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Keywords: *oyster mushroom, substrates, yield and nutritional value.*

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Abstract- Mushrooms are important fungi that play a great role for food self-sufficiency and to fill the gap of protein deficiency in most cereal crops, vegetables and fruits. Moreover, they are known for their flavor enhancing property, medicinal value a well as cholesterol free food. However, Mushroom production in Ethiopia is still less known due to multiple problems which include high input cost, lack of quality of spawn, lack of proper production skill, and lack of information on substrates. In view of this, the experiment was conducted at Arsi University, College of Agriculture and Environmental Science during 2013/14 Cropping years with the objectives of evaluating different organic substrates and pasteurized methods on Pleurotus ostreatus yield, biological efficiencies and nutritional values. Factorial combination of seven substrates (Dried faba bean stalk, Maize Stalk, Wheat straw, Teff straw, Eucalyptus Sawdust, Wheat straw + Teff straw+ Faba bean stalk and Saw dust + Faba bean stalk) and two pasteurization methods (Cold water treatment and Hot water) were used. The experiment was arranged in RCBD with three replications by using the box constructed from the wood timber as blocks in cropping room. Carbon content, nitrogen content and water holding capacity of the substrates were analyzed at spawning and harvest of mushrooms and significant difference among the substrates and pasteurization methods (Hot and cold water) were observed. Phenological data including days to onset of mycelium invasion, days to appearance of pin heads and days to maturation of fruiting bodies of mushrooms and cap diameter and different nutritional analysis of mushroom for crude protein, organic carbon, organic matter and carbohydrates contents were analyzed and recorded and the significant difference among the substrates have been observed. Non-significance difference between pasteurization methods was observed on pin head formation, fruiting body formation, cup diameter, biological efficiency, Production rate, moisture content and dry matter content. To give general recommendation from this research for the producers, the highest yield of oyster mush room (1.58kg/4 kg of substrate) was recorded from maize stalk treated with hot water and the lowest was recorded from saw dust treated with hot water (0.50 kg/4 kg of substrate). The highest protein content was recorded from faba bean stalk Pasterualized with both cold and hot water (27.38%) and the lowest was recorded in case of wheat straw treated with hot water (17.03%).

Keywords: oyster mushroom, substrates, yield and nutritional value.

I. Background and Justification

Today as the challenge on world, the 20th century began with a world population of 1.6 billion and ended with six billion, and projected to be nearly nine billion by 2050, with most of the growth occurring in the developing country [1]. According to Population Institute of Ethiopia (2008), the Ethiopian population is 82.8 million and projected to be 149.5 million by mid of 2050. However, land in agricultural use has increased by only 11% from the early late seventeenth to the late twentieth century, meaning the land per capital declined by about 40%. Therefore, in the near future it will become increasingly difficult to produce the food required to feed the ever-growing human population [2]. Ethiopia is among few countries that failed to participate in the general thrust towards increasing average food consumption level. In 2010/2011 production season total crop production in Ethiopia was about 22.5 million tones on 13.5 million ha of land [3], making Ethiopia agriculture one of the least productive all over the world with productivity of less than 2t/ha. The food deficit of the under nourished Ethiopia people is 340 K cal/person/day and the share of cereals, root and tubers in total dietary energy supply is 79%. This problem is further aggravated by recurrent droughts [4].

Food grains are the principal dietary source of calories, proteins and other nutrients but their dietary supply is not sufficient and proteins supply is not in the required amount. Besides, economic and health factors make animal protein inaccessible to the majority of the people [5]. Developing countries like Ethiopia are caught in vicious circle of poverty, shortage of food and nutritional disorder. Erratic rainfall, shortage of land, low yield of traditional crops and low status of most crops

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especially in protein are among the causes of the three mentioned problems above. To tackle this problem, the Ethiopia government has been engaged in improving the agriculture sector through different initiatives. One of them being transforming the sector from subsistence to commercially oriented one by encouraging the production of high value diversified native and exotic crops.

[6] Reported productivity of fresh mushroom per kg of dry substrate was 0.8 to 1 kg. Dawit reported 7.3 kg of fresh Oyster mushroom produced from 10 kg dry cotton waste supplemented with 1% wheat bran. [7] Also grew Oyster mushroom on paddy straw, maize and sugarcane reported yield of 0.28 to 0.39 kg per kg of dry substrate.

Cultivation of oyster mushroom represents one of the major current economically profitable biotechnological process for the conversion of plant residues or a non-edible plant biomass in to nutritious / protein rich food, which will help in overcoming protein malnutrition problem in developing countries [8]. Time is, therefore ripe for developing new technologies for commodities like mushroom which increase production and productivity without using arable land. Such have great role to pay in achieving food self-sufficiency. It has wide range of substrate growth and temperature adaption. Furthermore, it is fast growing, requires no casing, less fragile than others and has market in the dry form as well as fresh form.

Mushrooms are highly perishable mostly due to moisture loss, wilting, color changes and poor preservative methods. Improving the shelf life and quality characteristics of mushroom will enhance marketability and add value in agricultural chain. Evolving preservation methods that are cheap and affordable by farmers has become imperative in order to have food sustainability [9]. The food processing industry provides a wide range of canned and processed mushroom products, including frozen, sterilized, dried, pickled, marinated, and salted mushrooms; mushroom powder; paste; and concentrates and extracts [10].

Mushrooms are a cash crop, the market for which is growing worldwide and in Ethiopia it is a new activity. In most countries, there is a well-established consumer acceptance for cultivated mushrooms. Even though, the first small research project on Oyster mushroom (Pleurotus ostreatus) was started in 1993 at Addis Ababa University Ethiopia, the mushroom production in Ethiopia is still less known due to many factors which include high in put cost, lack of quality spawn, lack of proper production skill and lack of information on substrate for mushroom cultivation.

Almost all producers use cotton hull as substrate, which not available for most farmers and comparatively too costly compared to other agricultural wastes. So, identification of different substrates from different types of crops and method of pasteurization is the critical for successful mushroom cultivation.

II. Objectives

1. To evaluate different Organic residues (substrates) and their combinations for Oyster Mush room cultivation in terms of yield, biological efficiency and nutritional values.
2. To assess the possibility of using different pasteurization methods of substrates on growth performance of oyster mushroom, yield and nutritional value.

III. Materials and Methods

a) Experimental Site and Cropping House Construction

The experiment was conducted at Arsi University, College of Agriculture and Environmental Science. Mushroom-growing house with a length (12 m), height (4m) and width (2.5m) has been constructed from locally available material, the wall lined with bamboo tree and wooden flat, the roof covered by iron sheet and the floor was cemented. The growing media/box with a length 70cm and width 50cm was constructed from wooden flat.

b) Experimental materials

The mushroom spawn produced from pure cultures of Pleurotus ostreatus (sajor-caju) has been collected from Ethio-mushroom and Spawn Enterprise Company.

c) Treatments and Experimental Design

The experimental treatments have been including Seven organic substrates (Sawdust, Faba bean stalk, Maize stalk, Wheat straw, Teff straw, Wheat straw + Teff straw and Saw dust + Faba bean stalk) and two Pasteurization methods (Cold treatment and Hot water treatments). Fourteen treatments have been laid out in a randomized complete design (RCBD), in a complete factorial arrangement with three replications.

d) Preparation of the Substrates for Seeding

All substrates were dried before shredding and the dried bean stalk, maize stalk, wheat straw, teff straw and saw dust were shredded or chopped into small pieces of approximately pieces of 2-4 cm size with axes. All naturally dried substrates were subjected to three days sun drying after shredded as correction factor to balance the natural difference in percentage water holding capacity.

The prepared required amount of each substrate has been subjected to different treatments as followed. For cold water treatment, the substrates used in this study were separately soaked in fresh tap water for 24 hrs using 100 liter aluminum cans and achieved sufficient imbibitions. Similarly, pasteurization of shredded substrates has been done by soaking in hot water at 70°C for one hour using aluminum cans.

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The Pasteurized substrates separately kept on wire mesh tables and allowed to drain for 4 hours. The water content of the substrates was checked by squeezing in the palm and substrates were used for spawning when the water not dripped on squeezing.

e) Seeding of Substrates and Spawn Running

The pasteurized substrates were weighted in to small portion each weighing 4Kg dry weight basis and then filled into 50x70cm^2 box cemented by plastic sheet. The boxes arranged in a completely randomized block design with three replications keeping 5-10cm spacing between each box. Each box was inoculated with 50g of Spawn of mushroom strain and closed with plastic sheet to prevent possibility of contamination. The inoculated boxes were placed in spawn running room (part of the mush room house from which light excluded) until the substrate in each box fully colonized by mycelium. Mycelium invasion in the boxes visually followed in all substrates. The box has been opened and moved to the fruiting room (Mush room house fitted with fluorescent light) and allowed the development of fruiting bodies.

f) Mushroom Crop Management and Harvesting

The bags or substrates blocks were opened by cutting the polythene bags from sides and the plastic was removed. The exposed substrate blocks are kept on their original position as before. The substrate blocks was sprayed twice a day on the bags with water in order to maintain the relative humidity in the range of 80-85% during Cropping period till pin heading and subsequent fruiting when the condition was not fulfilled. Temperature of the cropping rooms was maintained at about above 15^{o}C and monitored by using a thermometer and also sufficient ventilation was provided during fructification to keep low CO_2 level. The fruiting bodies were harvested when the cap has opened up, the gills of the mushroom were visible and when the edge of the cap was slightly rolled inwards.

g) Analysis of substrates

All the substrates were analyzed for organic carbon, percentage nitrogen and water holding capacity before spawning and after harvesting of mushroom. Water holding capacity of the substrates was determined by soaking a weight amount of cold water and hot water treated substrates for 24 hrs. The moisture content was determined by weighing the pressed substrate before drying and also after drying in an oven at 70^{o}C to constant weight. The percent moisture content of substrate was determined as given below [11].

Percent water holding capacity = (wet weight - dry weight/wet weight of substrate) X 100

Organic carbon was determined using Walkley and Black Method (Walkley and Black, 1934). In this method after adding different chemicals on ground sample it was tittered by H_2SO_4 and the organic carbon was calculated by taking the volume difference. Percent nitrogen in tissue was determined using micro Kjeldahl method [12]. In this method a ground sample was digested, distilled and tittered and nitrogen was determined by taking the volume difference during titration.

h) Data collected

i. *Phenological observations*

Days to the completion of invasion of mycelium (MI) on different substrates, appearance of pin heads formation (PHF), and days to maturation of fruiting bodies formation (FBF) and Cap/pileups diameter from the day of spawning in different substrates were recorded.

ii. *Yield and yield related traits*

a. *Total yield*

Weight of fresh fruiting bodies was harvested from each substrate blocks and measured using sensitive balance. Data on weight of mushrooms from each substrate blocks at first, second and third flush harvesting stages were recorded separately and their total weight was considered as total yield which was also used for calculating biological efficiency.

b. *Biological efficiency*

The weight of each dry substrate and total fresh mushroom weight per box was recorded separately and then the biological efficiency (BE) of oyster mushrooms in each substrate was calculated as bellow:

$$BE = \frac{\text{Weight of fresh mushrooms}}{\text{Weight of dry substrate}} X .100$$

c. *Production rate*

On the basis of biological efficiency on each substrate and the time taken in days from spawning to harvesting, the production rate (PR) of oyster mushrooms in each substrate was calculated as below: PR = BE / Time

i) Nutritional Analysis of Mushroom

i. *Crude protein*

Ground and dried samples of fruiting bodies of oyster mushroom was analyzed for crude protein (CP) using Kjeldahl method. In this method a sample was digested, distilled and tittered and nitrogen was calculated by taking the volume difference during titration. In order to determine protein, percentage nitrogen was multiplied by 6.25.

ii. *Total ash and Organic matter*

Total ash (TA) content was estimated by heating 2gm of ground mushroom sample at 550^{0}C for 5 hrs. At this temperature all the organic matter burns off as CO_2

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and the remaining matter was inorganic ash. The ash content was measured according to following formula:

% Total ash = (W3-W1 / W2-W1) x 100

Where, W1 = Weight of crucible

W2 = Weight of crucible + Sample

W3 = Weight of crucible + Ash

Based on percentage total ash, percent organic matter will be calculated by subtracting each value from 100.

iii. *Percentage moisture content and dry matter*

The moisture content of the fruiting bodies was determined as loss in weight which resulted from drying fresh mushroom samples at 70°C to constant weight and this was calculated using the following formula:-

$$\text{Percentage moisture content of fruiting body} = \frac{\text{Fresh weight} - \text{Oven dry weight}}{\text{Fresh weight}} \times 100$$

Based on moisture percentage, the **dry matter percentage** was calculated by subtracting each value from 100.

j) Statistical Data Analysis

All the data collected during the study were subjected to analysis of variance (ANOVA) using SAS statistical software. LSD was used to separate the treatment means at five percent probability level and Simple correlation between the treatments was computed.

IV. Results and Discussion

a) Results

The present study was conducted to evaluate the effect of different organic substrates and their pasteurization methods on yield and quality of oyster mushroom. Seven substrates (sawdust, dried faba beanstalk, maize stalk, wheat straw, tef straw, the combination of Saw dust + faba bean stalk and wheat straw+ tef straw+ faba bean stalk) and treated with hot water and cold water as check were evaluated using RCBD design in a factorial combination with three replications.

Carbon and nitrogen content and water holding capacity of the substrates were recorded before and after harvesting of mushrooms. Phenological observations like days to complete mycelium invasion, appearance of pin head and days to maturation of fruiting bodies of mushrooms were recorded. Yield and yield related attributes such as biological efficiency and production rate and quality attributes of mushroom such as percentage moisture content, crude protein (CP) and percent total ash and organic carbon were also estimated.

Table 1 : Effect of substrate type and pasteurization methods on Water holding capacity of substrates at spawning

	Moisture (%) at spawning						
	Substrates						
Pasteurization	Saw Dust	Fababean Stalk	Maize Stalk	Wheat Straw	Teff Straw	WTF Straw	Saw Dust & Fababean
Cold water	1.50n	34.20d	22.94h	16.26i	10.62k	30.78f	7.25l
Hot water	6.20m	32.77e	54.87a	37.17c	23.85g	39.88b	15.88j
LSD(0.05)				0.32			
CV (%)				0.82			

Means with in column followed by the same letter are not significantly different at 5% probability level

Table 2 : Effect of substrate type and pasteurization methods on water holding capacity of substrates at harvest

	Moisture(%) at harvest						
	Substrates						
Pasteurization	Saw dust	Fababean Stalk	Maize Stalk	Wheat straw	Teff straw	WTF straw	Saw dust& Faba bean
Cold water	6.50n	39.13d	27.98h	21.26i	15.62k	35.78f	12.25l
Hot water	11.20m	37.57e	59.96a	42.11c	28.85g	45.00b	20.96j
LSD(0.05)				0.28			
CV(%)				0.52			

Means with in column followed by the same letter are not significantly different at 5% probability level

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Table 3 : Carbon and nitrogen content of substrates at spawning and harvest

Substrates	OC(%) at Spawning	OC(%) at harvest	N(%) at Spawning	N(%) at harvest	C:N at spawning	C:N at harvest
Saw Dust	74.97a	73.32a	0.08g	0.07f	937.18a	1024.98a
Fababean Stalk	44.85g	43.41f	1.32a	1.02a	33.93f	42.29e
Maize Stalk	50.11c	48.71c	0.65d	0.60c	77.14d	82.02d
Wheat straw	46.13d	44.64d	0.31f	0.27e	147.5b	165.54b
Teff straw	45.37f	43.98e	0.44e	0.39d	103.05c	113.89c
WTF straw	45.89e	44.44d	0.85b	0.74b	60.06e	67.23d
Saw & Faba.	59.75b	58.40b	0.80c	0.73b	74.73d	79.12d
LSD(0.05)	0.108	0.31	0.01	0.03	2.7	24.46
CV(%)	0.17	0.51	1.42	4.69	1.11	9.16

Means with in column followed by the same letter are not significantly different at 5% probability level. WTF straw =Wheat +Teff + Faba bean straw, Saw & Faba = Saw + Faba bean stalk.

Table 4 : Effect of substrate type and pasteurization methods on Growth, BE and PR of oyster mushroom

Substrates	MI(days)	PHF(days)	FBF(days)	CD(cm)	BE(%)	PR
Saw Dust	12.50cd	55.00b	68.66b	2.50b	36.10cd	0.55cd
Fababean Stalk	16.50a	56.66b	66.83bc	7.16a	51.94bcd	0.84bcd
Maize Stalk	12.00d	45.00b	54.83c	7.00a	70.87ab	1.40a
Wheat straw	16.50a	72.83a	84.83a	6.63a	64.70ab	0.79bcd
Teff straw	12.50cd	52.50b	62.50bc	6.66a	32.44d	0.51d
WTF straw	14.00b	54.33b	65.50bc	8.50a	74.59a	1.16ab
Saw & Fab.	13.00c	54.16b	64.00bc	7.16a	53.72bc	0.93bc
LSD(0.05)	0.74	11.92	13.16	3.03	20.31	0.41
Pasteurization methods						
Hot water	13.57b	53.33	64.28	7.03	55.77	0.91
Cold water	14.14a	58.23	69.19	6.00	54.05	0.85
LSD(0.05)	0.39	NS	NS	NS	NS	NS
CV(%)	4.53	18.01	16.62	39.20	31.17	39.03

Means with in column followed by the same letter are not significantly different at 5% probability level;

NS = Non- significant, Biological efficiency, Production rate, WTF = Wheat + Teff + Fababean, Mycelium invasion, Pin head formation, fruiting body formation ,cup diameter,

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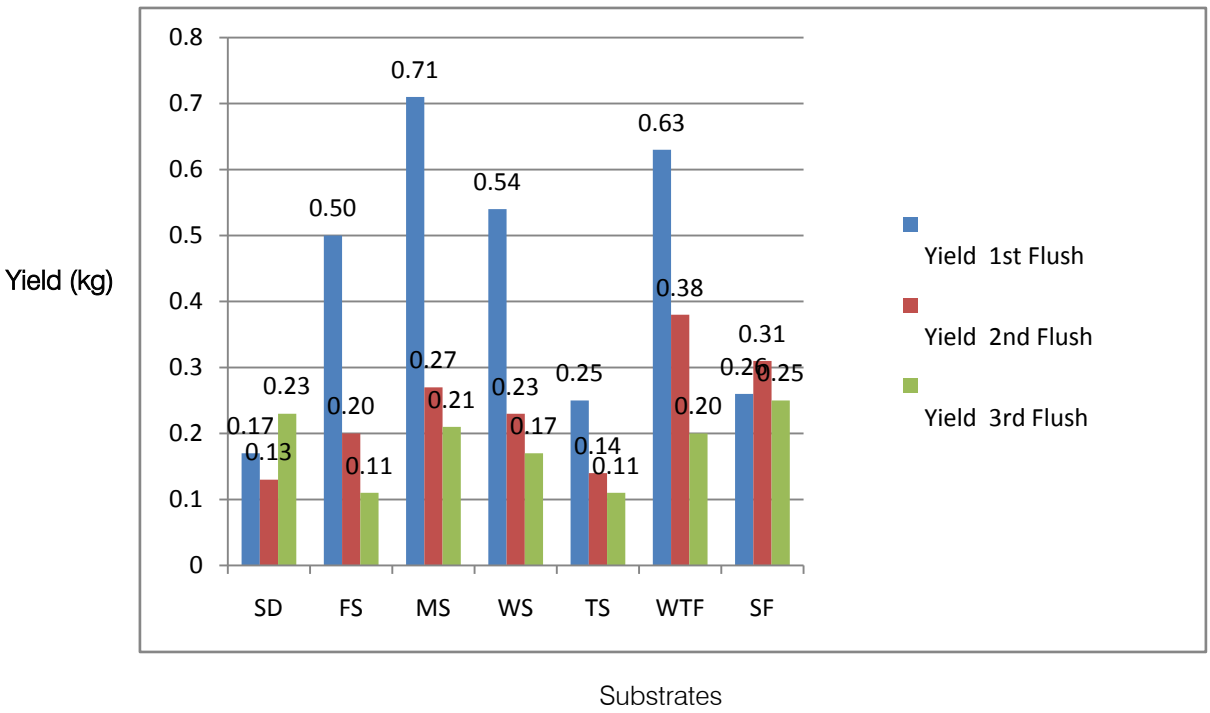

Fig.1 : Yield of Oyster mushrooms at different flushes

(SD= saw dust, FS= faba bean stalk, MS= maize stalk, WS= wheat straw, TS =tef straw, WTF= wheat + teff + faba bean, SF = saw dust + faba bean stalk; the unit of first, second and third flushes are in kg/ 4 kg dry substrate.)

Table 5 : Effect of substrate type and pasteurization methods on Total yield of oyster mushroom

	Total Yield (Kg)						
	Substrates						
Pasteurization Methods	Saw Dust	Faba bean Stalk	Maize Stalk	Wheat straw	Teff straw	WTF straw	SD+ FB
Cold water	0.58l	0.66j	0.83g	1.23c	0.35n	1.40b	0.86f
Hot water	0.50m	0.96e	1.58a	0.67i	0.62k	1.03d	0.80h
LSD(0.05)				0.01			
CV(%)				3.02			

Means with in row and column followed by the same letter are not significantly different at 5% probability level; WTF straw= Wheat + Teff + Faba bean stalk, SD+ FB+= Saw dust +Faba bean

Table 6 : Effect of Substrate type and Pasteurization methods on Protein content of oyster mushroom

	Protein Content (%)						
	Substrates						
Pasteurization methods	Saw Dust	Faba bean Stalk	Maize Stalk	Wheat straw	Teff straw	WTF straw	Saw & Fab.
Cold water	18.56h	27.38a	24.34c	18.03j	18.37i	27.37a	23.00e
Hot water	21.56f	27.38a	20.69g	17.03k	20.69g	26.47b	24.19d
LSD(0.05)				0.05			
CV(%)				0.45			

Means with in column followed by the same letter are not significantly different at 5% probability level

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Table 7 : Effect of Substrate type and Pasteurization methods on some quality of oyster mushroom

Substrates	Total Ash(%)	Organic matter(%)	Moisture Cont.(%)	Dry matter cont.(%)
Saw Dust	8.40d	91.59b	85.46ab	14.53bc
Fababean Stalk	8.00d	91.99b	86.09ab	13.74bc
Maize Stalk	7.25e	92.74a	90.34a	8.99c
Wheat straw	11.08a	88.91e	88.27a	11.72bc
Teff straw	10.51b	89.48d	78.41cb	19.91bc
WTF straw	9.94c	90.06c	86.52ab	13.47bc
Saw &Fab.	8.45d	91.54b	73.73c	26.26a
LSD(0.05)	0.49	0.49	8.72	8.26
Pasteurization methods				
Hot water	9.32a	90.67b	84.86	14.61
Cold water	8.86b	91.13a	83.37	16.43
LSD(0.05)	0.26	0.26	NS	NS
CV(%)	4.58	0.45	8.74	44.87

Means with in column followed by the same letter are not significantly different at 5% probability level

V. Discussion

a) Substrate Characteristics

i. *Water holding capacity*

Results of interaction effect of different substrates in terms of water holding capacity showed that the mean highest and the lowest water holding capacity of the substrates at spawning was for maize stalk (54.87%) and saw dust (1.5%) when treated with hot and cold water, respectively which were significantly different from other treatments (Table 1). Similarly, significantly highest and lowest water holding capacity at harvest was also observed for maize stalk treated with hot water (59.96%) and saw dust treated with cold water (6.5%). This could be due to the soft tissue of maize stalk and woody nature of saw dust, respectively (Table 2).

All substrates were significantly different from one another in terms of water holding capacity both at spawning and harvest. The water holding capacity of all substrates increased after growing mushroom which could be due to the frequent watering of substrates and loosening effect on tissue (Table1 and Table 2).

The main effects of treatment methods on water holding capacity of substrates indicated that substrates treated with hot water showed significantly more moisture at spawning and harvesting as compared to cold water except in the case of fababean stalk, it was the reverse. This could be due to the ability of hot water to make the tissues of substrates more soft in order to observe more water and due to the decay of substrate by unwanted fungi on substrates treated with cold water (Table 1 and Table 2).

b) Chemical composition of the substrates

i. *Carbon content of the substrates*

The results of carbon content of the substrates measured before spawning and after harvest are presented in Table 3. Maximum carbon was recorded for saw dust at spawning (74.97%) and after harvesting (73.32%) which was significantly higher than all other treatments. Similar results for saw dust were reported [13]. The lowest carbon content at spawning (44.85%) and after harvesting (43.41%) was observed for faba bean stalk. In all organic substrates a slight reduction of organic carbon was observed from spawning to harvest which may be due to the utilization of carbon by the growing mushroom. Cold water treatment and hot water treatment did not significantly influence the carbon content of the substrates.

ii. *Nitrogen content of the substrates*

Results of measurement of nitrogen in different substrates showed maximum nitrogen content was recorded for the faba bean stalk at spawning (1.32%) and harvest (1.02) which was significantly higher than nitrogen content of all other substrates (Table 3). This result is in line with [14] who reported the nitrogen content of leguminous substrates was higher than that of non-leguminous substrates. In contrast to the nitrogen content of dried faba bean stalk, the significantly lower nitrogen content was recorded for saw dust both at spawning (0.08%) and harvest (0.07%). All substrates are significantly different from each other in terms of their nitrogen contents at spawning. Significant difference was not observed between the combination of wheat + teff straw + fababean stalk and saw + faba bean stalk in terms of nitrogen content at harvesting. In all organic substrates a reduction in

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nitrogen content was recorded from spawning to harvesting of mushrooms which may be attributed to the utilization of nitrogen by the growing mushroom. Because of high carbon content and low nitrogen, C:N ratio for saw dust was found to be widest and that of dried faba bean stalk was narrowest both at spawning and harvest (Table 3). These results were in line with Richard who reported a C:N ratio of 600:1 and 16:1 for sawdust and dried bean pod husk, respectively. Significant difference was not observed between maize stalk and saw dust + faba bean in terms of C:N ratio at spawning and between wheat +teff + faba bean and Saw dust + faba bean at harvest statically. In all organic substrates a significant increase in C:N ratio was recorded from spawning to harvesting.

c) *Effect of Substrates and Pasteurization Methods on Growth and Yield*

i. *Mycelium invasion*

The fastest days to mycelium invasion (MI) of different organic substrates was recorded for maize stalk (12.00 days) however it was statistically at par with saw dust and bean straw (Table 4). Faba bean stalk and wheat straw were found to be invaded by inoculated mushroom spawn at the slowest rate (16.50 days).

Hot water treated substrates had significantly earlier invasion by mycelium than cold water. This could be due to the release of easily available nutrients from substrates and the reduction of unwanted green mould by effective hot water pasteurization. The pasteurization methods recorded significant differences with regard to days to mycelium invasion (Table 4). Interaction of substrates with pasteurization methods was non-significant for days to mycelium invasion.

ii. *Pin head formation*

With respect to the effect of different substrates on pin head formation, the slowest pin head formation (PHF) after spawning was observed in wheat straw (72.83 days). Significant difference was not observed among other substrates statically but Maize stalk recorded early pin head formation compared to others.

Significant difference was not observed between cold water and Hot water methods with regard to pin head formation statically (Table 4). But Hot water treated substrates had significantly earlier pin-head formation than cold water. This could be due to effect of hot water on chemical composition of substrates which may have increased availability of essential nutrients to the growing mushroom. There is no interaction effect between different substrates and pasteurization methods on pin head formation of the mushroom.

iii. *Fruiting body formation*

There was olso no interaction effect between the substrates and pasteurization methods on fruiting body formation of the mushroom. The fastest fruiting body formation (FBF) after spawning was observed on maize stalk (54.83 days) followed by teff straw however; teff straw was statistically at par with the other substrates except wheat straw. The slowest fruiting body formation was recorded on wheat straw (84.83 days). No significance difference was observed between Hot water and cold water treated substrates statically but numerically when it was observed Hot water treated substrates had significantly earlier ($p<0.05$) fruiting body formation than cold water (Table 4). This could be due to the effective pasteurization and increase in availability of nutrients due to hot water treatment of substrates.

iv. *Yield of oyster mushroom*

Harvesting of the mushrooms growing in different substrates was completed in three consecutive harvesting operations till all the mushrooms were harvested from each substrate. The yield of mushrooms in each flush and the total yield for each substrate are presented in Fig.1. The highest yield of mushrooms in first flush was obtained in maize stalk (0.71kg), in second flush was obtained in, Wheat + Teff + Fababean stalk (0.38 kg) and in third flush was obtained in saw dust + faba bean stalk (0.25kg). The lowest yield during the first and second flush was obtained for mushroom grown on saw dust 0.17 and 0.13 kg respectively, whereas the lowest yield in third flush was obtained for mushroom grown on faba bean stalk (0.11kg). In all cases yield showed a declining trend from the first flush to third flush. This could be due to the decreasing nutrient content of the substrate consumed by mushroom during growth from one flush stage to the next. But in the case of saw dust and saw dust + fababean stalk the highest yield was obtained in third and second flush respectively. This could be due to the hardness and less consumable of saw dust in the first flush.

v. *Total yield, biological efficiency and production rate*

Significant ($p<0.05$) variation was observed in total yield of oyster mushrooms as affected by the interaction of different substrates and Pasterualization methods used in this study (Table 5). The maize stalk Pasterualized with hot water gave significantly the highest yield (1.58 kg/ 4kg of dry substrate) while teff straw treated with cold water resulted in the lowest yield (0.35 kg/ 4kg of dry substrate). The highest yield of mushroom grown on maize stalk treated with the hot water and the lowest yield grown on teff straw treated with cold water may be due to the narrow C:N ratio of the maize stalk which provided enough nutrients and could be due to the effective pasteurization of the substrate with hot water in order to protecting unnecessary fungal pathogens that may occurred during the growing season of mushrooms.

Biological efficiency of various treatment combinations was calculated on the basis of percentage fresh mushroom production per unit dry weight of

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substrate. Significantly ($p<0.05$) higher biological efficiency (74.59%) was recorded for mushrooms grown on the combination of wheat + teff + faba bean stalk (Table 4). This value is close to that mentioned by Kumar [15] who observed a biological efficiency in the range of 80 to 150% for oyster mushrooms grown in leguminous substrates. Similarly a Biological efficiencies range of 73 to 100 was reported by Tripathi. Significantly lower biological efficiency (32.44%) was recorded for the mushrooms grown on teff straw (Table 4). High value of biological efficiency was observed for mushrooms grown on narrow C:N ratio substrates such as faba bean stalk and Maize stalk and low value of BE was observed for mushrooms grown on wide C:N ratio substrates such as saw dust (937:1). This implies that nitrogen is also important for improving the yield (Table 4).

Results from main effect of pasteurization methods revealed that no-significant difference among pasteurization methods was observed in terms of BE statically. But hot water (55.77%) proved to be superior to cold water (54.05%) treatments in terms of BE (Table 4). This may be due to the effective pasteurization and increasing the availability of nutrients due to hot water treatment which contributed towards high biological efficiency.

Significantly ($p<0.05$) the higher production rate (1.40) was recorded for maize stalk substrate followed by the mixture of wheat + tef straws + faba bean substrates (Table 4). The lowest production rate (0.51) was recorded for teff straw. However, significant difference was not observed between the wheat straw, faba bean stalk and the mixture of saw dust + faba bean stalk in terms of production rate statically. Teff straw, Saw dust and wheat straw showed lower PR as compared to the others. There was no significance difference ($p<0.05$) was observed between pasteurization methods statically but hot water treatment gave significantly ($p<0.05$) higher production rate compared to cold water (Table 4). This could be due to the effective pasteurization which reduced competition for the growing mushrooms and also the release of available nutrients due to hot water treatment.

d) Effect of Substrates and Pasteurization Methods on Quality Parameters of Oyster Mushrooms

In the present study, crude protein, total ash, organic matter, moisture content and dry matter percentage of oyster mushrooms at harvest were measured to assess quality of mushrooms grown on different substrates treated /Pasterualized with hot water and cold water.

i. *Crude protein*

The interaction effect (Table 6) of substrates and pasterualization methods on crude protein content of oyster mushroom in this study showed that the protein content (27.38%) of mushrooms grown on dried faba bean stalk treated by cold water and cold water were the highest and significantly ($p<0.05$) different from other combinations except in the case of the mixture of wheat, teff and faba bean stalk treated with cold water statically. The nitrogen contents of substrates directly correlated with the protein content of the oyster mushroom (refer Table 3 and 6). Similar values for crude protein of oyster mushrooms have been reported [16]. The positive correlation of high protein content with high nitrogen content of substrates implies that adequate nitrogen in the substrate is essential for synthesis of protein in mushroom fruiting bodies. The lowest value of crude protein was observed in the cold water treated saw dust (18.56%). Significant differences were also not observed between hot water treated maize stalk and teff straw.

ii. *Ash content*

The highest ash content (11.08%) was recorded for mushrooms grown on wheat straw followed by ash content of mushroom grown on tef and the mixture of wheat, teff and faba bean straw, respectively. This value was close to the value mentioned [17] who reported 11.5% ash for mushrooms grown on wheat straw. Contrary to the ash value of mushroom grown on wheat straw, lower ash content (7.25%) was recorded for mushroom grown on maize stalk (Table 7). There was no significance difference observed among ash content of mushroom grown on saw dust, faba bean and the mixture of fababean and saw dust. The ash content of oyster mush room recorded in this study ranged from 7.25% to 11.08%. In contrary, [18] reported 6.20% and 5.90% ash for oyster mushrooms grown on paddy straw and corn stalk, respectively. This difference could be due to straw type and method of analysis. On the other hand the result of this study is in agreement with [19] who reported ash content of 8.80% for oyster mushroom and 7.20% for P. sajor-caju respectively. Significant difference was observed among substrates.

Hot water treated substrates produced mushroom with significantly ($p<0.05$) higher ash content compared to cold treatment ones. It may be due to increased availability of mineral elements because of hot water treatment (Table 7).

iii. *Percentage organic matter*

Organic matter content of oyster mushrooms was significantly ($p<0.01$) affected by different substrates and their pasteurization methods. The highest organic matter (92.74%) was recorded for mushrooms grown on maize stalk substrate whereas the lowest organic matter (88.26%) was recorded for mushroom grown on wheat straw (88.91%). This may be due to highest and the lowest ash content of mushroom grown on maize stalk and wheat straw respectively (Table 7). No-significant difference was observed among the substrates such as saw dust, faba bean stalk

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and the mixture of saw dust + faba bean stalk in relation to organic matter (Table 7).

Cold water treatment resulted in the highest organic matter (91.13%) while hot water treatment was the lowest (90.67%). This may be due to highest and the lowest ash content of mushroom treated by hot water and cold water, respectively (Table 7).

iv. *Percentage moisture content*

Percentage moisture content of oyster mushrooms grown in different substrates ranged from 73.73-90.34%. The highest moisture content of fresh mushrooms (90.34%) at harvest was recorded for mushroom grown on maize stalk which was significantly higher than other substrates which was at par with wheat straw (Table 7). The moisture content obtained in this study is close to the value mentioned [20], who reported 89% moisture for mushrooms grown on paddy straw. The lowest moisture content (73.73%) was recorded for mushroom grown on the substrate mixture of saw dust and faba bean stalk. These results are in line with that of [21] who reported a range of 70-90.9% moisture.

Non-significant difference was observed for moisture content of mushroom between the pasteurization methods statically but the mean value for moisture content of mushrooms under pasteurization methods of substrates resulted in higher moisture (84.86%) was recorder for mushrooms grown on hot water treatments (Table 7).

v. *Dry matter percentage*

The highest dry matter percentage of oyster mushroom (26.26%) was recorded for mushroom grown on substrates mixture of saw dust and faba bean stalk (Table 7). This may be due to the lowest moisture content of fresh mushroom (73.73%) grown on this substrate. The lowest percentage dry matter (8.99%) was found for mushroom grown on maize stalk in apart with other substrates. The dry matter percentage of oyster mush room found in this study ranged from 8.99 - 26.26 g. These results are in agreement with [22] who reported a range of 5.3-14.8g dry matter percentage.

The mean value for dry matter percentage of mushrooms under two pasteurization methods of substrates showed that non-significant statically but cold treatment resulted in the highest percentage dry matter (16.43%) while hot water treatment was the lowest (14.61%).

VI. Summary and Conclusions

Growth, yield and quality of mushrooms are substantially influenced by the substrate type on which it is grown. Studies on the effect of locally available substrates along with pasteurization methods on yield and quality of mushroom through systematic research are vital for optimizing productivity and choice of best quality mushrooms. In view of this, the present research work was conducted at the Arsi University, College of Agriculture and Environmental Science Research and Training Center during the year 2013-2014. A factorial experiment involving two treatment methods (cold water, and hot water) and seven substrates (sawdust, dried faba bean stalk, shredded maize stalk, wheat, tef straw, wheat + teff + faba bean stalk, and Saw dust + faba beanstalk + Saw dust) in a randomized complete block design with three replications was used.

All substrates were dried before shredding and the dried bean stalk, maize stalk, wheat straw, teff straw and saw dust were shredded or chopped into small pieces of approximately pieces of 2-4 cm size with axes. The required amount of each substrate has been subjected to different treatments such as cold water treatment, the substrates used in this study were separately soaked in fresh tap water for 24 hrs using 100 liter aluminum cans and achieved sufficient imbibitions. Similarly, pasteurization of shredded substrates has been done by soaking in hot water at 70°C for one hour using aluminum cans.

After proper draining, the pasteurized substrates were weighted in to small portion each weighing 4Kg dry weight basis and inoculated with oyster mushroom spawn made on wheat grains and then filled into 50x70cm^2 box cemented by plastic sheet. The temperature of the cropping room was maintained around 15-20°C and the relative humidity (80-85%) was adjusted by frequently spraying water over the wall of cropping room and on the boxes.

Substrates were analyzed for water holding capacity before and after growing the mushroom, Organic carbon was determined by Walkley and Black Method (Walkley and Black, 1934) and percent nitrogen in tissue was determined using micro Kjeldahl method (Black, 1965) both before and after growing mushroom. Days to complete mycelium invasion, appearance of pin heads, and onset of fruiting bodies in different substrates were recorded. Yield parameters such as biological efficiency (BE) and production rate (PR) of oyster mushrooms in each substrate was calculated after weighing fresh fruiting bodies using weight balance and dividing by dry weight of substrates. Quality parameters such as crude protein (CP), and total ash (TA) content were analyzed using Kjeldahl method and by heating known weight of grinded mushroom sample at 550°C for 5 hrs, respectively. Data were subjected to analysis of variance (ANOVA) using SAS statistical software. LSD was used to separate the treatment means at five percent probability level and Simple correlation between the treatments was computed.

The highest and the lowest water holding capacity of the substrates were recorded in maize treated with hot water (54.87 % and 59.96%) and saw dust treated with cold water (1.5% and 6.5%) both at spawning and harvesting respectively. Maximum carbon content was recorded for saw dust both at spawning

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(74.97%) and harvest (73.32%) while lowest carbon at spawning (44.85%) and at harvest (43.41%) were observed for dried faba beanstalk while lowest nitrogen (0.08%) at spawning and harvest (0.07%) were observed for saw dust. Therefore widest C:N ratio was recorded for saw dust at spawning (937.18:1) and harvest (1024.98:1) while the narrowest C:N ratio was for faba bean stalk at spawning (33.93:1) and harvest (42.29:1).

The fastest mycelium invention (12 days), pin head formation (45 days) and fruiting body formation (54.83 days) was recorded for maize stalk straw while the lowest mycelium invention (16.50 days), pin head formation (72.83 days) and fruiting body formation (84.83 days) was recorded in wheat straw. The highest cup diameter (8.50cm) and the lowest (2.50cm) was observed in the substrate mixture of wheat+ teff+ faba been stalk and saw dust respectively. Hot water treated maize stalk followed by cold water treated the mixture substrates of wheat +teff+ faba bean stalk gave significantly higher total yield of (1.5 kg/ 4 kg of dry substrate) and (1.4 kg/4 kg of dry substrate), respectively, while the lowest yield was obtained on cold water treated sawdust (0.35 kg / 4kg of dry substrate). The mixture of substrates of wheat +teff +faba bean stalk was significantly better (74.59%) compared to all other substrates in terms of biological efficiency and the lowest biological efficiency (32.44%) was recorded in the case of teff straw. S Significantly the highest production rate (1.40) was recorded in maize stalk, while the lowest production rate was observed in teff straw (0.51).

The protein content of mushrooms grown on bean pod husk treated with cold and hot water (27.38%) was the highest in par with the substrate mixture of wheat + teff + faba bean stalk treated with cold water statically and significantly different from other treatment combinations. The lowest value of crude protein was observed in the hot water treated wheat straw (17.03%).

Ash content of mushrooms grown on wheat straw was the highest (11.08%) and significantly different from other substrates. Whereas, the lowest ash content (7.25%) was observer in mushroom grown on the maize stalk and the hot water treated substrates gave the higher ash content (9.32%) as compared to the cold water treated substances (8.86).

Organic matter content of mushrooms grown on maize was highest (92.74%) and significantly different from other substrates. While the least value (88.91%), was observed in mushroom grown on wheat straw. The mushroom grown on cold water treated substrates gave the higher organic matter content as compared to hot water treated substrates. Moisture content of fresh mushrooms grown on maize stalk was the highest (90.34%) and significantly different from other substrates. The lowest significantly different moisture content (73.73%) of fresh mushrooms was observed in substrate mixture of saw dust + faba bean stalk and inversely the highest (26.26%) percentage dry matter of mushrooms grown this substrate was recorded and significantly ($p<0.05$) different from other substrates and substrate combination. The lowest significantly ($p<0.05$) different percentage dry matter (8.99%) was observed in mushroom grown on maize stalk.

In general, results of the present study showed that hot water pasteurization of the substrates followed was effective to reduce competent micro organisms. Organic residues having wide C:N ratio and excessive WHC reduced the yield and quality of mushrooms, however, the level of contamination in substrates with wide C:N ratio was relatively less. On the other hand, crop residues with narrow C:N ratio such as dried faba bean pod stalk and maize stalk had a positive correlation with yield and quality of mushrooms but with some risk of relatively higher contamination due presence of readily available nutrients. Thus, a sort of compromise should be reached for yield and selecting different substrates for growing mushrooms, so as to obtain economical yield without much effect on the quality. The present study was conducted with a limited number of substrates. However there is a lot of scope for conducting similar studies on different substrates which are easily available locally to the farmers interested to grow mushrooms under different agro climatic conditions across the country.

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