EFFECT OF ORGANIC SUBSTRATES AND THEIR PASTEURIZATION METHODS ON YIELD AND QUALITY OF OYSTER MUSHROOM (Pleurotus sajor caju)

M.Sc.Thesis
BY
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1.INTRODUCTION

- World population growth surpass food production capacity therefore, in the near future it will become increasingly difficult to feed the ever growing human population (FAO,2003)
- Shortage of land and food, erratic rain fall, nutritional disorder, low yield and nutritional status of traditional crops specially in proteins among others have been problems of poor countries.
- **Food grains are the principal dietary source of calories and proteins but their protein content is very small. On the other hand economic and health factors make animal protein inaccessible to the majority of people(Dawit,1998).**

Introduction (Continued)

- **Food self sufficiency can be brought about through diversification of production and consumption, therefore new crops like mushroom need to be tried out under local condition**

Mushroom

- **Is a cholesterol free plant**
- **Can grow on non arable land**
- **Its production is free from natural calamities**
- **Environmentally friendly technology**
- **High yielder as compared to other crops**

  According to Cooke cited in Dawit (1998) 3000-6000kg/ha/annum grain yield but 2,000,000kg/ha/annum mushroom can be produced.
- **Has high protein content**
- **Has high quality protein**

Has excellent flavor and taste

Has medicinal properties

Introduction (Continued)

Despite its paramount importance, very little work has been reported and most have not provide statistical analysis. It has been suggested that applied research, which provide basic data on substrate , mushroom type and other appropriate technology must be worked out (Dawit,1998)

Accordingly, the present study was undertaken with the following specific objectives:-

- to assess the effect of different methods of pasteurization of organic substrates for growing oyster mushroom.
- to evaluate different organic substrates in terms of yield and quality of oyster mushroom.

3.MATERIAL AND METHODS

- 3.1 Experimental site
- The study was conducted at Mushroom Research, Production and Training Laboratory of Haramaya University from Sept(2005) to Feb(2006).Located $42^{0}3^{l}$E longitudes and $9^{0}26^{l}$N latitude and at an altitude of 1980m a.s.l (AUA,1996)

- During the study period mean Max. and Min. T^{0} were $23.9^{o}C$ and $7.05^{0}C$ respectively. And RH was in the range of 29.9-69.9%

3.2.Experimental material and cultivation methods

3.2.1.Source of mushroom

- Pure culture of *Pleurotus sajor- caju* was obtained from mushroom R,P and T laboratory of Haramaya University.
- Stock culture was prepared using PDA and incubated at 25 ^{o}C for 10-12 days to get pure mycelium growth.

3.2.2 Spawn preparation

-Clean and disease free wheat grains were boiled for 40 min.

-The grain being intact allowed to remain socked in hot water for 15-20min.

-water was allowed to drain off on sieve for over night.

3.2.2.1. Preparation and sterilization of spawn substrates

-Next day12 g of Ca SO_4.2 H_2O and 3g Ca CO_3 were mixed with each 900g of boiled wheat.

The grain was filled in 500ml conical flask up to 2/3rd level.

plugged with cotton and covered with aluminum foil.

Sterilized in pressure cooker at 121^{0}C, 1kg/cm^{2} pressure for 30 min and cooled

3.2.2.2.Inoculation of spawn substrate

- Inoculation is done by transferring a bit of agar with mycelium from stalk culture to substrate flask under aseptic condition over a flame.

- Kept in Incubator at 25^{o}C, shaken at 7th day and the grain were fully covered in 15 days

3.2.2.3. Multiplication of spawn

Grain to grain mycelium exchange from master stock culture to newly sterilized flasks as described in 3.2.2.2

3.3. Treatments and Experimental Design

- The experiment was laid out in factorial combination of three pasteurization methods and six organic substrates in a randomized complete block design with three replications as given below:

- Pasteurization Methods Substrates

- T_1 = Cold water treatment
- T_2 = Hot water treatment
- T_3 = Formalin treatment

S_0 = Sawdust
S_1 = Bean pod straw
S_2 = Shredded Maize stalk

S_3 = Chat leaves
S_4 = Wheat straw
S_5 = Tef straw

3.4.Seeding of substrates and Spawn running

- The substrates treated with various pasteurization methods were seeded uniformly with a wheat based spawn @ 2%
- 18 small holes with diameter of 1 cm and 10cm gaps were made and seeded straws were then filled in to poly-bags by light hand pressing.
- The bags were arranged in RCBD with 3- replication in 15cm spacing between them.
- Temperature was tried to maintain in 25^0C and RH was tried to adjusted then bags were kept in dark with minimum ventilation
- Depending up on types of substrates in a period of 15-20 days the bags were covered with growth of the mycelium

3.5.Management Activities for Primordial and Fruiting body Formation

- Misting was a little bit increased.
- Light was given using florescent 4hrs/24hrs.
- Ventilation was done by opening the door 3hrs/24hrs

3.6.Data Collection

3.6.1. Analysis of substrates

Moisture percentage of substrates was measured

$$\text{Moisture \%} = \frac{\text{wet weight- Dry weight}}{\text{wet weight of substrate}} \times 100$$

Organic carbon was determined by walkley and Black method (walkley and black ,1934).

Percentage nitrogen in tissue was determined using micro kjeldal method (Black,1965)

3.6.2. Phenological observation

- Days to the completion of invasion of mycelium
- Appearance of pin head
- Days to fruiting body formation from day of spawning in different substrates were recorded

3.6.3.Yield of mushroom

Total yield= the sum of three flushes

- $BE = \frac{\text{Weight of fresh mushrooms}}{\text{Weight of dry substrate}} \times 100$

PR= BE/ Time

3.6.4.Quality parameters

3.6.4.1. Crude protein (CP)

- Ground and dried samples of fruiting bodies of *pleurotus sajor caju were analyzed for CP using Kjeldahl method. In order to determined protein, % N was multiplied by 6.25*

3.6.4.2. Crude fiber (CF)

- Crude fiber was measured using conventional CF analysis techniques.

$$\%CF = \frac{(\text{Weight of silica crucible with dry residue before ashing}) - (\text{Weight of silica crucible with ash after ignition})}{(\text{Weight of fat free sample used for estimation})} \times 100$$

3.6.4.3.Total ash (TA)

- Total ash (TA) content was estimated by heating 2gm of grinded mushroom sample at 550^0C for 5 hrs.

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

  Weight of crucible = W1

  Weight of crucible + Sample = W2

  Weight of crucible + Ash = W3

❖ Based on percentage total ash, percent organic matter was obtained by subtracting each value from 100.

3.6.4.4. Percentage moisture content (MC)

- The moisture content of the fruiting bodies was determined as loss in weight which resulted from drying a samples at 70^0C to constant weight and this was calculated using the following formula:-

- Percentage MC of fruiting body = $\frac{\text{Fresh weight – Oven dry weight}}{\text{Fresh weight of mushroom}} \times 100$

- Based on moisture percentage, the dry matter percentage was obtained by subtracting each value from 100 (Singh, 2003).

3.7. Statistical Analysis

- Data were analyzed as factorial combination of RCBD in 3-replication.
- Mean separation was done by DMRT at 1% probability level.
- Correlation between the treatments was also computed using SPSS computer program.

4. Results and Discussion

Table1. Effect of organic substrates and pasteurization methods on percentage moisture content of substrates at spawning

	Moisture (%) at spawning						
	Substrates						
Pasteurization method	S_0	$S_{1(B)}$	$S_{2(M)}$	$S_{3(C)}$	$S_{4(W)}$	$S_{5(T)}$	Mean
T_1	50.33j	68.27g	85.77a	58.60i	78.37c	75.86e	69.53a
T_2	49.37k	61.62h	85.50a	62.10h	77.46cd	77.19d	68.87c
T_3	49.80jk	61.83h	83.20b	57.70i	77.22d	73.97f	67.29c
Mean	49.83f	63.91d	84.82a	59.47e	77.68b	75.67c	
	Substrates		**Pasteurization**		**subst.X past.**		
SE	0.33		0.33		1.84		
CV%	0.82		0.82		0.82		

Table 2. Effect of organic substrates and pasteurization methods on percentage moisture content

Moisture (%) at harvest							
	Substrates						
Pasteurization method	S0	S1	S2	S3	S4	S5	Mean
T_1	48.67j	66.73e	75.36b	57.60h	68.63d	68.60d	64.27b
T_2	47.67jk	60.10g	79.60a	60.50g	74.27d	67.53e	64.95a
T_3	47.33k	60.70g	68.80d	56.37i	75.70b	66.30f	62.53c
Mean	47.89f	62.51d	74.59a	58.16e	72.87b	67.48b	

	Substrates	Pasteurization	subst.X past.
SE	0.35	0.35	1.95
CV%	0.95	0.95	0.95

Substrates	%C at spawning	%C at harvest	%N at spawning	%N at harvest	C:N at spawning	C:N at harvest
S_0	79.33a	78.07a	0.13e	0.12d	600.00a	650.60a
S_1	44.11d	42.60d	2.75a	2.41a	15.50e	17.67e
S_2	49.97bc	48.77bc	0.75c	0.68b	65.75c	71.72c
S_3	53.84b	51.73b	2.71b	2.36a	19.89d	21.92d
S_4	46.46cd	45.06cd	0.58d	0.53c	79.78b	85.02b
S_5	46.03cd	45.44cd	0.58d	0.53c	79.67b	85.74b
Mean	53.29	51.95	1.25	1.105	143.43	155.45
S.E	4.21	3.45	0.08	0.18	9.33	8.7
CV	10.96%	8.10%	3.91%	2.89%	7.96%	7.99%

Table 4 . Effect of organic substrates on days to mycelium invasion of oyster mushroom

Treatments	Mycelium invasion (Days)
Substrates	
S_0	14.89b
S_1	10.78cd
S_2	11.89c
$S_{3(C)}$	16.33a
S_4	10.56d
$S_{5(T)}$	10.44d
Mean	12.48

Table 4 . Effect of pasteurization methods on days to mycelium invasion of oyster mushroom

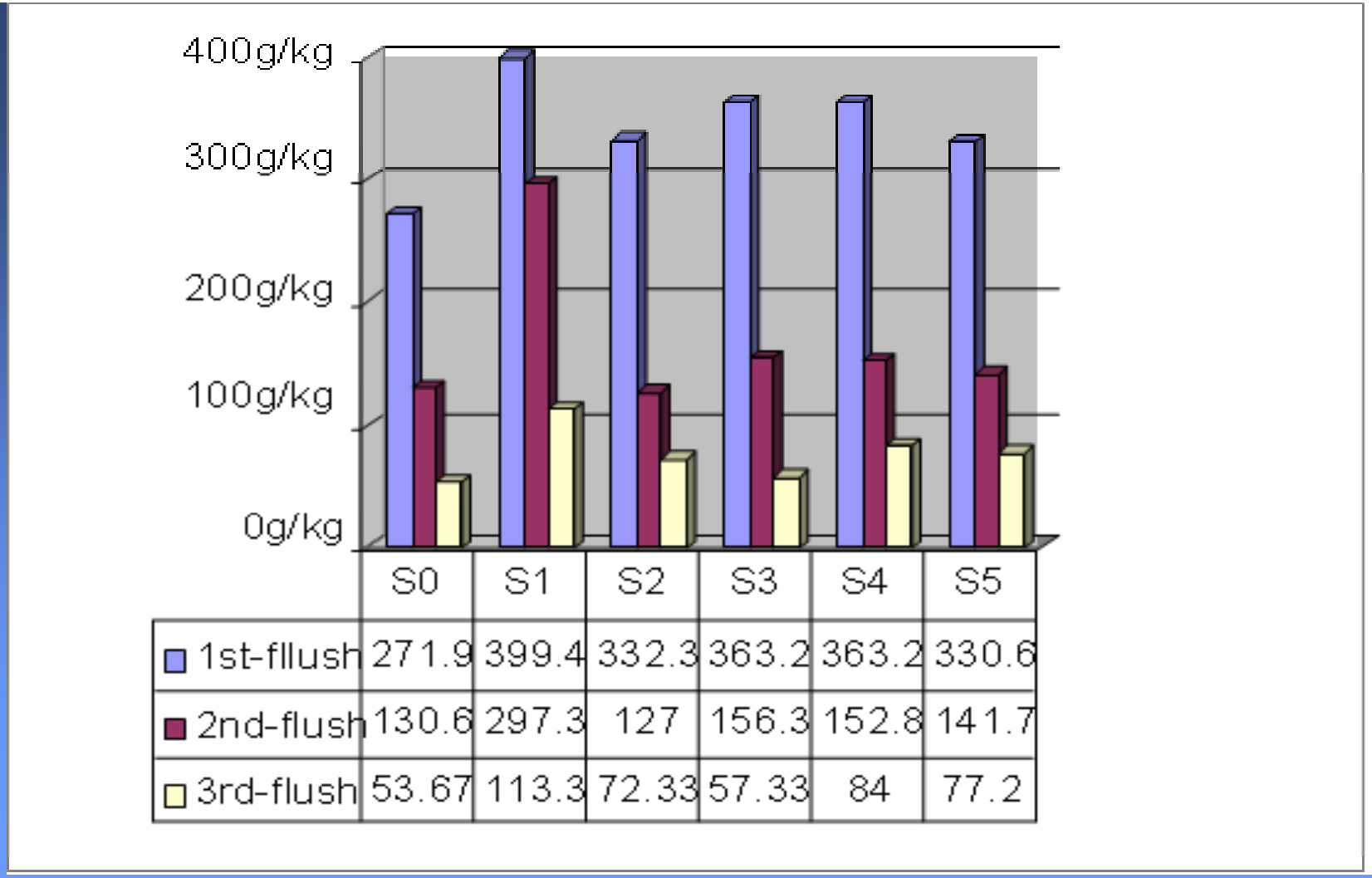
Pasteurization method	Mycelium invasion (Days)
T_1	13.33a
T_2	11.67c
T_3	12.44b
Mean	12.48
S.E	0.47
LSD(0.05)	0.55
C.V(%)	6.54

Table 5. Effect of organic substrates and pasteurization methods on pin head formation of oyster mushrooms

Days to pin heading							
	Substrates						
Pasteurization method	S_0	$S_{1(B)}$	$S_{2(M)}$	$S_{3(C)}$	$S_{4(W)}$	$S_{5(T)}$	Mean
T_1	19.00b	17.67c	15.33de	21.00a	14.67e	14.67e	17.06a
T_2	16.33d	18.33bc	14.33e	17.67c	14.33e	13.00f	15.67b
T_3	18.67bc	18.33bc	15.33de	19.00b	14.33e	14.33e	16.67a
Mean	18.00b	18.11b	15.00c	19.22a	14.44cd	14.00d	
	Substrates		**Pasteurization**		**subst.X past.**		
SE	0.45		0.45		0.86		
CV%	4.74		4.74		4.74		

Table 6. Effect of organic substrates and pasteurization methods on fruiting body formation of oyster mushrooms

Pasteurization method	Days to fruiting body formation						
	Substrates						
	S_0	$S_{1(B)}$	$S_{2(M)}$	$S_{3(C)}$	$S_{4(W)}$	$S_{5(T)}$	Mean
T_1	22.33bc	22.33bc	18.33f	24.00bc	18.33f	18.00fg	20.56a
T_2	19.00ef	22.67abc	16.33hi	20.33de	16.67ghi	15.33i	18.36c
T_3	21.33cd	23.00ab	17.67fgh	22.00bc	16.67ghi	16.67ghi	19.56b
Mean	20.86b	22.67a	17.44c	21.11a	17.22cd	16.67d	
	Substrates		**Pasteurization**		**subst.X past.**		
SE	0.47		0.47		0.79		
CV%	4.61		4.61		4.61		



	S0	S1	S2	S3	S4	S5
1st-fllush	271.9	399.4	332.3	363.2	363.2	330.6
2nd-flush	130.6	297.3	127	156.3	152.8	141.7
3rd-flush	53.67	113.3	72.33	57.33	84	77.2

Fig.1 Yield of Oyster mushrooms (*Pleurotus sajor caju*) at different harvesting operations

Table7. Effect of substrates and pasteurization methods on total yield of oyster mushrooms

Total yield

Substrates

Pasteurization method	S_0	$S_{1(B)}$	$S_{2(M)}$	$S_{3(C)}$	$S_{4(W)}$	$S_{5(T)}$	Mean
T_1	417.70i	675.00c	450.00hi	478.00fgh	501.70f	456.70g	496.50c
T_2	487.00fg	918.30a	587.30de	678.70c	602.30d	603.30d	646.20a
T_3	458.70gh	837.00b	557.70e	574.00de	580.00de	588.30de	599.30b
Mean	454.40e	810.10a	531.70d	576.90b	561.30bc	549.40cd	

	Substrates	**Pasteurization**	**subst.X past.**
SE	12.07	12.07	34.64
CV%	3.6	3.6	3.6

Table 8.Effect of substrates and pasteurization methods on biological efficiency of oyster mushrooms

Biological efficiency (%)							
	Substrates						
Pasteurization method	S_0	$S_{1(B)}$	$S_{2(M)}$	$S_{3(C)}$	$S_{4(W)}$	$S_{5(T)}$	Mean
T_1	13.92h	67.80c	45.00g	47.80fg	50.10f	45.67g	45.00c
T_2	16.21h	91.83a	58.73de	67.50c	60.23d	60.33d	59.20a
T_3	15.29h	83.70b	55.77e	57.40de	58.00de	58.83de	54.83b
Mean	15.14e	81.01a	53.17d	57.69b	56.11bc	54.94cd	

	Substrates	Pasteurization	subst.X past.
SE	1.19	1.19	4.09
CV%	3.88	3.88	3.88

Table 9. Effect of substrates and pasteurization methods on production rate of oyster mushrooms

Production rate							
	Substrates						
Pasteurization method	S_0	$S_{1(B)}$	$S_{2(M)}$	$S_{3(C)}$	$S_{4(W)}$	$S_{5(T)}$	Mean
T_1	0.56h	2.60e	2.28f	1.82g	2.54e	2.29f	2.02c
T_2	0.78h	3.67a	3.33b	3.00cd	3.27b	3.61a	2.94a
T_3	0.66h	3.26b	2.94d	2.42ef	3.17bc	3.22bc	2.61b
Mean	0.67e	3.18a	2.85c	2.41d	2.99b	3.04b	

	Substrates	Pasteurization	subst.X past.
SE	0.08	0.08	0.21
CV%	5.51	5.51	5.51

Table10.Effect of substrates and pasteurization methods on CP content of oyster mushrooms

Crude protein							
	Substrates						
Pasteurization method	S_0	$S_{1(B)}$	$S_{2(M)}$	$S_{3(c)}$	$S_{4(W)}$	$S_{5(T)}$	Mean
T_1	20.40l	36.60a	25.20f	32.63c	23.60i	25.20f	27.27a
T_2	20.10m	22.43k	23.30j	26.50e	23.20j	24.27h	23.30c
T_3	20.20m	34.87b	24.50g	31.40d	23.67i	25.10f	26.62b
Mean	20.23f	31.30a	24.33d	30.18b	23.49e	24.86c	
	Substrates		**Pasteurization**		**subst.X past.**		
SE	0.07		0.07		2.98		
CV%	0.46		0.46		0.46		

Table 11. Effect of substrates and pasteurization methods on crude fiber content of oyster mushrooms

Crude fiber content							
	Substrates						
Pasteurization method	S_0	$S_{1(B)}$	$S_{2(M)}$	$S_{3(C)}$	$S_{4(W)}$	$S_{5(T)}$	Mean
T_1	10.60i	15.80c	14.90d	8.57j	13.50ef	12.40h	12.63b
T_2	10.53i	17.40a	13.73e	8.80j	12.77g	12.50gh	12.62b
T_3	10.70i	16.67b	15.17d	10.80i	13.20f	12.70gh	13.21a
Mean	10.61e	16.62a	14.60b	9.39f	13.16c	12.53d	
	Substrates		**Pasteurization**		**subst.X past.**		
SE	0.1		0.1		0.67		
CV%	1.42		1.42		1.42		

Table 12.Effect of substrates and pasteurization methods on ash content of oyster mushrooms

Ash content							
	Substrates						
Pasteurization method	S_0	$S_{1(B)}$	$S_{2(M)}$	$S_{3(C)}$	$S_{4(W)}$	$S_{5(T)}$	Mean
T_1	8.78f	7.69i	6.95j	11.42b	10.82c	10.15d	9.29c
T_2	8.23g	7.87h	7.86h	12.32a	11.49b	10.70c	9.74a
T_3	8.84ef	8.25g	7.88h	11.47b	8.930e	10.75c	9.35b
Mean	8.62d	7.90e	7.56f	11.74a	10.41c	10.53b	
	Substrates		**Pasteurization**		**subst.X past.**		
SE	0.05		0.05		0.67		
CV%	0.79		0.79		0.79		

Table 13. Effect of substrates and pasteurization methods on percentage organic matter of oyster mushroom

Percentage organic matter							
	Substrates						
Pasteurization method	S_0	$S_{1(B)}$	$S_{2(M)}$	$S_{3(C)}$	$S_{4(W)}$	$S_{5(T)}$	Mean
T_1	91.22e	92.40b	93.05a	88.58i	89.18h	89.81g	90.71a
T_2	91.77d	92.13c	92.14c	87.68j	88.51i	89.30h	90.26c
T_3	91.16ef	91.75d	92.12c	88.53i	91.07f	89.25h	90.65b
Mean	91.38c	92.10b	92.44a	88.26f	89.59d	89.45e	
	Substrates		**Pasteurization**		**subst.X past.**		
SE	0.32		0.32		0.61		
CV%	0.64		0.64		0.64		

Table 14. Effect of substrates and pasteurization method on percentage moisture content of oyster mushroom

Percentage moisture content							
	Substrates						
Pasteurization method	S_0	$S_{1(B)}$	$S_{2(M)}$	$S_{3(C)}$	$S_{4(W)}$	$S_{5(T)}$	Mean
T_1	89.57f	90.17e	91.50d	93.13c	94.40a	90.20e	91.49a
T_2	89.17g	89.17g	88.80h	93.33c	94.33ab	89.43fg	90.71b
T_3	90.40e	88.30i	85.47j	94.00b	90.50e	90.27e	89.82c
Mean	89.71d	89.21e	88.59f	93.49a	93.08b	89.97c	
	Substrates		**Pasteurization**		**subst.X past.**		
SE	0.46		0.46		0.8		
CV%	0.88		0.88		0.88		

able 15. Effect of substrates and pasteurization methods on dry matter percentage of oyster mushroom

Dry matter percentage							
	Substrates						
Pasteurization method	S_0	$S_{1(B)}$	$S_{2(M)}$	$S_{3(C)}$	$S_{4(W)}$	$S_{5(T)}$	Mean
T_1	10.43f	9.83g	8.5j	6.87k	5.6m	9.8g	8.51c
T_2	10.83d	10.83d	11.2c	6.67kl	5.67m	10.57e	9.29b
T_3	9.6gh	11.7b	14.53a	6.00l	9.5i	9.73h	10.18a
Mean	10.29c	10.79b	11.41a	6.51f	6.92e	10.03d	

	Substrates	Pasteurization	subst.X past.
SE	0.41	0.41	0.7
CV%	7.65	7.65	7.65

Table 16. Mean square of parameters studied

Parameter	Mean square Substrate	Mean square pasteurization	Mean square SubxPast
Moisture % of substrate	1535.99**	23.95**	10.15**
Carbon content of substrate	1571.8**	23.97^{ns}	17.51^{ns}
Nitrogen content of substrate	12.24**	0.001ns	0.02ns
Days to mycelium invasion	57.14**	12.52**	0.74^{ns}
Days to pin- head formation	44.95**	9.24**	1.89**
Days to fruiting body formation	65.19**	21.17**	1.86*
Yield of oyster mushroom	91.09**	26.91**	3.33**
Total yield of oyster mushroom	130213.2**	105485.9**	3600.12**
Biological efficiency	4056.10**	952.58**	50.2**
Production rate	8.04**	3.97**	0.14**
Crude Protein content of mushroom	159.77**	81.72**	26.56**
Crude fiber content of mushroom	62.05**	2.02**	1.33**
Total ash content of mushroom	25.16**	1.102**	1.36**
Percentage organic matter	2.8**	1.25*	0.901**
Percentage moisture content	4.316**	3.73**	1.923**
Dry matter percentage			

Table 16. Correlation coefficient for different substrates and yield parameters of mushrooms

	MI	C	N	C:N	PHF	FBF	BE	PR	WHC
MI	1	0.51**	0.20	0.37**	0.75**	0.60**	-0.49*	-0.67**	-0.62**
C		1	-0.36*	0.87**	0.30*	0.20	-0.79**	-0.82**	-0.65**
N			1	-0.58	0.57**	0.64**	0.67**	0.34*	-0.23
C:N				1	0.19	0.12	-0.84**	-0.90**	-0.61**
PHF					1	0.95**	-0.09	-0.46*	-0.75**
FBF						1	0.03	-0.38*	-0.71**
BE							1	0.9	0.38*
PR								1	0.45**
WHC									1

Table 17. Correlation coefficient for different substrates and quality parameters of mushrooms

	C	N	WHC	%CP	%CF	%TA	C:N
C	1	-0.36*	-0.65**	-0.43*	-0.52**	-0.12	0.87**
N		1	-0.23	0.78**	0.14	0.15	-0.58**
WHC			1	-0.02	-0.46*	-0.10	-0.61**
%CP				1	0.18	0.09	-0.59**
%CF					1	-0.65**	-0.40*
%TA						1	-0.23
C:N							1

5.Summary and conclusion

- The highest and the lowest water holding capacity of the substrates were recorded in maize (84.82 %) and saw dust (49.83%) respectively.
- Cold water treated substrates at spawning and hot water treated substrates at harvest showed better WHC.
- Maximum carbon content was recorded for saw dust both at spawning (79.33%) and harvest (78.07%) while lowest carbon at spawning (44.11%) and at harvest (42.60%) were observed for bean pod straw
- Lowest nitrogen (0.13%) at spawning and harvest (0.12%) were observed for saw dust. And highest N- was for bean pod straw both at spawning and harvest

- The fastest mycelium invasion, pin head and fruiting body formation was observed in tef straw while the slowest was in chat leaves.
- Hot water treated substrates were the fastest and cold water treated substrates were the slowest in the afore mentioned parameters

Bean pod straw treated with hot water gave the highest TY, BE and PR while Saw dust treated with cold water gave the lowest.

Cold water treated bean pod straw gave the highest crude protein content while hot water treated saw dust was the lowest

Hot water treated bean pod straw gave the highest crude fiber content while cold water treated chat leaves was the lowest

- The highest ash content of mushroom was recorded for hot water treated chat leaves while the lowest was for cold water treated maize stalk

  The reverse is true for percentage organic matter.
- The highest moisture content of mushroom was recorded for cold water treated wheat straw while the lowest was for hot water treated maize stalk

  The reverse is true for percentage dry matter

Conclusion

- In general, results of the present study showed that

- Organic residues having wide C:N ratio and excessive WHC reduced the yield and quality of mushrooms, however, the level of contamination was relatively less.
- On the other hand, crop residues with narrow C: N ratio had a positive correlation with yield and quality of mushrooms but with some risk contamination Thus, a sort of compromise should be reached.

- pasteurization of the substrates either with hot water or formalin was equally effective.

THANK YOU