

Original Research Article

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Comparative Studies of Span Growth on Different Grains Substrate in Three *Pleurotus* spp. (*Pleurotus florida*, *Pleurotus flabellatus* and *Pleurotus sapidus*)

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ABSTRACT

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Mushroom is a popular nutrient and protein-rich food of the world. Oyster mushroom is the second most popular mushroom after button mushroom all over the world. Oyster mushrooms are the important and least expensive to grow, they are well known for conversion of crop residues to food proteins. Growing medium of the mushroom is generally known as substrate. It is the base of mushroom cultivation same as soil for crops. The present investigations were carried out on Comparative studies of span growth on different grains substrate in three *Pleurotus* spp. (*Pleurotus florida*, *Pleurotus flabellatus* and *Pleurotus sapidus*). The results of present investigations showed that the spawn improvement maximum mycelial growth of strains *P. florida*, *P. flabellatus* and *P. sapidus* on maize and sorghum grains. *P. sapidus* maximum mycelium growth was observed in Maize grain (90 mm) and *P. florida* shows maximum mycelium growth was observed in Maize grain (89.25 mm) while minimum mycelium growth was found in Wheat grain substrate (54.5) and in case of *P. flabellatus* maximum mycelium growth was observed in Maize grain (86.50 mm). Based on the results obtained, maize grain would be recommended as most suitable substrate for the cultivation of all three *Pleurotus* spp.

Introduction

Mushrooms are fruiting bodies of fungi. They are classified in Kingdom of Fungi, which is also called Kingdom of Mycetae. The use of mushrooms as food is an old age practice and there is cultivation record in 60 A.D. (Chang and Miles, 2004). Mushrooms are considered as a source of high quality protein and crops possessing high medicinal and economical values. Mushroom farming has two inseparable phases; spawn production and

fruiting body cultivation. Spawn is the planting seed of mushroom and technically, it is an expanding mushroom mycelium colonizing a given substrate media. Currently, albeit mushrooms are cultivated in more than 100 countries (Chang S-T, 2006). Cultivated species of mushroom, *Pleurotus* exhibits much diversity in the adaptability to varying climates, and this flexible nature makes it a more cultivated species than any other cultivated mushroom. Out of 28 species reported from India, more than a dozen are

under cultivation in different part of the country (Verma, 1998).

Spawn is the vegetative mycelium from a selected mushroom grown on a convenient medium like wheat, pearl millet, sorghum, etc for raising mushroom crop. It essentially involves preparation of pure culture of mushroom from tissues/ spores that is generally maintained in any agar medium, followed by culturing on sterilized grains and further multiplied on grains. The spawn thus comprises of mycelium of the mushroom and a supporting medium which provides nutrition to the fungus during its growth. From 1652 to 1894 A.D. spawn was gathered from the wild rather than made. Before the advent of grain spawn, different kinds of spawn used were natural or Virgin spawn (from the pastures & meadows), Flake spawn (breaking of beds through which mushroom mycelium has run), Mill track spawn (bricks dried and made from mixture of horse dung, cow dung and loan soil) and manure spawn (on sterilized horse manure or compost manure).

Thus, Production of good quality spawn requires a strict laboratory procedure in which maintaining sanitation and purity of the spawn are critical importance. The quality of spawn affects both yield and quality of cultivated mushrooms (Mbogoh, 2011). Spawn production is a fermentation process in which the mushroom mycelium will be increased by growing through a solid organic matrix under controlled environmental condition. In almost all cases the organic matrix will be sterilized grain e.g. wheat, maize, sorghum etc (Jain, 2005).

Species of *Pleurotus* are grown on a more limited scale (Royse and Schisler, 1980). *P. florida* was isolated from subtropical Florida in U.S.A. and commercially exploited (Zadrazil, 1980). *P. flabellatus* was grown successfully in India (Bano and Srivastava,

1962) and Philippines (Quimio, 1978). Thus, the objective of this study was mainly to assess the effect of different locally available grain substrates for spawn production of Oyster mushroom (*Pleurotus florida*, *Pleurotus flabellatus* and *Pleurotus sapidus*).

Materials and Methods

Experimental site

The experiments were conducted during 2015-2016 in Mushroom Laboratory Department Plant of Pathology, S. V. P. University of Agriculture and Technology, Meerut, UP, India, which is situated on the Western side of the Delhi-Dehradun high way (NH-58) at a distance of 10.0 km away in the north of Meerut city. The district Meerut is situated between 29° 01'N latitude and 77° 45'E longitude at an altitude of 237 meters above the mean sea level.

Establishment of pure culture

Culture of *Pleurotus* spp. (*P. florida*, *P. flabellatus* and *P. sapidus*) were purified and maintained by single hyphal tip method. For this purpose, the culture was grown in sterilized Petri plates on Potato Dextrose Agar Medium (PDA) for 8-10 days. Single branched hyphae from the periphery of the growing colony were marked under low power (10x) in the compound microscope and transferred to PDA slants. These tubes were incubated at 24±⁰C for about a week, again sub cultured on PDA and then stored in a refrigerator at 5±⁰C for further use.

Spawn Improvement

Spawn was prepared on healthy and clean wheat grains in half litre capacity wide mouthed glass bottles. After that, the grains were soaked overnight in clean water and then washed. They were boiled in water for 15

minutes taking care that grains should not split but remain slightly hard after boiling. The boiled grains were spread in thin layer over a wire net to remove excessive water and enable them to cool about $25\pm 1^{\circ}\text{C}$. The cooled grains were then mixed with 1.2 percent commercial grade gypsum (CaSO_4) and 0.3 percent calcium carbonate (CaCO_3). Gypsum prevents the sticking of wheat grains together and calcium carbonate maintains the pH 5.5-7.5. The grains were then filled in clean glass bottle up to $2/3^{\text{rd}}$ of its capacity. The bottles were plugged with non-absorbent cotton and covered with butter paper. These bottles were then sterilized at 121°C (15 lbs pressure) for 2 hours on two consecutive days. Sterilized bottles were taken out from the autoclave, while still hot and were shaken to avoid clumping of grains. Sterilized bottles were inoculated with few 9 mm disc of a vigorously grown 10 days old culture of *Pleurotus* spp., Inoculated bottles were incubated at $24\pm 1^{\circ}\text{C}$ and shaking was done after 7 days. Entire grains were covered with fine mycelial growth after 20 ± 1 days. This spawn is known as mother spawn or master spawn which were used for the study of present investigation.

Effect of different grains on spawn growth

In this experiment, different types of grains were used as substrate (i.e. sorghum, oat, barley, pearl millet, maize and wheat). The Spawn was prepared as described under 3.6. The grains were filled up to (90 mm) in the bottle for six treatments in four replicates. *Pleurotus* spp. (*P. florida*, *P. flabellatus* and *P. sapidus*) were inoculated by 9 mm disc in individual bottle under laminar air flow. The spawn bottles were incubated without shaking at $24\pm 1^{\circ}\text{C}$ in B.O.D incubator and observations were recorded for the spawn on 4th, 8th & 12th day till the first bottle completely covered by mycelial growth (i.e. 90 mm) in anyone grains used in the spawns bottles.

Experimental design

In the experiments complete randomized design (CRD) with three replicates of each. Sorghum spawn, oat spawn, barley spawn, pearl millet spawn, maize spawn and wheat spawn, in all six types of substrates.

Data analysis

Data were analyzed using the analysis of variance (ANOVA) procedure by O. P. STAT.

Results and Discussion

Effect of different grain substrate on spawn growth

The maximum spawn growth (34.75 mm) was found in maize grain followed by oat grain (21.00 mm) on 4th day which was significantly lower than maize while minimum mycelium growth were observed in wheat grain (17.50 mm) followed by pearl millet (19.50 mm) which was significantly similar to wheat grain. In case of *P. flabellatus* on 4th day maximum mycelial growth (32.50 mm) was recorded in maize grain followed by oat grain (21.50 mm) which was significantly lower than maize while minimum mycelium growth were recorded in wheat grain (16.25 mm) followed by pearl millet and sorghum (18.50 mm) both which was significantly similar to barley grain. In case of *P. sapidus* on 4th day maximum mycelial growth (36.50 mm) was recorded in maize grain followed by sorghum grain (29.25 mm) which was significantly lower than maize while minimum mycelium growth were recorded in pearl millet grain (20.00 mm) followed by oat grain (23.75 mm) which was significantly higher than pearl millet grain.

On 8th day, maximum spawn growth (62.00 mm) was found in maize grain followed by barley grain (45.50 mm) which was

significantly lower than maize while minimum mycelium growth were recorded in wheat grain (32.00 mm) followed by pearl millet (32.25 mm) which was significantly similar to wheat grain in case of *P. florida* while in case of *P. flabellatus* on 8th day maximum mycelial growth (60.75 mm) was observed in maize grain followed by oat grain (43.50 mm) which was significantly lower than maize while minimum mycelium growth were recorded in wheat grain (31.5 mm) followed by pearl millet (32.25 mm) which was significantly similar to wheat grain. In case of *P. sapidus* on 8th day maximum mycelial growth (60.75 mm) was recorded in maize grain followed by barley grain (46.25 mm) which was significantly lower than maize while minimum mycelium growth were recorded in pearl millet grain (40.50 mm) followed by wheat grain (44.75 mm) which was significantly higher than pearl millet grain.

On 12th day maximum spawn growth (89.25 mm) in *P. florida* was found in maize grain followed by oat grain (76.25 mm) which was significantly lower than maize while minimum mycelium growth were recorded in wheat grain (54.50 mm) followed by pearl millet (58.50 mm) which was significantly higher than wheat grain.

In case of *P. flabellatus* on 12th day, maximum mycelial growth (86.50 mm) was recorded in maize grain followed by oat grain (71.50 mm) which was significantly lower than maize while minimum mycelium growth were recorded in wheat grain (50.75 mm) followed by pearl millet (59.50 mm) which was significantly higher than wheat grain. In case of *P. sapidus* on 12th day maximum mycelial growth (90.00 mm) was recorded in maize grain followed by barley grain (89.00 mm) which was significantly lower than maize while minimum mycelium growth were recorded in pearl millet grain (69.00 mm) followed by barley grain (76.00 mm) which

was significantly higher than pearl millet grain. Regarding spawn growth rate (mm/day) in all three *Pleurotus* species maximum spawn growth rate was found in maize grain, in case of *P. florida* (7.44 mm/day) followed by oat grain (6.35 mm/day) and minimum growth rate was found in Wheat grain (4.54 mm/day) followed by pearl millet (4.88 mm/day).

In case of *P. flabellatus* maximum spawn growth rate was found in maize grain (7.21 mm/day) followed by oat grain (5.96 mm/day) and minimum growth rate was found in wheat grain (4.23 mm/day) followed by pearl millet (4.96 mm/day). In case of *P. sapidus* maximum spawn growth rate was found in maize grain (7.50 mm/day) followed by sorghum grain (7.42 mm/day) and minimum growth rate was found in pearl millet (5.75 mm/day) followed by barley grain (6.33 mm/day).

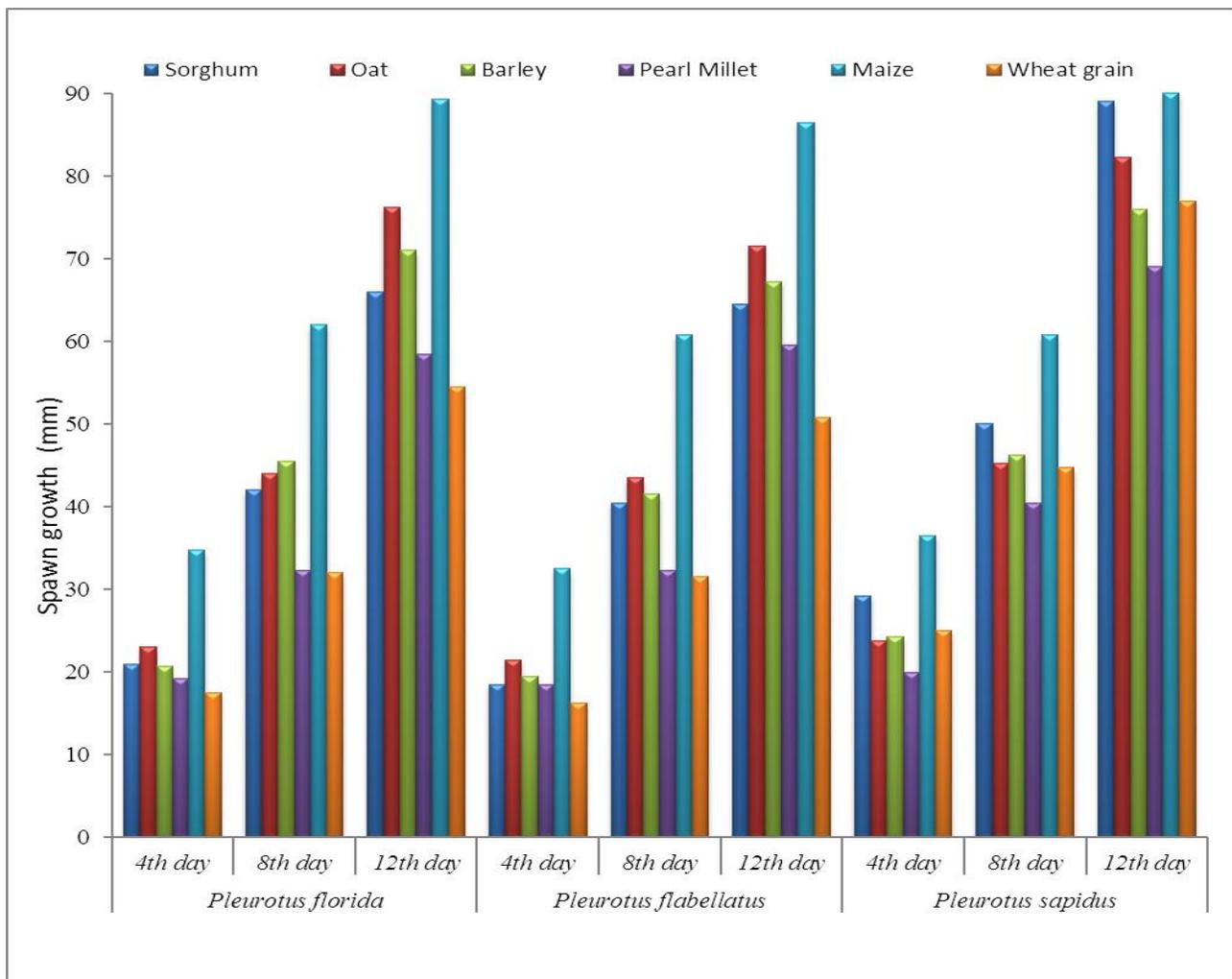
In the present investigation, all *Pleurotus* species i.e. *P. florida*, *P. flabellatus* and *P. sapidus* grown on different grain substrates in spawn bottles, took different time to complete mycelial growth (90 mm). Full mycelial growth of strains *P. florida*, *P. flabellatus* and *P. sapidus* in maize and sorghum grain was obtained on 12th days in spawn bottles which is significantly superior to other grain spawn. Among the other grains mycelial growth of maize were followed by sorghum (89 mm) and oat (82.25 mm) from the strain *P. sapidus* at 12th day observations while in case of *P. florida*, mycelial growth of maize were followed by oat (76.25 mm), barley (71 mm) but in case of *P. flabellatus* mycelial growth of maize (86.5 mm) were followed by oat (71.5 mm), Barley (67.25 mm). Minimum mycelial growth was observed in wheat grain in *P. florida* (54.5 mm) and *P. flabellatus* (50.75 mm) but in case of *P. sapidus*, minimum mycelium growth was observed in pearl millet grain (69 mm) on 12th day respectively.

Table.1 Spawn growth (mm) and growth rate (mm/day) of different *Pleurotus* spp.

Grains	<i>Pleurotus florida</i>				<i>Pleurotus flabellatus</i>				<i>Pleurotus sapidus</i>			
	4 th day	8 th day	12 th day	Growth rate (mm/day)	4 th day	8 th day	12 th day	Growth rate per day	4 th day	8 th day	12 th day	Growth rate per day
Sorghum	21.00	42.00	66.00	5.50	18.50	40.50	64.5	5.38	29.25	50.00	89.00	7.42
Oat	23.00	44.00	76.25	6.35	21.50	43.50	71.5	5.96	23.75	45.25	82.25	6.85
Barley	20.75	45.50	71.00	5.92	19.50	41.50	67.25	5.60	24.25	46.25	76.00	6.33
Pearl Millet	19.25	32.25	58.50	4.88	18.50	32.25	59.5	4.96	20.00	40.50	69.00	5.75
Maize	34.75	62.00	89.25	7.44	32.50	60.75	86.5	7.21	36.50	60.75	90.00	7.50
Wheat grain	17.50	32.00	54.50	4.54	16.25	31.50	50.75	4.23	25.00	44.75	77.00	6.42
CD at 5%	3.02	2.557	2.27	-	2.168	2.27	1.98	-	1.466	1.655	1.044	-
SE (m)	1.009	0.854	0.759	-	0.724	0.759	0.661	-	0.489	0.553	0.349	-

Average of four replication

Fig.1 Different grain on spawn growth (mm) and growth rate (mm/day) of three *Pleurotus* spp.



Similar observations were also reported by Pathmashini *et al.*, (2008), revealed that the efficacy of four different types of grain spawns viz; kurakkan, maize (*Zea mays*), sorghum and paddy on oyster mushroom (*Pleurotus ostreatus*) production. Four types of spawns were tested on a medium based on sawdust. Highest mean numbers of sporophore (fruiting bodies) were noticed in the harvests obtained from sorghum spawn (7.67+ or -0.66). The kurakkan spawn significantly enhanced biological efficiency and increased size and yield, when compared with other spawn types viz; maize, sorghum, and paddy.

Mbogoh, *et al.*, (2011) reported that the spawn is pure culture of mycelium growing on a solid substrate such as cereal grain. Maize, wheat and millet grains were used as substrates for production of grain mother spawns of *Pleurotus ostreatus*. Linear mycelium extension was measured.

Bhadana (2014) also reported that the best grain spawn with maximum spawn growth observed in the maize substrate which was the best, followed by wheat, then millet for the spawn growth.

Singh (2015) reported that, in case of *P. djamor* the maximum mycelial growth was found in chickpea while the minimum mycelial growth was recorded in barley grains, followed by wheat grain respectively.

Many workers worked on development of different grain spawns and their effect on yield. Pathmasini *et al.*, (2008) used locally available grains of kurakkan (*Eleusine coracana*), maize (broken), sorghum (*Sorghum bicolor*) and paddy (*Oryza sativa*) for spawn production. According to him the kurakkan spawn produced an acceleration of spawn running, pin head formation, fruit body formation and increased yield compared with

other types of spawn viz. maize, sorghum and paddy. Shah *et al.*, (2004) took three types of grain for spawn production kurakkan (*Eleusine coracana*), maize (broken) (*Zea mays*), sorghum (*Sorghum bicolor*) and reported the spawn run appear earlier in kurakkan. Arulnandhy and Gayathri (2007) obtained a mean yield of 24 gm on sawdust medium. Chaubey, (2010) used wheat, maize and sorghum grain spawn for the cultivation of oyster mushroom. Thulasi *et al.*, (2010) reported spawn production of oyster mushroom on different substrates. Khan *et al.*, (2011) reported different spawning methods of oyster mushrooms on cotton waste. It is concluded from the present study that all the three spp. On all six grains are suitable for spawn production but Maize grain is more efficient on for the spawn production of *Pleurotus sapidus*.

In case of spawn production maximum mycelial growth of strains *P. florida*, *P. flabellatus* and *P. sapidus* in maize and sorghum grain was obtained on 12th days in bottles. In case of *P. sapidus* maximum mycelium growth was observed in maize grain (90 mm) while minimum mycelium growth was found in Wheat grain substrate (77 mm), In case of *P. florida* maximum mycelium growth was observed in maize grain (89.25 mm) while minimum mycelium growth was found in wheat grain substrate (54.5 mm) and in case of *P. flabellatus* maximum mycelium growth was observed in maize grain (86.50 mm) while minimum mycelium growth was found in Wheat grain substrate (50.75 mm).

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