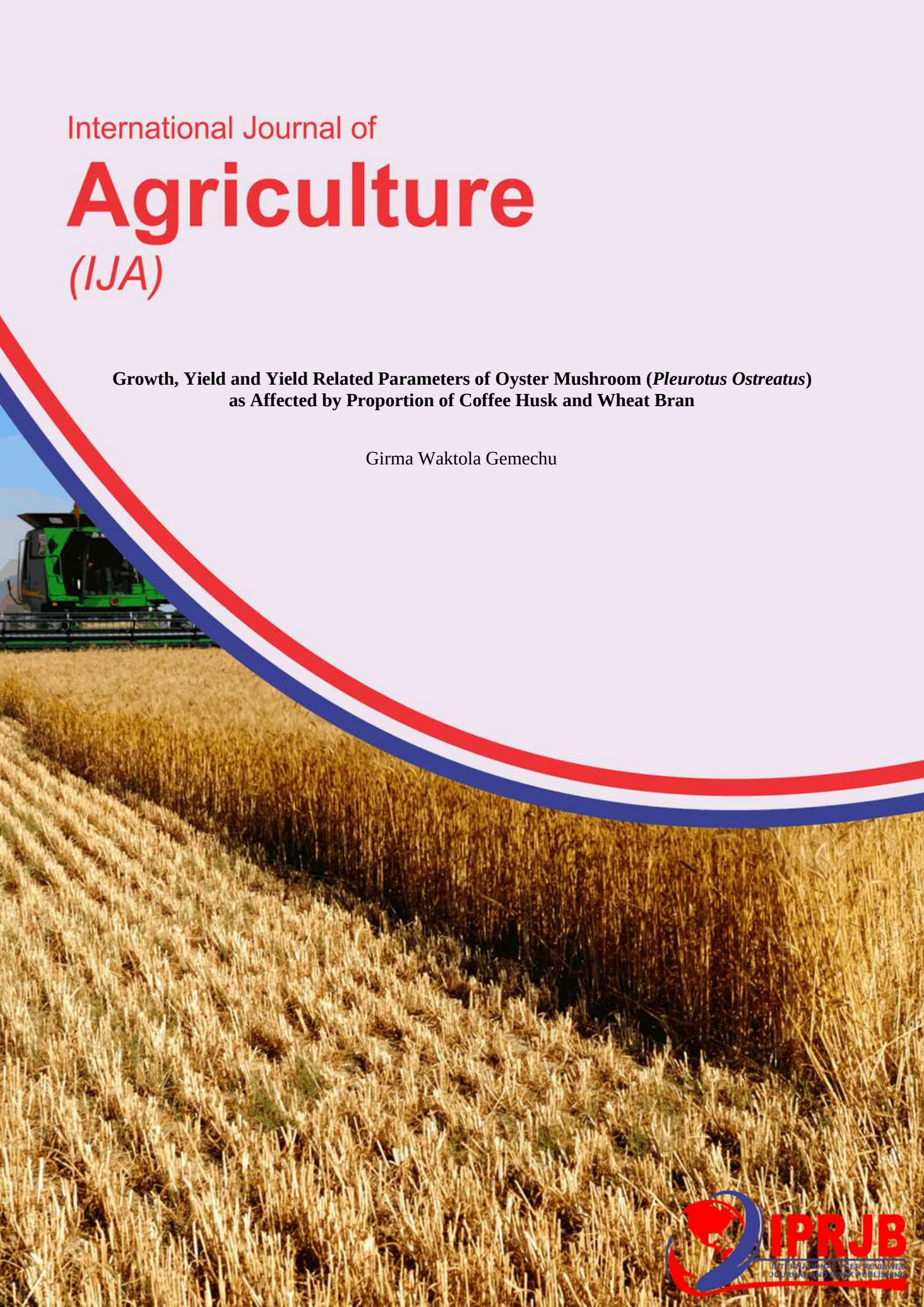

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Growth, Yield and Yield Related Parameters of Oyster Mushroom (*Pleurotus Ostreatus*) as Affected by Proportion of Coffee Husk and Wheat Bran

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Abstract

Purpose: This study was conducted in order to evaluate growth, yield and yield related parameters of oyster mushroom as affected by proportion of coffee husk and wheat bran.

Methodology: The mushroom culture was grown on malt extract agar and the spawn was prepared on yellow color sorghum grain. The substrate was sterilized and inoculated with (10%) spawn. The experiment was laid in completely randomized block design in triplicate. The data collected were analysed by using SPSS software version 20.0 and were compared by LSD at ($p \leq 0.05$). The shortest days for complete mycelium colonization, primordia formation and first maturation of oyster mushroom after substrate inoculation were (19), (21.66) and (28.33) respectively from T3RI. The largest cap diameter (12.18cm) was observed from T3RI, maximum number of bunches (4.66) recorded from T5RI. T3RI. The longest incubation to 1st harvest was recorded for T7RI (44.33) days. In this study, the fresh weight of the 1st harvest was (354g) to (393g). The highest total flush weight was (806.33g/500g) T3RI and highest biological efficiency (161. 26%) T3RI.

Findings: The finding of this study put forward that Pleurotus ostreatus grow on locally accessible agro processing by products and has the potential to secure food self insufficiency of low income community.

Unique Contribution to Theory, Practice and Policy: In this experiment, converting coffee husk alone or together with different proportion of wheat bran resulted in highest growth, yield, yield related parameters and biological efficiency of the oyster mushroom. Form all the substrates and substrate composition tested in this investigation T3RI, coffee husk, wheat bran in the ratio of 90:10 gave maximum in all the parameter evaluated; as a result this composition of substrate mix ratio need to be evaluated for farm, pilot and large scale production of oyster mushroom in the dry coffee processing areas.

Keywords: *Biological Efficiency, Coffee Husk, Spawn, Wheat Bran, Yield*

INTRODUCTION

Mushrooms are the fleshy and corpulent spore-bearing fruiting body of a fungus usually produced above the ground (Takele *et al*., 2018). The mushroom cultivation practice can elevate the income of country within sustainable use of natural resources it can improve food security (Semwal *et al*., 2014; Tsegaye, 2015). Cultivation of oyster mushrooms has recently become universal practise throughout the world because of their abilities to grow at a broad assortment of temperatures utilizing various lignocellulosic substrates (Minten *et al*., 2014). It is more widely cultivated and acceptable throughout the world (Knop *et al*., 2015). The most attractive features are its adaptability to a wide range of substrate material. Compatible substrates include but, are not limited to living trees, dead logs, paper, straw, wood, seeds and coffee grounds. Even toxic compounds often fail to inhibit growth of these persistent fungi. *P. ostreatus* has demonstrated an ability to break down the core molecular components of oil, creosote and pesticides (Pozdnyakova *et al*., 2008; Purnomo *et al*., 2010). Coffee residue (byproduct) is generated from coffee processing stations and is disposed into arable land and surface water leading to environmental pollution and environmentally friendly disposal method is needed in a useable way (Henok and Tenaw, 2014; Mensah, 2015). Waste conversion towards mushroom cultivation is not only for environmental sustainability but also for contribution of food self sufficiency by producing mushroom fruiting bodies.

MATERIALS AND METHODS

Organism and Culture Conditions

The fungal strain, oyster mushroom (*P. ostreatus)* was obtained from Biology laboratory of Ambo University. The pure culture of *Pleurotus ostreatus* was transferred on malt extract agar (MEA), agar 20g and chloramphenicol 0.2g in 1000ml of water. After pure culture was inoculated to medium it was incubated at 28^{o}C. The growth of the culture and presence of contamination were visually inspected at three day intervals.

Spawn Production

The spawn of *Pleurotus ostreatus* was produced on yellow colored sorghum grain, wheat bran and calcium sulfate in the ratio of 88:10:2 respectively. The sorghum grain was weighed and soaked overnight in a sufficient amount of water. After removing the excess water from the grain, 10% and $CaSO_4.2H_2O$ were added and transferred to 1000ml glass bottle and autoclaved at 121^{0}C temperature for 45min. After cooling, each bottle were inoculated with 15 day old mushroom culture and incubated for 21 days at 28^{0}C until the substrate was fully colonized and the mycelia invasion was inspected at five day intervals.

Source of Substrate and Its Preparation

Coffee husks were soaked overnight in sufficient water. After excess water in the substrate was removed the substrate was mixed with conditioner substrates. The substrate was mixed with wheat bran in varying proportion and 1% of $CaCO_3$ then transferred to plastic bag in order to sterilize the substrate.

Experimental Design

The experiment was designed in a Completely Randomized Design (CRD) with three replications involving a 7x3 factorial arrangement for coffee husk and wheat bran. For 21 treatments spawn were prepared by mixing that substrate with 1% of $CaCO_3$ in all treatments.

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Table 1: The Composition of Different Treatments of Coffee Husk and Wheat Bran

TRTS	CH 'g'	CH %	WB 'g'	WB%	Total 'g'	Rrk
T1RI	500	100	_____	_____	500	Pure
T2RI	475	95	25	5	500	Mix
T3RI	450	90	50	10	500	Mix
T4RI	425	85	75	15	500	Mix
T5RI	400	80	100	20	500	Mix
T6RI	375	75	125	25	500	Mix
T7RI	350	70	150	30	500	Mix

TRTS=treatments CH=Coffee Husk WB =wheat bran Rrk=Remark

Substrate Sterilization, Inoculation and Incubation

The prepared substrates were autoclaved at 121^0C temperature and 15lb pressure for 1hr. Then after, 10% of the *Pleurotus ostreatus* spawn was added. The open ends the bags were tied with rubber bands and some holes were made by using sterilized needle to allow air exchange of bags (Dawit, 2008). All inoculated bags were incubated at the room temperature and placed at 15cm apart in a completely randomized design in a clean and disinfected dark room.

Mushroom Production and Product Running

After fully colonization, the bags were transferred to the mushroom house, whose environment is kept through the improvised windows and a temperature about 28°C and humidity 75% to 85% were maintained by sprinkling the mushroom house with water twice a day.

Data Collection

Mycelium Colonization

The number of days of the mycelium fully colonized on the substrate after the days of spawn inoculation mycelial colonization was recorded by observing the change day to day until the substrate change to white color. The colonization was seen when the mycelia was formed throughout the substrate within the transparent bags.

Pin Head Formation

After the bags were slit open, the formation of primordia was observed every two days intervals and the number of days it took for first primordia formation was observed and recorded for all treatments.

Harvesting Date

After emerged primordia were changed to the matured fruits the date of maturation was recorded starting from first flush to the last flush and the mean was calculated.

Yield parameters

Number of fruit bodies was expressed directly by counting the number of fruit bodies on each substrate. The height of fruit bodies was measured in cm using transparent ruler from the base of the stipe to the cap. Diameter of the cap was also measured in cm with ruler from one edge of the cap across the stripe to the other edge or was measured from the tip to the tip by crossing the center of the cap.

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Total Fresh Weight and Biological Efficiency

The total weight of fruiting bodies harvested from the substrates from the first to the fourth flushes was collected as total yield of the mushroom and described by gram. Total fresh weight of fruit bodies harvested from various treatments was measured by using an electronic balance.

The fresh mushroom biological efficiency is directly related to nutritional composition of the substrate used for growing mushrooms as described in (Khan *et al*., 20012; Tsegaye, 2015). The biological efficiency was calculated as the following formulae (Frimpon-Manso *et al*., 2011).

$$\text{Biological efficiency} = \frac{\text{Weight of freish mushroom}}{\text{Weight of dry substrates}} \text{x}100\%$$

Data Analysis

The collected data was analyzed by (ANOVA) using SPSS version 20 software to analyses data through qualitative. Means collected data were considered for significant difference by using LSD at P≤5%.

RESULTS AND DISCUSION

Vegetative growth of oyster mushroom on coffee husk and wheat bran

Days for Mycelial Complete Colonization

The days taken for complete mycelial colonization after substrate inoculation for the different substrates composed from coffee husk supplemented with different proportion of wheat bran had significant differences at (P≤0.05) within the treatments. To compare the days taken for mycelium colonization within treatments, T3RI took the shortest incubation period (19) days to fully colonize the substrate while T7RI took the longest days (34) to complete mycelial colonization. The remaining treatments were intermediate between the shortest and the longest incubation period for complete mycelia colonization (Fig.1).

The mean of incubation period of mycelium fully colonization of this study was in line with report of Dagnew and Abel, (2018) that conducted on sawdust alone 19.87 days while sawdust + sugarcane bagasse took 17 to 19 days. The length of day taken to fully colonize the substrates for this study had similar incubation period with the result reported by Gan *et al*., (2011) that the mycelial fully colonization took 22.4 days to 26 days. The incubation period for mycelium extension of *P. ostreatus* of this study was not in line with the report of Baysal *et al*., (2003) that complete mycelium colonization took 15.8 days which is fast when compared with the data in this study.

Days of Primordial Formation

Days of primordial formation of oyster mushroom had significant differences at (p≤0.05) within the treatments. T3RI took the shortest (21.66) days after substrate inoculation while T7RI took the longest (37) days after substrate inoculation. The remaining treatments were intermediate between the fastest and slowest in primordial formation after substrate inoculation (Fig.1).

The length of days taken for primordial initiation for this experiment was in line with the length of days reported by Sharma *et al.*, (2013) for substrates of wheat straws, waste paper and rice straws taken 4days to 5.6 days 26.4 to 31.6 days from substrate inoculation to primordia formation. As the investigation of Zenebe *et al*., (2016) the interim period of pin-

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head formation varied with substrates ranging from 17 to 33 days after spawn seeding. Primordial formation occurred on cotton seed took 17days followed by sawdust 29 days while wheat straw took relatively longer incubation of 32.66 days.

Days of Maturation of Oyster Mushroom

Oyster mushroom first harvest was highly significant at ($p \leq 0.05$) within the treatments and the shortest days from inoculation to the first maturation was (28.33) days observed from treatment T3RI while T7RI took longer days from inoculation to first maturation (44.33) days. The remaining treatments were took intermediate days between the shortest and the longest days from substrate inoculation to the first harvest (Fig.1).

In this experiment, the recorded results were closely related with report of Sharmila *et al*., (2015) *P. ostreatus* cultivated on areca nut husk + topsoil initiated in 28 days, Bamboo shoot + topsoil took 30 days as well as areca palm leaves + topsoil that initiated after 34 days. The first maturation of *Pleurotus ostreatus* cultivated on coffee husk and wheat bran was in line with the days of first harvest that took 32.4 to 37.8 days Sharma *et al*., (2013).

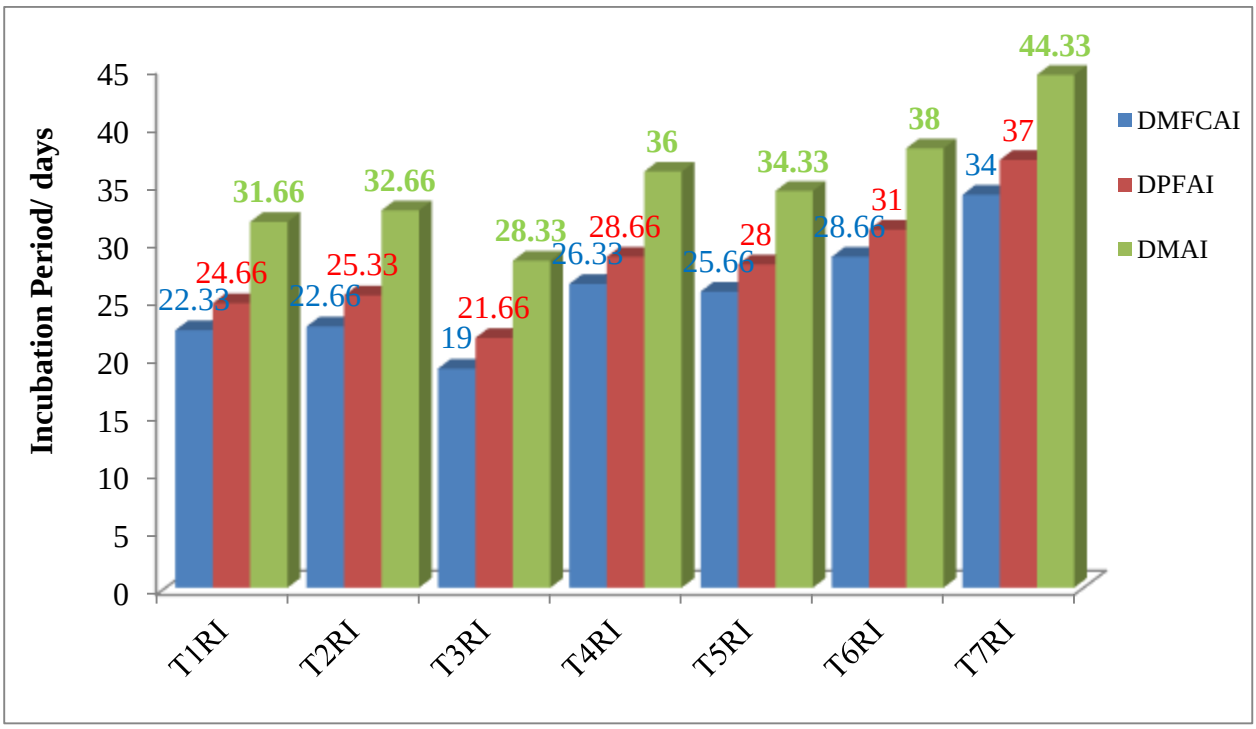

Figure 1: Days of Mycelium Fully Colonization, Primordia Formation and First Maturation

Number of Fruits, Bunches and Aborts

The effect of coffee husk supplemented with wheat bran on numbers of fruiting bodies, number of bunches and number of aborts had significance differences at ($p < 0.05$) within the treatments. The maximum number of fruits (52) was collected from treatment T3RI while small numbers of fruits were recorded from T7RI (30.66). On the other hand the maximum number of aborts (16.66) were recorded from treatment T6RI while the maximum number of bunches (4.66) were recorded from treatment T5RI. However, the minimum number of these parameters were recorded as abort (8.66) from T3RI and number bunches (3) from T7RI and

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all the remaining treatments showed intermediate maximum and minimum for these parameters (Table-2).

The result of this study was in line with the report of Asefa and Geda, (2014b) who reported number of aborting16-110, fruiting 22-72 and bunches 3-5. In other case, the result of this study was quite different from the report of Asefa, (2018) that the number of abort 60 to 91 while the number of bunches 7 to 15 of *P. ostreatus*. However, the mean number of fruiting bodies reported by Bhatti *et al*., (2007) showed similar consistency when compared with the fruiting bodies recorded in this study.

Table 2: Effects of Coffee Husk and Wheat Bran on Number of Fruits, Bunches and Aborts

Treatments	Growth parameters		
	NFB	**NB**	**NA**
T1RI	42.66^{d}	4.00^{c}	12.00^{h}
T2RI	45.66^{c}	3.33^{d}	13.66^{i}
T3RI	52.00^{a}	4.33^{b}	8.66^{f}
T4RI	41.00^{e}	4.00^{c}	12.00^{h}
T5RI	47.00^{b}	4.66^{a}	10.33^{g}
T6RI	38.66^{f}	3.33^{d}	16.66^{k}
T7RI	30.66^{g}	3.00^{e}	15.33^{j}
Mean	42.52	3.80	12.66
St Dev.	6.29	0.55	2.57
Sign.	*	*	*

NFB = number of fruiting bodies NA = number of aborts NB = number of bunches
*=Significant Mean values with in a column sharing the same superscript letter(s) are not significantly different by using LSD test at $P \leq 0.05$

Cap-Diameter and Stipe Length

Cap-diameter and stipe length of oyster mushroom had significant difference at ($p<0.05$). The highest mean cap-diameter and stipe length of oyster mushroom grown on coffee husk supplemented with wheat bran were recorded from T3RI(12.18cm) and (4.9cm) from T5RI while the smallest cap-diameter (6.03cm) measured from T7RI. According to the result of this study, the shortest stipe length was measured from T7RI and T6RI were (2.76cm) and (2.96cm) respectively. The remaining treatments were intermediate between the longest and shortest values (Fig.2).

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The longest and shortest means of cap-diameters and stipe length in this study was in line with the results reported by Khan *et al.*, (2017) in his study under substrates of wheat straws and cotton wastes 4.50cm to 17.12cm for cap-diameters were measured. As well as 2.50cm to 5.24cm of stipe length was reported by Islam and Riaz, (2017). On the other hand, the results of this parameters were not match with the results reported by Sharma *et al.*, (2013) from substrates composed of wheat straws, paper and rice 6.11cm-7.4cm and 2.25cm-3.08cm respectively. The differences may be due to the nutritional contents of substrates as Chukowry *et al.*, (2009) reported in his studies the longest and wider stipe and cap-diameters of mushrooms were depend on the substrates on which the mushroom can be cultivated Masevhe *et al.*, (2016).

Incubation Periods of Different Harvests

Mean incubation periods of mushroom flushes showed highly significant differences at ($P<0.05$) on the first flushes but, there is no significant difference ($p>0.05$) in the 2nd flushes. T3RI harvested in (28.33) days showed relatively shorter incubation period while T7RI took longer incubation to 1st flush in first and second flushes.

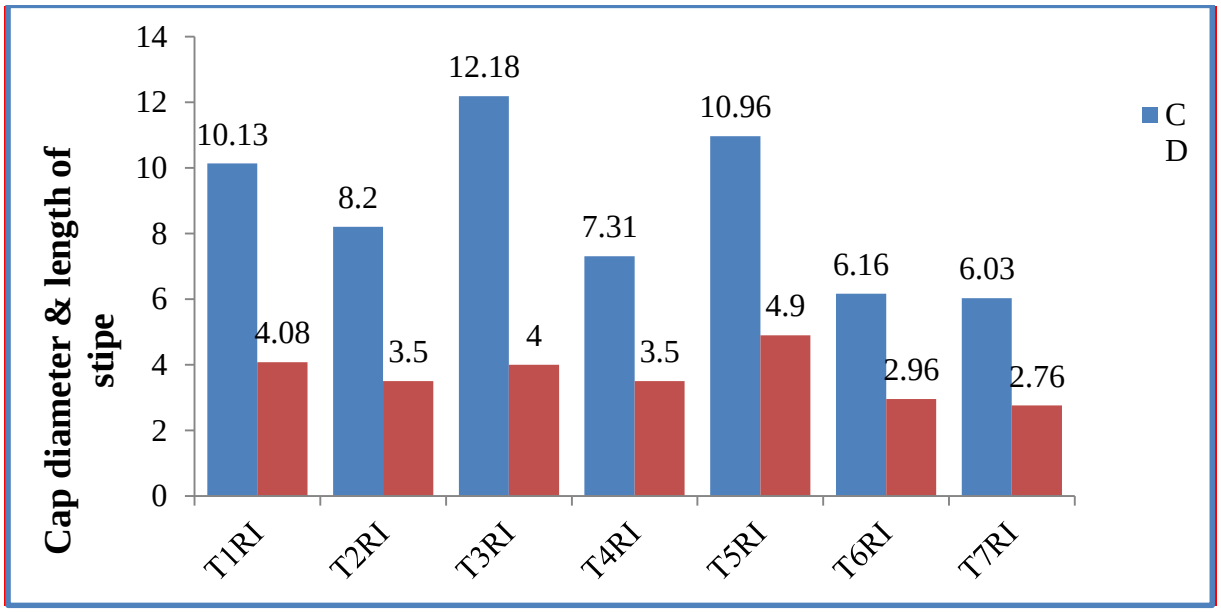

Figure 2: Effects of Coffee Husk and Wheat Bran on Cap-Diameter and Stipe Length of Oyster Mushrooms

All the rest treatments took incubation to 1st flush between the shortest and longest incubation periods. The incubation period taken from the 1st flush and the 2nd flushes took longer period were T7RI and T6RI that took (44.33) and (38) days respectively. The rest treatments took between the shortest and longest incubation period between each consecutive harvests in each treatments. Similar trends were observed for 2nd to 3rd and from 3rd to 4th flushes also there is no significant difference of incubation period between 3rd and 4th flushes The shortest production cycle was observed (65.33) days for treatment T3RI while the longest production cycle (85.66) days was recorded for treatment T7RI to complete the cycle of oyster mushroom production cultivated on coffee husk and wheat bran (Table-3).

The results of this experimental study considering incubation periods of different successive harvests from 1st to 4th flushes, the 2nd flush, 3rd flush and 4th flush were matched with the report

of Asefa and Geda, (2014b) that recorded from *Pleurotus ostreatus* cultivated on waste paper with supplement of wheat bran as 1st flush 40 to 55 days, 2nd flush 15-18 days, 3rd flush 13 to 16 days and 4th flush 12 to 13 days. Mekonnin and Semira, (2014) reported 17 days from cotton hulls which is more faster than the result of this study and 35 days for saw dust substrates which is closely related to the result of this study from substrate inoculation to the first flush However, the 1stincubation period from inoculation to first maturation was too long when compared with *Pleurotus ostreatus* grown on coffee husk supplement with wheat bran and also the length of days between consecutive harvests from 1st harvest to 4th harvests were not in line with report of Asefa, (2018). Considering the total days required to complete mushroom production cycle Asefa, (2018) reported that the production cycle from 100 days to111days is not in line with the incubation period to complete the cycle recorded from 65.33 to 85.66 days of this study.

Table 3: The Incubation Period (Days) for Flushes Harvested from Coffee Husk and Wheat Bran

Treatment	Inoculation to1st flush	1st to 2nd flush	2nd to 3rd flush	3rd to 4th flush	Total days
T1RII	31.66^{b}	17.66^{c}	14.33^{c}	13.00^{c}	76.33^{d}
T2RII	32.66^{c}	16.33^{b}	13.00^{a}	12.00^{a}	74.33^{b}
T3RII	28.33^{a}	15.00^{a}	13.00^{a}	12.00^{a}	65.33^{a}
T4RII	36.00^{e}	17.33^{c}	13.00^{a}	12.00^{a}	74.33^{b}
T5RII	34.33^{d}	16.66^{b}	14.00^{b}	12.00^{a}	75.00^{c}
T6RII	38.00^{f}	16.33^{b}	13.00^{a}	12.33^{b}	76.66^{e}
T7RII	44.33^{g}	18.00^{d}	13.33^{a}	12.00^{a}	85.66^{f}
Mean	35.04	16.75	13.38	12.19	75.37
St Dev.	4.76	0.93	0.51	0.34	5.49
Sign	*	ns	ns	ns	*

CH= Coffee husk WB= Wheat bran *=significant ns= Not significant Mean values with in a column sharing the same superscript letter(s) are not significantly different by using LSD test at P≤0.05.

Yield of Mushroom per Flushes

The flush weight of oyster mushroom grown on coffee husk and wheat bran under 1st, 2nd ,3rd and 4th flush had highly significant at ($p<0.05$) within the treatments. The maximum and minimum fresh weight of oyster mushroom under the 1st flush was (393g) and (354g) which were collected from T3RI and T7RI respectively. However, the highest and the lowest flush weight of oyster mushroom picked at the 2nd flush were (203.66g) and (151.33g) which were collected from T3RI and T7RI respectively. Regarding the 3rd flush, the maximum and minimum fresh weight of oyster mushroom was (116.33g) and (87.33g) which were harvested from T3RI and T7RI respectively. In case of the 4th flush, the maximum and minimum fresh weights of oyster mushroom picked were (86.67g) and (57.33g) which were collected from T3RIand T6RI respectively (Fig-3).

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Kimenju *et al*., (2009) reported that yields of mushroom in different substrates slightly declined from the first flush to the successive harvests. The crops of oyster mushroom harvested in four flushes and the maximum yield was obtained in the 1st flush than the 2nd, 3rd and 4th flushes and is related with this study. As a report of Dagnew and Abel, (2018) the mean weight of flush harvested oyster mushrooms were harvested for three consecutive flushes were decreased in consecutive harvests from 1st flush to 3rd flush and it is in line with this study.

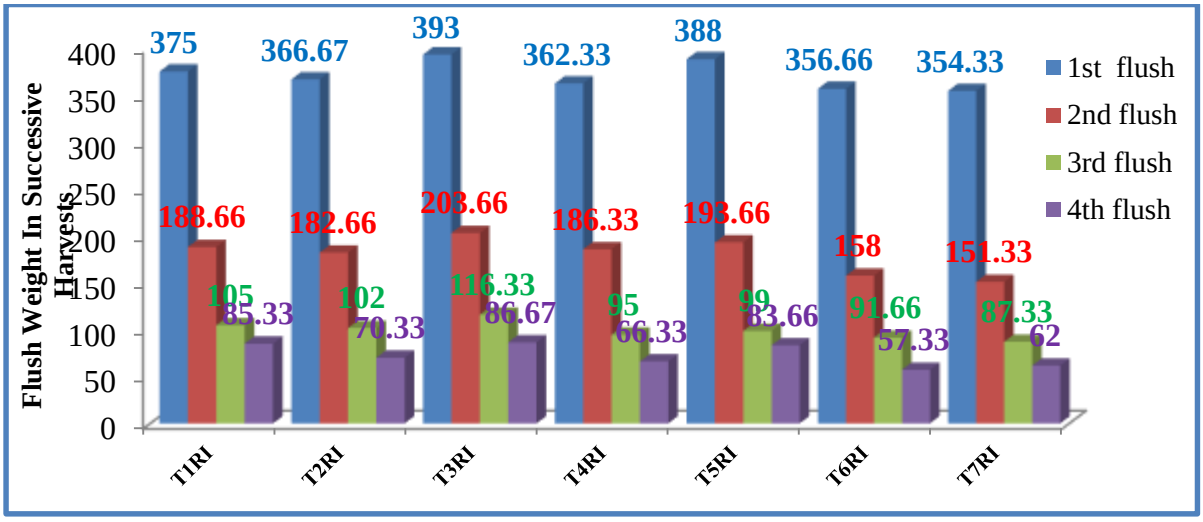

Figure 3: Flush Weight from 1st Flush to the 4th Flush Harvested from Coffee Husk and Wheat Bran

Total Fresh Weight

Total fresh weight of the oyster mushroom had significant differences at (P<0.05) within the treatments. The maximum total fresh weight of oyster mushroom grown on coffee husk and wheat bran by different mix ratio was (806.33g/500g) of dry substrate that was harvested from T3RI while the minimum total fresh weight was (679.66g/500g) collected from T7RI (Fig.4).

The total fresh weight was in line within the results reported by Sharm *et al., (*2013) which is found in the range of total biomass recorded in this study. The results verified in this study from coffee husk supplemented with wheat bran was 679g to 806g/500g was closely related with the report of Lakew and Asefa, (2016) that the highest total wet/fresh weight of mature mushroom recorded was 795g.

Biological Efficiency

The mean of biological efficiency of oyster mushroom grown on coffee husk and wheat bran was highly significant at (P<0.05) within the treatments. The maximum mean biological efficiency of oyster mushroom was (161.26%) T3RI and the minimum value of biological efficiency was (135.93%) recorded from treatment T7RI (Fig.5).

Considering biological efficiency, the report of Islam *et al*., (2017) 162.5% was closely related with the biological efficiency ranged from 135.93% to 161.26% of this study. On the other hand Takele *et al*., (2018) reported that, biological efficiency 83.62%, 72.8 to 87.5, 62.6 to 70% and 63.4 to 63.8% from different substrates were not in line with the results of this study. This difference may be due to substrate quality, substrate ratio of experimental design,

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cultivation season, low humidity and temperature those factors should be arranged and optimized for quality of mushroom cultivation and its yields.

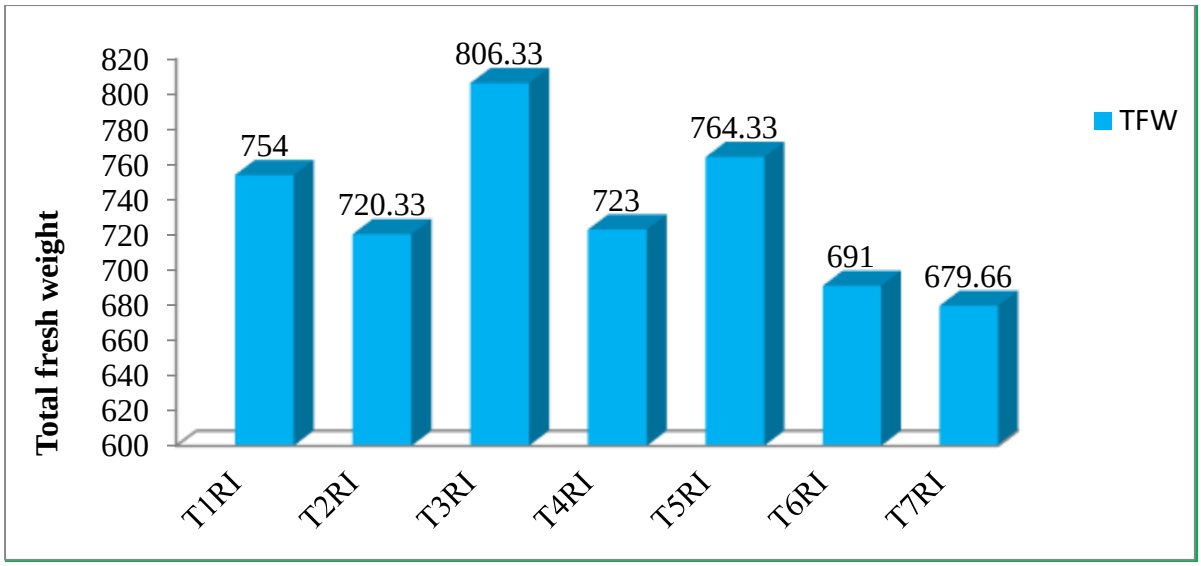

Figure 4: Total Fresh Weight of Oyster Mushroom Harvested From Coffee Husk and Wheat Bran

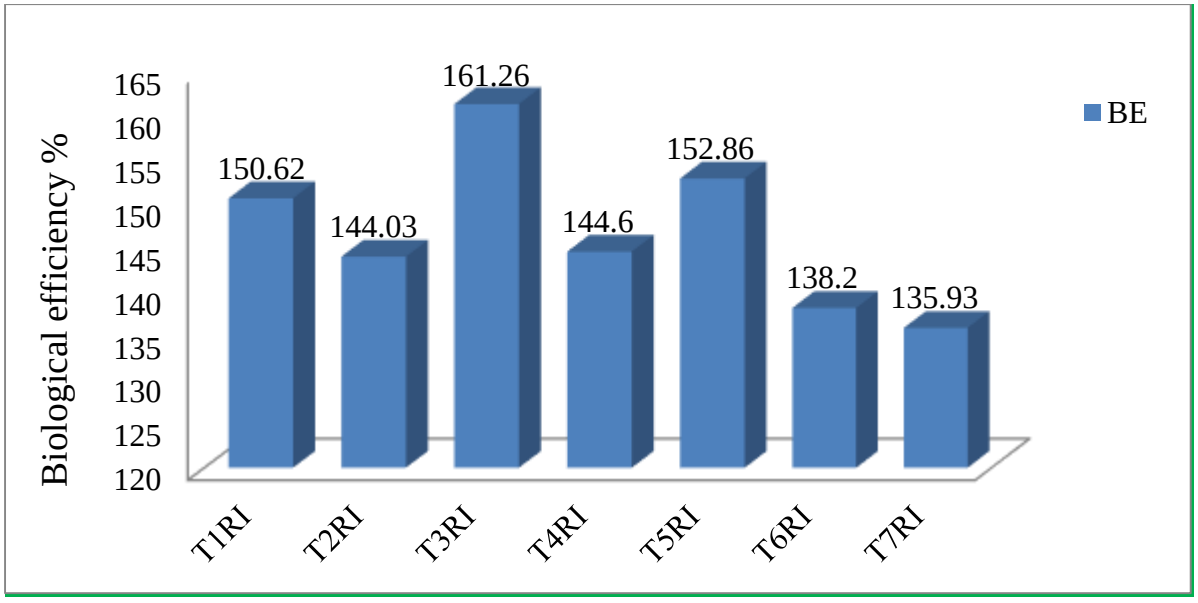

Figure 5: Effects of Coffee Husk and Wheat Bran on Biological Efficiency of Oyster Mushroom

Conclusion

Oyster mushroom has been well recognized for its easy adaptability to various substrate types and to a wide range of environmental conditions as compared to other mushroom varieties. Huge agricultural and agro processing by products were richly available particularly in the rural and semi urban areas of the Ethiopia which in some cases burned to minimizes it accumulation on the field and in the processes of preparing the plot for the next cycle of crop production.

In this experiment, converting coffee husk alone or together with different proportion of wheat bran resulted in highest growth, yield, yield related parameters and biological efficiency of the oyster mushroom. Form all the substrates and substrate composition tested in this investigation T3RI, coffee husk, wheat bran in the ratio of 90:10 gave maximum in all the parameter evaluated; as a result this composition of substrate mix ratio need to be evaluated for farm, pilot and large scale production of oyster mushroom in the dry coffee processing areas.

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