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Comparative study on growth parameters of *Pleurotus florida* cultivated on different substrates at Tawang, Arunachal Pradesh, India

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Abstract
The aim of the study was to evaluate effect of different substrates namely finger millet (*Eleusine coracana*) straw (FMS), paddy (*Oryza sativa*) straw (PS) and their combination (50% FMS + 50% PS) on growth parameters such as spawn run, pin head appearance, maturation of fruiting bodies, flushing intervals, cropping period, total yield (g) and biological efficiency (B.E %) of *Pleurotus florida* cultivated at Tawang, Arunachal Pradesh, India. A total of three treatments replicated five times were taken under complete randomize design. The minimum average interval between flushes was recorded on FMS (7.8 days) followed by 50% FMS + 50% PS (12.1 days) for *P. florida*. The total maximum yield and biological efficiency were noted on FMS (1155.40g and 92.43% respectively) which were significantly ($P \leq 0.5$) higher than other tested substrates. This was the first comparative study conducted to evaluate the efficiency of locally available finger millet straw with paddy straw (conventionally used) for the cultivation of oyster mushroom (*P. florida*) under the high altitude condition of Tawang, Arunachal Pradesh, India.

Keywords: *Eleusine coracana* (finger millet straw), *Pleurotus florida*, flushing interval, biological efficiency (%), Tawang (Arunachal Pradesh)

Introduction
Tawang district of Arunachal Pradesh is located in the extreme Northeast near to Indo-China border. The economy of Tawang district is basically agrarian in nature with more than 80% of the population is dependent on agriculture [1]. The farmers of the region mostly follow traditional method of cultivation practices which is subsistence in nature. Except few, many of the crops they go for cultivation with local varieties with low productivity. The major issues related to agriculture sector are mono – cropping, low adoption of scientific cultivation practices, rain fed agriculture, unpredictable weather conditions etc. In Tawang majority of the farmers belong to Buddhist religion and in their culture animal hunting is prohibited. Thus, protein in their diet comes from vegetables. These vegetables are transported from the nearby lower regions especially from Tezpur, Assam (India) and their transportation charges are very high. In this context mushroom production not only provides good source of protein and vitamins in the diet but is also an alternate source of livelihood particularly in the rural sector. Oyster mushroom cultivation has the potential of converting agricultural waste into protein rich nutritional products [2]. *Pleurotus florida* is an edible oyster mushroom and it belongs to class the Basidiomycetes, subclass Hollobasidiomycetidae and order Agricals. These mushrooms can be produced from locally available agricultural waste products such as rice straw, paddy straw, finger millet straw, maize cobs, coconut fiber, bean fiber and saw dust etc. These substrates contain lignocellulose, which help in oyster mushroom growth. Finger millet (*Eleusine coracana*) crop is mostly cultivated by the farmers of the Tawang region and its by-products are usually left to rot in the field or are disposed through burning. These agro by – products particularly it straw can be used for the cultivation of high protein rich oyster mushrooms.

The average temperature of Tawang in the month of July to September is usually 18 °C ± 2 °C which is favourable for the cultivation of *Pleurotus* spp. However, the mushroom farmers are challenged with numerous difficulties which include shortage of quality spawn, lack of technical skill in mushroom cultivation technology and most importantly the lack of information about the substrates for cultivation. Therefore, identification of suitable substrate is critical for successful mushroom cultivation (Kurtzman & Zandrazil, 1982; Shah *et al.*,

2004) [3, 4]. The objective of the present study was to evaluate the bio efficiency of locally available agro waste (finger millet straw) as a substrate in comparison to traditional substrate (paddy straw) and their combination on the growth of *Pleurotus florida* under the native condition of Tawang, Arunachal Pradesh.

2. Materials and Methods

2.1 Study site

The investigation was carried out at Defence Research Laboratory R & D Center, Tawang, Arunachal Pradesh during July 2019 to September 2019. The average temperature during this period was 18 °C ± 2 ºC (approx.)

2.2 *Pleurotus* Strain

Pleurotus florida (strain 3308) was procured from ICAR-Directorate of Mushroom Research, Solan, India. Pure culture was maintained on potato dextrose agar (PDA, Himedia Laboratories Pvt. Ltd., India) at 4 °C.

2.3 Mother spawn preparation

The wheat grains were thoroughly washed in sufficient water to remove soil debris and weeds. Washed grains were then soaked in sufficient water for 15 -20 minutes and boiled in a container for 15 -20 minutes until the grains get soft and the same was tested by pressing the grains. Excess water from the boiled grains was removed by spreading boiled grains on sieve made of fine wire mesh. Now the grains were mixed with gypsum (calcium sulphate) 2% and chalk powder (calcium carbonate) 0.5% of dry weight basis. About 250g treated grains were filled in 500 ml glass bottles and plugged with non – absorbent cotton. The plugs were covered with brown paper. The bottles were autoclaved at 15 p.s.i. pressure for 15 minutes under 121 ºC temperature. Each bottle was inoculated with a piece of mycelium (pure culture) of *Pleurotus florida* and incubated for 15 days at 25 ºC until the substrate become fully colonized.

2.4 Spawn preparation

The wheat grains were processed as described earlier and 250g of treated grains were filled into polypropylene bags of 25 x 17 cm size, and packed tightly. The filled bag was plugged with non – absorbent cotton and covered with brown paper. The bags were then autoclaved for 15 minutes at 121 °C under 15 p.s.i. pressure. The bags were inoculated with mother spawn of *Pleurotus florida* and incubated for 15 days at 25 °C to achieve full growth of the mycelium. The fully colonized packets were used for spawning.

2.5 Substrate preparation

Finger millet straw (*Eleusine coracana*), paddy straw (*Oryza sativa*) and their combination (50-50%) were tested. The substrates were collected from local framers and chopped into pieces of about one centimeter in length, and then cleaned with running water. Chopped substrates were soaked in cold water for 16 - 18 hours. After cold treatment the substrates were taken out and excess water was drained in sun light until 25-30% moisture was retained.

2.6 Preparation of mushroom bags

The mushroom bags were prepared by using transparent polypropylene bags of size: 14 x 23 inches having 12 -15 small holes of 10 mm diameter all around. The bags were filled with 2.5 kg (wet weight) of respective substrate and combination. Five replicates were prepared for each treatment. Spawning was done in three layers and rate of spawning was 3% of wet substrate. The spawned bags were tied tightly and incubated in dark room at 18 °C ± 2 ºC for mycelial growth.

2.7 Cropping and Harvesting

When the spawned bags appeared white due to cottony growth of *P. florida* on the respective substrate, the bags were then shifted into another room called cropping room which was well ventilated with 200 lux light intensity provision. The bags were exposed for 5 – 7 hours daily for pin head appearance and subsequent maturation into the fruiting bodies. After the first harvest the polythene from each bag was removed using sterilized blade. Water was sprayed on the bags twice a day to maintain 85% – 9% humidity for the formation of healthy fruiting bodies. The bags were retained for upto three flushes.

2.8 Recording of Data

Days to spawn run completion, pin head formation, flushing interval, maturation of fruiting bodies and total cultivation time were recorded for each bag. Total yield (g) and biological efficiency (%) of the respective substrate and their combination for *P. florida* were also recorded. The biological efficiency was calculated by the formula given below (Chang *et al.* 1981) [5].

$$\text{Biological efficiency (B.E)} = \frac{\text{Fresh weight (g) of mushroom (three flushes)}}{\text{Dry weight (g) of substrate}} \times 100$$

2.9 Statistical analysis

All the data was analysed in 5 replicates (n =5) using Grap Pad InStat version 3.05 by one-way Analysis of Variance (ANOVA) followed by Tukey – Kramer multiple comparisons tests. All the statistical analysis was done at the 0.05 significance level. The results were expressed as mean values and standard deviation (SD).

3. Results

Table 1 indicates that spawn run completion of *Pleurotus florida* was relatively faster on PS (27.20 days) in comparison to 50% FMS + 50% PS (31.80 days) and FMS (36.40 days). The test species showed similar pattern for pin head formation i.e., 32 days, 38.60 days and 43.40 days on PS, 50% FMS + 50% PS and FMS respectively. The time taken to the maturity of pin heads into fruiting bodies was 39 days on the PS followed by 43.80 days and 48.40 days on 50% FMS + 50% PS and FMS respectively. However, the shortest flushing interval between one flush to the next was recorded on FMS (9 days) followed by 50% FMS + 50% PS (10.20 days) and PS (14.20 days). Similarly, the flushing interval between second flush to the third was recorded least for FMS (6.6 days) in comparison to 50% FMS + 50% PS (14 days) and PS (14.20 days). Moreover, no significant difference ($P \leq 0.5$) was recorded with respect to the cultivation time taken by *P. florida* on different substrates (PS and FMS) and their combination under the prevailing cool and temperate condition of Tawang.

Table 1: Effect of different substrates and their combination on growth factors of *P.florida*

Substrate	Completion of spawn run (days)	Pin head formation (days)	Maturation of fruiting bodies (days)	Flushing intervals (days)		Cultivation time (days)
				1-2*	2-3*	
Paddy straw (PS)	$27.20^{a} \pm 0.83$	$32^{a} \pm 1.22$	$39^{a} \pm 0.63$	$14.20^{ac} \pm 0.83$	$14.20^{ac} \pm 1.78$	$65^{a} \pm 3.08$
Finger millet straw (FMS)	$36.40^{b} \pm 1.14$	$43.40^{b} \pm 1.14$	$48.40^{b} \pm 0.48$	$9^{b} \pm 0.70$	$6.6^{b} \pm 0.57$	$64.20^{ab} \pm 1.92$
50% FMS + 50% PS	$31.80^{c} \pm 1.48$	$38.60^{c} \pm 1.14$	$43.80^{c} \pm 0.40$	$10.20^{b} \pm 0.83$	$14^{c} \pm 1.22$	$67.60^{abc} \pm 2.50$

*1-2: First to second flush; 2-3: second to third flushe. Data presented as mean ± SD of 5 replicates. Values in the same column not sharing common superscript letter (s) are significantly different at $P \leq 0.05$ by using Tukey – Kramer multiple comparisons test.

The maximum total yield (g) of *Pleurotus florida* was noted on FMS (1155.40g) followed by 50% FMS + 50% PS (1063.80g) and PS (947g) per 1250g dry weight of the respective substrates. The highest biological efficiency of *P. florida* was recorded on FMS (92.43%) followed by 50% FMS+ 50% PS (85.10%) and PS (75.76%). The total yield and biological efficiency of the test oyster species were significantly ($P \leq 0.05$) different for the substrates tested (Table 2)

Table 2: Yield and biological efficiency of *P. florida*

Substrate	Total yield (g)	Biological efficiency (%)
Paddy straw (PS)	$947^{a} \pm 18.82$	$75.76^{a} \pm 1.50$
Finger millet straw (FMS)	$1155.40^{b} \pm 21.52$	$92.43^{b} \pm 1.72$
50% FMS + 50% PS	$1063.80^{c} \pm 15.17$	$85.10^{c} \pm 1.21$

* Data presented as mean ± SD of 5 replicates. Values in the same column not sharing common superscript letter (s) are significantly different at $P \leq 0.05$ by using Tukey – Kramer multiple comparisons test.

4. Discussion

The findings of this study indicated that *Pleurotus florida* was cultivated efficiently on paddy (*Oryza sativa*) straw, finger millet (*Eleusine coracana*) and their combination (50:50) under the native condition of Tawang, Arunachal Pradesh. The mycelial growth of *Pleurotus florida* was noted faster on paddy straw (PS) as compared to finger millet straw (FMS) and their combination because the carbohydrates present in the substrate were efficiently utilized by the particular strain of *P. florida*. Since the beginning of 19th century, oyster mushrooms were successfully cultivated on paddy straw in many countries under natural condition (Quimio *et al*., 1990) [6]. The difference in days for complete spawn run of *P. florida* on different substrates was observed by several investigators in the past. Iqbal *et al.*, (2016) [7], reported that fastest spawn running occurred on wheat straw (26 days) followed by sorghum straw (34 days) and paddy straw (37 days) while the lowest spawn running was recorded on maize straw (39 days) and sugarcane bagasse (40 days) for *Pleurotus florida*. However, Patra and Pani (1995) [8], Jiskani *et al.*, (1999) [9] and Lalithadevi and Many (2014) [10] reported shorter spawn running time (13-25 days) for *Pleurotus* spp. on paddy straw. The difference in days for complete mycelial growth on different substrates might be due to variation in their chemical composition and C:N ratio as reported by Bhatii *et al.*, (1987) [11] and Shah *et al*., (2004) [12]. In the present study the complete mycelial run of *P. florida* on 50% FMS + 50% PS (31.80 days) was significantly ($P \leq 0.05$) shorter in comparison to FMS (36.40 days). The reason may be the amalgamation of soft and hard lignocellulosic substrates in which the softer one (PS) is facilitating the oyster species to extract nutrients easily for mycelial growth and thus increasing its biomass which in turn may be secreting optimum level of hydrolysing and oxidizing enzymes to break the other hard lignocellulosic material (FMS), thus resulting in fast and dense mycelial growth on the mixture of substrates.

The pinhead formation phase was recorded following complete mycelia growth. The pinheads of *P. florida* appeared earlier on paddy straw than other substrates. On *Eleusine coracana* straw pinheads appearance took longer time (43.40 days). The difference in the time required for the formation of pinheads on different substrates is well documented in various past studies. Ahmed (1998) [13] reported pinhead formation of oyster mushroom cultivated in different substrates in the range of 23 to 26 days from spawning, while Fan *et al*., (2000) [14], noted the pinheads between 20 to 23 days. Patra and Pani (1995) [8] recorded 20-24 days for pinhead appearance on paddy straw. Kimenju *et al.* (2009) [15] stated that initiation of pinning after ramification depends on the type of substrate used. Raw materials having higher lignin and cellulose contents take longer time to initiate pinheads appearance compared to the substrates with lower contents of lignin and cellulose.

The result indicated that minimum number of days was observed for transformation of pinheads into mature fruiting bodies on paddy straw followed by 50% FMS + 50% PS. The difference in time period for pinheads maturation was reported by many researchers in the past. Khan *et al.* (1981) [16] recorded 21-28 days for pinheads maturation on cotton boll locules. Tan (1981) [17] noted 30 days for the maturity of pinheads on cotton waste. Khanna and Garcha (1981) [18] reported 20-24 days for the maturation of pinheads on paddy straw. Tirkey *et al.* (2017) [19] recorded 21-29 days for the maturity of pinheads on different substrates and their combinations. Moreover, the pin heads maturation into healthy fruiting bodies critically relies on factors like O_2 concentration, CO_2 concentration (1000 ppm or 1%), humidity (80-90%) and light (200 lux intensity) which must be maintained and monitored in the cropping room.

In the present study, the flushing interval between the first flush to the second flush is significantly least ($P \leq 0.05$) on finger millet straw compared to paddy straw for *P. florida.* Further, the time interval noted for the second flush to the third flush on FMS (6.6 days) was significantly ($P \leq 0.05$) less compared to both PS (14.20 days) and 50% FMS + 50% PS (14 days). The data regarding flushes intervals were documented in some studies by the researchers. Iqbal *et al.* (2005) [20] reported 4.7 - 6.3 days minimum interval between the flushes on an average basis on paddy straw for *Pleurotus* species. However, the maximum time intervals for flushing were observed in chickpea straw (9.3 days) followed by wheat

straw (8.0 days). Similarly Bhatti (1984) [21] reported different flushes with an interval of 5-6 days for oyster species. In contrary to the previous studies, the current investigation noted on an average minimum interval between flushes for *Eleusine coracana* straw (7.8 days) compared to paddy straw (14.20 days). The initiation of pinning followed by maturation into healthy fruiting bodies chiefly depends on optimum concentration of moisture to be retained by the substrate during cropping. Finger millet straw is comparatively harder in comparison to paddy straw due to high lignocellulosic content thus able to retain the moisture for longer period of time, which in turn reduces the time period between two flushes.

The cultivation period (after three flushes) for *P. florida* was noted 64.20 days, 65 days and 67.60 days on FMS, PS and 50% FMS + 50% PS respectively under the native condition of Tawang. Usually, the longer cultivation period is attributed to the high altitude climatic conditions of Tawang, Arunachal Pradesh. The study confirmed that the use of different substrates brought about a significant ($P<0.05$) effect on yield and biological efficiency (B. E) of *P. florida.* The yield was maximum on FMS (1155.40g) with biological efficiency of 92.43% followed by 50% FMS + 50% PS (1063.80g) with B.E of 85.10% and PS (947 g) with B.E of 75.76%. Other workers also recorded difference in the total yield and biological efficiency with respect to different substrates. Raghav *et al.* (2016) [22] got yield in the range of 370g – 2160g on different substrates for *Pleurotus* spp. Graham and Clyde (1985) [23] recorded 80-120% B. E of *P. sajor-caju* on cotton waste. Nunez and Mendoza (2002) [24] observed that B.E. of substrates varied from to 50.8 to 106.2% for *P. ostreatus*. However, the yield and B.E of a particular oyster mushroom species depends on the quality of spawn, certain environmental factors, cultivation practices adopted and most importantly the physico chemical and nutritional characteristics of the substrate used.

5. Conclusion

Based on the data analyzed during the present study, it can be concluded that locally available substrate finger millet straw *(Eleusine coracana)* is the best substrate compared to paddy straw in terms of minimum flushing interval (days), yield (g) and biological efficiency (%) of *Pleurotus florida* cultivated under the high altitude condition of Tawang, Arunachal Pradesh. The study will be helpful for the local farmers of the Tawang and in around area for efficiently adopting the oyster mushroom cultivation technology using locally available substrate (*Eleusine coracana*).

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