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Growth and yield of oyster mushrooms (*Pleurotus* spp.) on organically amended agro wastes

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ABSTRACT

The present experiment was conducted to identify the best organic amendment on the growth and yield of five species of oyster mushrooms *viz.,Pleurotus florida, P. sajor-caju, P. eous, P. tuber-regium* and *Hypsizygus ulmarius* by using organic amendments like rice bran, dry azolla, *Neem* cake, vermiwash and dry biogas slurry at three different concentrations. The effect of organic amendments on the number of days for sporophore formation, number and weight of sporophores varied according to the mushroom species. Results revealed that except dry biogas slurry, all organic amendments had superior effect in reducing number of days for sporophore formation, increasing the number of sporophores and yield. Effect of organic amendments on the yield of oyster mushrooms showed that all organic amendments except dry biogas slurry performed well with more number and weight of sporophores. The number of days for sporophore formation varied between 16.5 to 20.8 days in *P. eous*, 19.5 to 39 days in *P. tuber-regium* and 17.5 to 36.8 days in *H. ulmarius*. In *P. florida* and *P. eous* highest yield of 350.3g and 379g, respectively obtained from paddy straw amended with 1 per cent *Neem* cake. *P. sajor-caju* gave the maximum yield of 405.3g in 5 per cent rice bran. The maximum yield of 134.8g was recorded in *P. tuber-regium* when treated with 4 per cent rice bran whereas paddy straw amended with 6 per cent dry azolla gave highest yield of 218.3g in *H. ulmarius*.

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INTRODUCTION

Oyster mushroom (*Pleurotus* spp.) is the second most important edible mushroom species grown in the tropics, accounting for 25 per cent of total world production of cultivated mushrooms. Mushroom cultivation is recognized as a profitable and popular agribusiness as the demand for this white vegetable having excellent flavour and taste is increasing day-by-day (Naraian *et al.*, 2014). *Pleurotus* spp. is one of most extensively studied white-rot fungi for its exceptional ligninolytic properties (Li and Shah, 2016). They are considered as a potential resource to convert agro waste substrates into protein rich food and regarded as one of the commercially important edible mushrooms throughout the world. Its popularity has been increasing due to its ease of cultivation, high yield potential and high nutritional value (Banik and Nandi, 2004). It consists of a number of different species including *P. ostreatus, P. cystidiosus, P. cornucopiae, P. pulmonarious, P. tuber-regium, P. citrinopiliatus* and *P. flabellatus* and the most commonly cultivated *Pleurotus* spp. in India are *P. djamor, P. citrinopiliatus, P. flabellatus, P. eous, P. sajor-caju* and *P. florida*. It degrades most of the lignocellolosic agro-wastes and is an efficient means for the conversion of worthless wastes into valuable proteins (Mukhergee and Nandi, 2001). The use of different types of substrate by fungus will depend on its capacity to secrete oxidative (ligninase, laccase, manganese peroxidase) and hydrolytic (cellulase, xylanase and tannase) enzymes which are involved in utilizing lignocellulosic substrates (Singh and Singh, 2012). Oyster mushroom species can produce lignolytic and hydrolytic enzymes and are therefore adapted for growth within a wide variety of lignocellulosic wastes (Mikiashvili *et al.*, 2006). Several workers have reported that addition of organic amendments could enhance the yield of mushrooms. For example, supplementing the growth substrate with oil seed cakes greatly influenced the production of mushroom and doubled the yield. Keeping these in view, a study was conducted to assess the efficiency of different organic amendments such as rice bran, *Neem* cake, dry azolla, vermiwash and dry biogas slurry in the growth and yield of *Pleurotus* spp.

MATERIAL AND METHODS

The investigation was conducted at College of Horticulture, Vellanikkara, Kerala during 2006 to 2009 to find out the effect of organic amendments such as rice bran, *Neem* cake, dry azolla, vermiwash and dry biogas slurry on the growth and yield of five species of oyster mushrooms *viz*., *Pleurotus florida, P. sajor-caju, P. eous, P. tuber-regium* and *Hypsizygus ulmarius*. Paddy straw was used as the substrate. Good quality paddy straw was chopped into bits of 4 cm to 5 cm length and used for mushroom cultivation. The species of oyster mushrooms were obtained from Department of Plant Pathology, College of Horticulture, Vellanikkara, Thrissur, Kerala. Five different *Pleurotus* spp. were established by tissue culture technique and used for the production of spawn.

Chemical sterilization was followed for sterilizing the paddy straw (Sameera, 2007). The substrates were transferred to gunny bags and steeped in a solution of carbendazim (75ppm), formaldehyde (500ppm) and calcium carbonate (0.2%) for 18 h. After draining off the excess water, the substrate was spread on a clean floor for drying. The moisture content of the paddy straw was maintained at optimum level and was used for bed preparation. The standard compact polybag method described by Bhaskaran *et al.* (1978) was used for bed preparation. Poly bags of size 30x 60cm with 150- 200 guage thickness was used. About 30 holes of 0.5mm size were made on each polythene bag and the bottom was tied with a twine. Organic amendments at three different concentrations were used for the experiment *viz*. T_1 (4% rice bran), T_2 (5% rice bran), T_3 (6% rice bran), T_4 (1% *Neem* cake), T_5 (3% *Neem* cake), T_6 (5% *Neem* cake), T_7 (4% dry azolla), T_8 (5% dry azolla), T_9 (6% dry azolla), T_{10} (5% vermiwash), T_{11} (10% vermiwash), T_{12} (15% vermiwash), T_{13} (paddy straw and dry biogas slurry @ 1:0.25 ratio), T_{14} (paddy straw and dry biogas slurry @ 1:0.5 ratio), T_{15} (paddy straw and dry biogas slurry @ 1:1 ratio) and T_{16} (control- without amendments). Rice bran, *Neem* cake and dry biogas slurry were sterilized in an autoclave at a pressure of 1.05 per cm^2 for 15 minutes. Azolla was dried to optimum moisture level and sterilized by steaming for 20 minutes in a pressure cooker. Vermiwash was also sterilized by steaming. The sterilized paddy straw was thoroughly mixed with these organic amendments and used for the preparation of beds; whereas vermiwash was sprayed on the sterilized paddy straw using a hand sprayer. The bags were filled upto 5 cm height using the sterilized substrate and pressed with hand for making it even. Then 20-25g spawn was sprinkled over the filled straw along the peripheral region. A second layer of sterilized straw was filled and spawned as above. This process was repeated four times. Finally, the bag was tied tightly with a twine. For filling one bed 125g of spawn was used. The inoculated bags were incubated in a dark room for spawn run. The room temperature and relative humidity were maintained at 25-28^{0}C and 80-90 per cent, respectively by spraying water inside the room. After spawn run, the beds were transferred to cropping room where the temperature and relative humidity were maintained 25-28^{0}C and 80- 90 per cent, respectively.

Experiment was laid out in Completely Randomized Design with four replications. Observations on the time taken for spawn run, time for sporophore production as well as number and weight of sporophores were recorded. Analysis of variance was performed on the data collected in the experiments using statistical package of MSTAT (Freed, 1986). Multiple comparisons of the means were done using DMRT.

RESULTS AND DISCUSSION

In all the treatments, spawn run was not influenced much by the treatment with organic amendments (Table 1). However, in treatment with dry biogas slurry, all the oyster mushroom species except *P. eous* showed late spawn run. The poor performance of dry biogas slurry may be attributed to the restricted ability of the fungus to degrade and utilize the substrate due to an undesirable shift in C:N ratio as reported by Purkayastha *et al.* (1981) in the case of *Volvariella volvacea*.

The effect of organic amendments on the number of days for sporophore formation varied according to the mushroom species (Table 2). It is evident from the data that in *P. florida* and *P. sajor-caju* addition of organic amendments had no significant effect on the early sporophore production compared to control. However, in *P. florida* and *P. sajor-caju* early sporophore formation of 27 days and 24 days were noticed in T_2 (paddy straw+5% rice bran) and T_4 (paddy straw+1% *Neem* cake), respectively. Early sporophore formation of 16.5 days was noticed in *P. eous* grew on paddy straw amended with 4 per cent rice bran, that is seven days earlier than that of control. In this species, all concentrations of organic amendments except dry biogas slurry showed significant effect in reducing the number of days for sporophore formation ranging from 16.5 to 20.8 days. Paddy straw amended with 4 per cent dry azolla gave early sporophore formation of 19.5 days, and also significantly reduced the days (29 days) required for sporophore formation in *P. tuber-regium*. Early sporophore formation of 17.5 and 18.3 days observed in *H. ulmarius* in paddy straw amended with 4 per cent and 5 per cent rice bran (28 days earlier than control). In general, organic amendments exhibited a positive effect on the time taken for sporophore formation in *P. eous, P. tuber-regium* and *H. ulmarius*. It is also noted

Table 1: Effect of organic amendments on the time for spawn run of oyster mushrooms

Treatments	Time for spawn run (days)				
	P. florida	*P. sajor- caju*	*P. eous*	*P. tuber-regium*	*H. ulmarius*
T_1	14	12	12	12	14
T_2	14	12	12	12	14
T_3	14	12	12	12	14
T_4	14	12	12	12	14
T_5	14	12	12	12	14
T_6	14	12	12	12	14
T_7	14	12	12	12	12
T_8	14	12	12	12	12
T_9	14	12	12	12	12
T_{10}	13	12	12	13	12
T_{11}	13	12	12	13	12
T_{12}	13	12	12	13	12
T_{13}	16	15	12	15	17
T_{14}	16	15	12	15	17
T_{15}	16	15	14	15	17
T_{16}	16	15	12	15	17

that the supplementation of dry biogas slurry generally increased the days taken for sporophore formation in all species of *Pleurotus* compared to the other additives tested. Paddy straw supplemented with 2 per cent *Neem* cake hastened the maturity of sporophores of *P. sajor-caju* (Hazarika, 1998). Srivastava and Singh (1999) observed an early harvesting of *P. citrinopileatus* grown on paddy straw amended with rice bran and *Neem* cake. Kumar and Singh (2002) have also obtained same result during the cultivation of *Volvariella diplasia*. The inferior performance of dry biogas slurry may be because of the undesirable shift in the C: N ratio of the substrate that could be inhibitory to the mushroom growth as reported by Purkayastha *et al*. (1981).

Maximum number of fruiting bodies of *P. florida* were obtained in T_4 (paddy straw +1% *Neem* cake) (Table 3). With the all other organic amendments it produced a lesser number of sporophores compared to control, indicating organic amendments do not have a positive effect in increasing the number of sporophores in *P. florida*. In T_6 (paddy straw+5% *Neem* cake) *P. sajor-caju* produced the maximum number of sporophores followed by paddy straw supplemented with 4 per cent rice bran and 10 per cent vermiwash. In the case of *P. eous* all treatments except dry biogas slurry gave more number of sporophores compared to control. In the case of *P. tuber-regium*, treatments with rice bran, *Neem* cake, dry azolla and vermiwash recorded the maximum number of sporophores compared to control. The organic amendments did not resulted in markable increase in the number of sporophores. The maximum number of sporophores of *H. ulmarius* was noticed in paddy straw without supplementation indicating that the addition of organic amendments had not influenced the number of sporophores in this species.

Studies on the effect of organic amendments on the yield of oyster mushrooms revealed that all organic amendments except dry biogas slurry performed well with maximum weight of sporophores (Table 4). *P. florida* and *P. eous* recorded maximum yield of 350.3g and 379g, respectively when paddy straw was amended with 1 per cent *Neem* cake. *P. sajor-caju* gave the maximum yield of 405.3g in paddy straw supplemented with 5 per cent rice bran. The highest yield of *P. tuber-regium* (134.8g) was obtained from paddy straw amended with 4 per cent rice bran. Paddy straw supplemented with 6 per cent dry azolla gave the highest yield of 218.3g in *H. ulmarius*. It is evident from the data that, among the treatments the lowest yield was obtained with dry biogas slurry at all concentrations (T_{13}, T_{14} and T_{15}) for all species of oyster mushrooms.

Among the five species of oyster mushrooms

Table 2: Effect of organic amendments on the number of days for sporophore production

Treatments	Number of days for sporophore production				
	P. florida	*P. sajor- caju*	*P. eous*	*P. tuber-regium*	*H. ulmarius*
T_1	28.0^{ABc}	30.0^{Aba}	16.5^{Ba}	37.8^{Aab}	17.5^{Bc}
T_2	27.0^{Ac}	27.5^{Aa}	18.5^{Aa}	38.0^{Aab}	18.3^{Ac}
T_3	31.7^{ABbc}	25.0^{Aba}	19.0^{Ba}	39.5^{Aab}	27.5^{ABbc}
T_4	27.8^{BCc}	24.0^{BCa}	17.5^{Ca}	39.0^{ABab}	29.0^{BCbc}
T_5	28.0^{Ac}	28.5^{Aa}	17.0^{Aa}	32.3^{Abc}	24.5^{Abc}
T_6	27.8^{ABc}	26.3^{Aba}	17.5^{Ba}	40.8^{Aab}	25.8^{ABbc}
T_7	28.8^{Ac}	28.3^{Aa}	18.5^{Aa}	19.5^{Ad}	36.5^{Aab}
T_8	29.5^{Ac}	26.8^{Aa}	20.8^{Aa}	22.0^{Acd}	28.8^{Abc}
T_9	30.5^{Ac}	24.3^{Aa}	19.8^{Aa}	24.8^{Acd}	36.5^{Aab}
T_{10}	28.0^{Bc}	25.0^{Ba}	19.0^{Ba}	48.5^{Aa}	36.8^{ABab}
T_{11}	29.5^{ABc}	24.5^{Ba}	19.0^{Ba}	46.0^{Aa}	36.0^{ABab}
T_{12}	27.8^{Bc}	25.8^{Ba}	19.0^{Ba}	46.5^{Aa}	30.8^{ABbc}
T_{13}	46.0^{Aa}	26.8^{Ba}	27.3^{Ba}	48.3^{Aa}	47.3^{Aa}
T_{14}	42.8^{ABab}	33.8^{Aba}	27.3^{Ba}	50.8^{Aa}	48.0^{Aa}
T_{15}	46.3^{Aa}	26.8^{Ba}	27.5^{Ba}	46.0^{Aa}	47.5^{Aa}
T_{16}	27.8^{Bc}	25.0^{Ba}	24.3^{Ba}	48.8^{Aa}	48.5^{Aa}
	31.8^{C}	26.8^{D}	20.5^{E}	39.3^{A}	34.9^{B}

studied, *P. sajor-caju* produced maximum yield (262.8g) whereas *P. tuber-regium* gave lowest for all the treatments studied. Addition of organic amendments to the substrate to increase the yield of oyster mushrooms is dependent on the strain and type of organic amendments. *P. sajor-caju* gave the maximum yield among the five species of oyster mushrooms (Table 4). Compared to other oyster mushroom species, *P. sajor-*

Table 3 : Effect of organic amendments on number of sporophores of oyster mushrooms

Treatments	Number of sporophores				
	P. florida	*P. sajor- caju*	*P. eous*	*P. tuber-regium*	*H. ulmarius*
T_1	85.0^{Bb}	153.0^{Aab}	56.8^{BCbcd}	24.0^{Cb}	19.8^{Cb}
T_2	63.0^{Bbcd}	110.8^{Acd}	70.3^{ABabc}	43.3^{BCa}	13.8^{Cb}
T_3	51.8^{BCbcdef}	112.5^{Acd}	62.0^{Bbcd}	24.5^{BCb}	15.8^{Cb}
T_4	122.0^{Aa}	78.3^{Bef}	96.8^{Aba}	19.8^{Cb}	10.5^{Cb}
T_5	72.5^{Bbc}	126.0^{Abcd}	59.3^{BCbcd}	20.0^{Cb}	16.5^{Cb}
T_6	55.5^{Bbcdef}	179.0^{Aa}	48.8^{Bbcd}	21.8^{Bb}	31.3^{Ba}
T_7	57.8^{Bbcde}	134.3^{Abc}	63.3^{Bbcd}	14.0^{Cb}	43.5^{BCa}
T_8	52.5^{ABbcef}	74.3^{Aef}	59.8^{Abcd}	10.3^{Bb}	40.8^{Aba}
T_9	28.3^{Bef}	95.0^{Ade}	82.8^{Aab}	11.8^{Bb}	32.3^{Ba}
T_{10}	44.3^{ABcdef}	75.5^{Aef}	52.3^{ABbcd}	19.3^{Bb}	33.5^{Aba}
T_{11}	64.3^{Bbcd}	155.0^{Aab}	55.8^{Bbcd}	24.5^{Cb}	29.0^{Cb}
T_{12}	54.8^{ABbcdef}	48.5^{ABf}	82.3^{Aab}	22.5^{Bb}	26.8^{Bb}
T_{13}	33.0^{Bdef}	94.8^{Ade}	35.0^{Bd}	14.8^{Cb}	18.8^{Cb}
T_{14}	22.8^{Af}	19.0^{Ag}	34.0^{Ad}	11.8^{Ab}	14.0^{Ab}
T_{15}	22.0^{Af}	12.8^{Ag}	31.0^{Ad}	11.8^{Ab}	15.8^{Ab}
T_{16}	80.5^{ABb}	104.3^{Acde}	47.5^{BCcd}	11.8^{Cb}	44.0^{BCa}
	56.9	98.3^{A}	58.6^{B}	19.1^{C}	25.4^{C}

Table 4 : Effect of organic amendments on the yield of oyster mushrooms

Treatments	Yield (g/500g substrate)				
	P. florida	*P. sajor- caju*	*P. eous*	*P. tuber-regium*	*H. ulmarius*
T_1	336.0^{Aa}	349.3^{Aabc}	269.3^{Acd}	134.8^{Ba}	105.8^{Bbcd}
T_2	189.0^{BCcde}	405.3^{Aa}	270.8^{Bcd}	131.0^{CDa}	57.3^{Dcde}
T_3	144.3^{Bdef}	351.3^{Aabc}	295.3^{Abcd}	112.5^{Ba}	49.8^{Bde}
T_4	350.3^{Aa}	246.3^{Bef}	379.0^{Aa}	113.5^{Ca}	120.3^{Cbcd}
T_5	251.3^{Abc}	284.8^{Acdef}	285.3^{Acd}	76.3^{Bab}	59.5^{Bcde}
T_6	227.3^{Abc}	317.0^{Abcde}	225.5^{Adef}	111.8^{Ba}	57.8^{Bcde}
T_7	265.5^{BCb}	382.8^{Aab}	338.3^{ABabc}	67.3^{Dab}	179.8^{Cab}
T_8	201.0^{Bbcd}	256.0^{Aef}	271.3^{Acd}	59.8^{Cab}	211.0^{Ba}
T_9	127.5^{BCef}	217.3^{Bf}	368.8^{Aa}	58.3^{Cab}	218.3^{Ba}
T_{10}	207.5^{ABCbcd}	215.0^{ABf}	264.3^{Acde}	113.3^{Ca}	126.3^{BCbc}
T_{11}	247.8^{ABbc}	332.5^{Abcd}	223.8^{Bdef}	110.3^{Ca}	158.8^{BCab}
T_{12}	335.3^{ABa}	262.0^{Bdef}	359.5^{Aab}	118.3^{Ca}	161.5^{Cab}
T_{13}	99.3^{ABf}	104.8^{Ag}	127.0^{Agh}	91.5^{ABab}	28.8^{Be}
T_{14}	80.5^{ABf}	114.3^{ABg}	157.3^{Afgh}	74.5^{ABab}	22.8^{Be}
T_{15}	83.8^{ABf}	142.5^{Ag}	112.3^{ABh}	32.0^{Bb}	29.0^{Be}
T_{16}	206.5^{Abcd}	224.0^{Af}	193.8^{Aefg}	74.0^{Bab}	147.8^{ABab}
Mean	209.5^{B}	262.8^{A}	258.8^{A}	92.4^{C}	108.4^{C}

caju had better degradation ability as evident from the higher yield of the species in rice bran, *Neem* cake and dry azolla. Sangeetha *et al*. (2004) showed that the increased protein content of substrates due to the addition of gram powders enhanced enzymatic degradation by the fungus and increased the yield of paddy straw mushroom. Srivastava and Singh (1999) obtained the maximum yield of *P. citrinopileatus* grown on paddy straw amended with rice bran and *Neem* cake. Shashirekha *et al.* (2002) observed the maximum yield of *P. sajor-caju* grown on rice straw supplemented with cottonseed powder. They found that oil seed cakes enhanced the secretion of lignolytic enzymes, aided break down of lignin and create easier accessibility for the degradation of other carbohydrates. The degraded carbohydrates served as energy source for the formation of fruiting bodies. Organic amendment, dry biogas slurry was inferior in mushroom production. Singh and Singh (2014) reported enhanced biological efficiency, protein and essential amino acids of oyster mushroom grown on paddy straw substrate supplemented with different vegetable waste including pea pod shell, cauliflower leaves, radish leaves and brassica straw. Purkayastha *et al.* (1981) made such a report on amendment of paddy straw with nitrogenous wastes.

Organic amendments in general reduced the number of days for sporophore formation and increased the number of sporophores. Thus, the addition of organic amendments to paddy straw increased the yield of oyster mushroom compared to control.

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