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Standardization of Shiitake Mushroom (Lentinula edodes (Berk.) Pegler) Spawn Production Technology in Meghalaya

Nandeesha. S. V.[1]* . Rajesh. T.[1] . Devi. R. K. T[2] . Thakur. N. S. A.[3]

School of Crop Protection, College of Post Graduate Studies in Agricultural Sciences, Central Agricultural University (Imphal), Umiam, Meghalaya -793 103

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ABSTRACT

Shiitake mushroom *Lentinula edodes* (Berk.) Pegler, is one of the six popular edible mushrooms and it is a wood rotting fungi capable of decomposing the cellulose and lignin structural components which is having several medicinal properties. Only few types of mushrooms have the particular bioactive compounds that can improve the human health in many ways. The compound existing in shiitake mushroom, "lentinan" is having immune modulating property and promotes the immune function of the human body against the cancer risk and tumour growth. The present study was undertaken to evaluate different locally available grain substrates *viz.,* paddy, maize, pearl millet, sorghum, wheat, saw dust and wood pieces of jack fruit, willow and pine tree for spawn production by using three strains, "DMR 388", "DMR 32" which were collected from Directorate of Mushroom Research (DMR), Solan and "Meghalaya Local" strain was collected from Department of Agriculture (DOA), Upper Shillong, Meghalaya. The results revealed that maximum mycelial growth on PDA media was observed in the strain, Meghalaya Local (89.74 mm) at 20th day after inoculation whereas the minimum mycelial growth was seen in DMR 388 (85.40 mm). Among three strains and 12 different substrates used for spawn production, the fastest growth of mycelium was observed in sorghum grains (14.40 days) for strain Meghalaya Local whereas the highest number of days taken by saw dust of pine (60.00 days) for strain DMR 388 compared to other strain. It can be concluded that sorghum grains were found to be the ideal substrate for spawn production of shiitake mushroom, followed by wheat grains compared to other different substrates. Among strains, Meghalaya Local was the best strain for spawn production of shiitake mushroom. The economics involved for spawn production from 10 kg of grains/substrates, the cost benefit ratio is 1:3 for the production of mushroom spawn.

1. Introduction

Mushrooms are the eatable fungus belongs to the phylum Basidiomycota. These are multicellular, eukaryotic, macro-fungi, bearing spores and achlorphyllus as saprophytes on decomposed matter. They also depend upon other substrates for their survival which are rich in lignin content. Shiitake mushroom (*Lentinula edodes*) is one among the six popular edible mushrooms in the world accounting 17 per cent production in terms of tonnes (Chang and Miles, 2004; Miles and Chang, 1997). The name shii-take was originated from Japanese word: "shii" means the wood of *Castanopsis cuspidata* and mushroom is the meaning of "take". It is a white wood rotting fungi capable of decomposing the cellulose and lignin structural components (Jong, 1989). This mushroom is also called as golden oak mushroom, oak wood mushroom, black mushroom and black forest mushroom. This mushroom is fat free and containing less cholesterol, low gluten and sodium content. Besides this, in fruiting

Correspondence author: ksumanth29@gmail.com

bodies elements such as potassium, phosphorous, magnesium, iron, copper, zinc, calcium and manganese are plenty in nature. It is rich in proteins, lipids, carbohydrates, fibres, ergosterols, antioxidants and vitamins like provitamin-D which are not found in other food supplements.

Spawn is nothing but the seed material for mushroom production and there are two kinds of spawns one is mother spawn and another is commercial spawn. Mother spawn is obtained by tissue culture method that the tissue or a particular mushroom bit is inoculated to selected growth media and mycelium will cover all over the media. Then, the mycelium is transferred to suitable substrate for growth and multiplication of spawn and this spawn is considered as a mother spawn. Later the commercial spawn is produced from the mother spawn packets. Now a days, mushroom technology is emerging everywhere in India, but there is lack of technology in producing spawn both in public and private establishments. Mushroom production mainly depends upon the spawn material and the yield also depends on the quality of the spawn material. Therefore, there is an urgent need of suitable method for getting good quality spawn within fewer periods of time and without any contamination. Such method should be made available to the mushroom growers for better production of fruiting bodies. Hence, different locally available substrates were used for quality spawn production within short period. Therefore this study made to get the better quality spawn by standardizing the best substrates among selected substrates and strains within a lesser time which is available as seeding material to the mushroom producers for better yielding of shiitake mushroom.

2. Materials and Methods:

The research work has done in the Microbial culture laboratory of Plant Pathology, School of Crop Protection, College of Post-Graduate Studies in Agricultural Sciences (CPGSAS), CAU (Imphal), Umiam, Meghalaya during the academic year 2018-2019. The materials and methods used in the study were described:

1. Collection of shiitake mother culture strains

In the past years, the mushroom strains were collected from various forests and identified using proper taxonomic for a particular species. There is no mushroom strains supply units in the country for the production of mushrooms. The forest trees are the original source for all commercial strains of shiitake mushroom. In this experiment, three strains *viz.,* "DMR 388" and "DMR 32" strains were obtained from the Directorate of Mushroom Research (DMR), Solan and one strain "Meghalaya Local" was obtained from the Department of Agriculture (DOA), Upper Shillong, Meghalaya.

2. Sub culturing of mother culture for multiplication

The pure culture strains "DMR 388", "DMR 32" and "Meghalaya Local" were sub-cultured (Plate1 and Plate 2) by using pre sterilized Potato Dextrose Agar (PDA) culture medium aseptically. The cultures were inoculated into petri plates containing medium by disc inoculation method. Then, the plates were incubated at 27 ± 1°C in BOD incubator for one to two weeks for mycelial growth. The plates with fully grown mycelium were kept in refrigerator at 5-10°C for further inoculation to substrates.

3. Multiplication of cultures by disc inoculation method

Inoculation of any culture was done aseptically under the laminar air flow chamber. Pure cultures were taken inside the laminar flow and sterilized the surface with ethanol, by switching on the blower and lamp as well as using spirit lamp for flame sterilization. The cork borer of 6.5 mm diameter was sterilized by dipping in spirit and flaming to spirit lamp till attaining red-hot stage and allowed to cool before picking fungal mycelium. Mycelial discs of pure cultures was picked up (Plate 3) by using cork borer (Alam, 2004).

With the help of inoculation loop, mycelial discs was taken out (Plate 4) and inoculated to the solidified PDA in the petri plates (Plate 5). Then inoculated plates were incubated in BOD incubator at 27 ± 1°C for one to two weeks for mycelium growth (Plate 6 and 7) and petri plates with fully grown fungal mycelium (Plate 8) were kept in refrigerator at 5-10°C for further inoculation and use.

The grain substrates such as paddy, maize, pearl millet, sorghum and wheat were collected from local farmers which were produced in their farm. Similarly the wood logs from trees of jack fruit, willow and pine were collected. From these wood logs, sawdust and wood pieces were prepared in sawmills which were then used as substrates for spawn production.

4. Collection of locally available substrates

The grain substrates such as paddy, maize, pearl millet, sorghum and wheat were collected from local farmers which were produced in their farm. Similarly the wood logs from trees of jack fruit, willow and pine were collected. From these wood logs, sawdust and wood pieces were prepared in sawmills which were then used as substrates for spawn production.

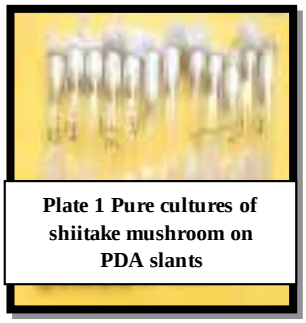


Plate 1 Pure cultures of shiitake mushroom on PDA slants

Plate 2 Pure cultures of shiitake mushroom on PDA petri plate

Plate 3 Making mycelial discs by using cork borer

Plate 4 Transferring mycelial discs for sub-culturing

Plate 5 Inoculation of mycelial discs into PDA media

Plate 6 Growth of mycelium after inoculation

Plate 7 Measuring mycelium growth culture

Plate 8 Fully grown mycelium in petri plate

5. Procedure for spawn production

5.1 Cleaning and washing of substrates

For spawn production, the healthy unbroken cereal grains free from pests and diseases were chosen. Old, insect damaged and black coloured grains were discarded. The grains were thoroughly washed in sufficient water 3-4 times for removing soil debris, straw particles, floating grains, undesirable seed of grasses, weeds etc. these washed grains were soaked in required quantity of water for 20-30 minutes and drained out the water for further boiling. Likewise, saw dust and wood pieces which were collected from sawmills cleaned from undesirable wood stalks, wood barks and soil particles. Then they were washed in sufficient water for 2-3 times for removing of floating materials and dust particles. The wood pieces and saw dust were soaked in sufficient water for 20-30 minutes and drained out the water with a sieve made of fine wire mesh so that the substrates were ready for further boiling.

5.2 Boiling of substrates and mixing of chemicals

Substrates after cleaning and draining out the water, were boiled in a vessel for 20-25 minutes until the substrates become soften, care was taken for the grains were not splitted apart and the saw dust and wood pieces were not over cooked. Excess water from the substrates were removed by spreading on sieve made up of fine wire mesh or bamboo basket. Then, the substrates were left as such for few hours on the bamboo basket so that the water on the surface could be evaporated. Later, the substrates were spread on clean surface under shade for drying. Next to drying, the substrates were mixed with chemicals such as Calcium Sulphate (gypsum) and Calcium Carbonate (chalk powder). For one kg of substrates, 20 g of Calcium Sulphate and 5 g of Calcium Carbonate were added (Sharma *et al.*, 2011). Calcium Sulphate was added to remove the excess moisture from the substrates (as a dessicant), whereas Calcium Carbonate was added to maintain the pH 7.0 to 7.8 and not to form clumps on the substrates. First gypsum and chalk powder were mixed separately and then thoroughly mixed to the substrates by spreading on over the clean surface after wearing gloves. The substrates were filled to the small packets then plugged with non-absorbent cotton and cover the cotton plug with newspaper tied neck of bag using rubber band and place all the packets in autoclavable plastic cover and tied with rubber band for sterilization.

5.3 Sterilization of substrates by autoclaving

Sterilization of substrates was carried in autoclave machine at 15 lbs psi pressure at 121^{o}C for 2 hours (Pandey and Tewari, 1990). The bags from autoclave were taken out after the pressure came down. After cooling down the substrates to room temperature, they will be used further.

5.4 Inoculation of cultures into sterilized substrates

Autoclaved bags after cooling down were mixed properly before inoculation so that water droplets were not accumulated inside the bags. The bags were kept inside the laminar air flow chamber under UV light for 20-30 minutes before inoculation. Mycelium discs from the healthy growing petri plates containing cultures were picked up by using sterilized cork borer of 6.5 mm size. The mycelial discs

were transferred to the bags containing substrates. The bags were shaken well to get the culture discs to be distributed equally. The strain number and date to inoculation were noted in the bags after inoculation.

5.5 *Incubation of polypropylene bags for mycelium growth*

Once the inoculation was done, the bags were then incubated in BOD incubator at 27± 1°C for mycelium growth. After 7-8 days of inoculation, the bags were gently shaken to spread mycelium and to cover all over the bags uniformly. During incubation the bags were regularly examined for mould infection and contaminated bags were removed immediately to avoid spreading of contamination to other healthy bags. The fully colonized bags with mycelia were kept in cold room at 4°C for future use.

5.6 Statistical analysis

The laboratory experiment was carried out under Completely Randomized Design (CRD) with five replications. Each substrate was treated as treatment and mushroom culture strains as well. The analysis of variance was performed by using SPSS version and means were compared by Duncan's multiple range tests at 5% level of probability for interpretation of results (Gomez and Gomez, 1984).

3. Results and Disscussions

The results showed that the number of days taken for mycelial growth in petri plate for three strains and spawn standardized of shiitake mushroom by using various grains, sawdust and wood pieces as substrates.

6.1 *Mycelial growth of shiitake mushroom strains on PDA media*

Growth of mycelium for three different strains of shiitake mushroom on PDA media was observed in petri plates till 20 days after inoculation at every 4 days intervals (Plate 9 [a] and [b]). The results showed that the maximum linear mycelial growth was observed in strain Meghalaya Local (89.74 mm) at 20th day (Plate 10) and the minimum mycelium growth was recorded in DMR 388 (17.10 mm) at 4th day as shown in Table 1 and Fig.1.

The growth of mycelium was observed maximum on 4 days after inoculation, in strain Meghalaya Local (17.80 mm), which was on par with the strain DMR 32 (17.70 mm) and the minimum growth was shown by DMR 388 (17.10 mm). The strain Meghalaya Local showed maximum mycelium growth (36.10 mm), followed by strain DMR 32 (35.00 mm) and the minimum growth was observed in DMR 388 (33.00 mm) on 8 days after inoculation.

Table. 1: Mycelial growth of three strains of shiitake mushroom on PDA media

STRAINS / DAI	Growth of mycelium (mm)*		
	DMR 388	DMR 32	Meghalaya Local
4	17.10	17.70	17.80
8	33.00	35.00	36.10
12	51.80	58.20	59.20
16	68.40	70.40	72.00
20	85.40	87.60	89.74
Mean	51.14	53.78	54.97
SE(m) ±	0.345		
CD @ 5%	0.976		

Note: *Values are the mean of five replications, DAI - Days After Inoculation.

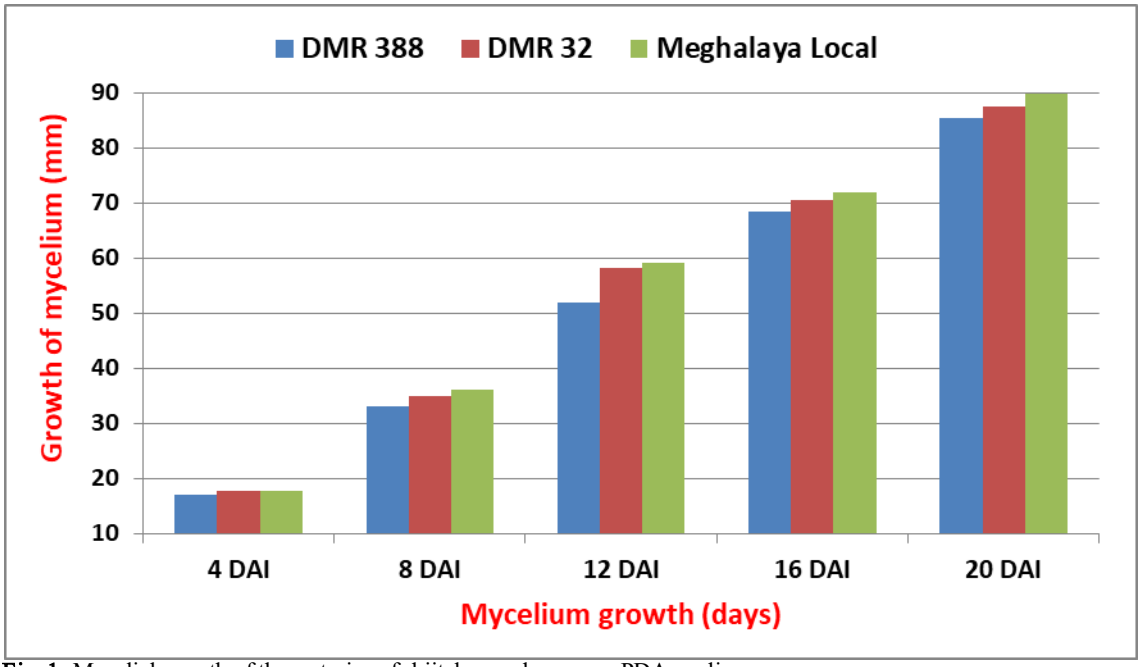
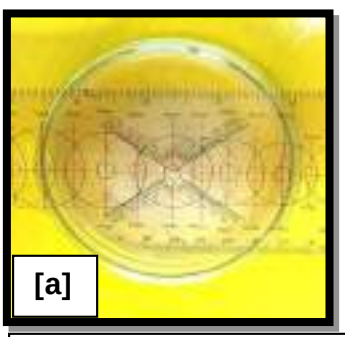


Fig. 1: Mycelial growth of three strains of shiitake mushroom on PDA media
Note: DAI - Days After Inoculation.



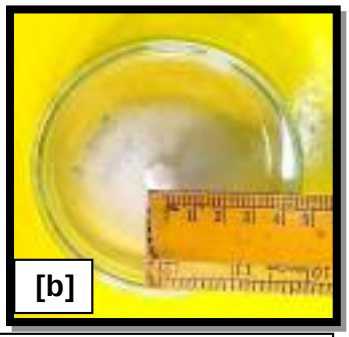


Plate 9 Measuring mycelial growth at different days interval of time [a] and [b]

Plate 10 Fully grown mycelium on 20 days after inoculation

The maximum mycelium growth was recorded in strain Meghalaya Local (59.20 mm) on 12 days after inoculation, followed by strain DMR 32 (58.20 mm) whereas, the minimum growth was observed in DMR 388 (51.80 mm). The strain Meghalaya Local showed the maximum growth of mycelium (72.00 mm), followed by strain DMR 32 (70.40 mm) and the minimum growth was seen in DMR 388 (68.40 mm) on 16 days after inoculation. The complete coverage of mycelium in the petri plates were noticed on 20th day of inoculation with the highest growth *i.e.* 89.74 mm, 87.60 mm and 85.40 mm in the strains Meghalaya Local, DMR 388 and DMR 32 respectively as represented in Fig.1.

The present results confirmed the earlier findings of (Gbolagade *et al.,* 2005) who recorded 92.7 mm of mycelial growth of shiitake mushroom on PDA media followed by 92.0 mm on yellow corn agar media.

Chittaragi *et al.* (2018) studied the mycelial growth of shiitake mushroom (*Lentinula edodes*) on different media. He found that the highest growth was obtained in sorghum meal agar for the strain OE-388S (69.5 mm) and the lowest growth rate was seen in strain LE-16-02 (46.25 mm). Further, he got similar results with wheat meal agar (WMA), PDA and bajra meal agar (BMA), where the highest growth rate was observed in the strain OE-388S (66.5 mm, 59 mm and 56.5 mm respectively) and the lowest in LE-16-02 (with 39.5mm, 46.5 mm and 49.25 mm respectively on PDA, WMA and BMA media). In the present work also, PDA media was evaluated for three different strains of shiitake mushroom for mycelial growth and found the best for the growth of all three strains.

Muthu and Shanmugasundaram (2015) carried out an experiment to analyse the growth performance of *Agrocyba*

aegerita mushroom cultures using different media. They found that the highest mycelial colony diameter of 88.2 ± 0.27 mm in malt extract agar (MEA) media followed by PDA media with 80.0 ± 0.86 mm.

6.2 *Number of days taken for production of spawn of shiitake mushroom strains in different substrates*

The total number of days taken by different substrates for spawn production of three different shiitake mushroom strains were evaluated *in vitro*. Results showed that among three strains and 12 different substrates used, the fastest growth of mycelium with lesser days was observed in sorghum grains (14.40 days) *i.e.* less number of days for growth for the strain Meghalaya Local. It was found that highest number of days taken for full mycelium growth was observed in sawdust of pine tree (60.00 days) for the strain DMR 388 as shown in Table 2 and Fig.2.

Out of 12 different substrates evaluated, sorghum grains took (31.00 days) lesser number of days for complete covering of spawn bags with mycelium growth, followed by wheat grains (32.20 days). Other grain substrates, pearl millet, maize, wood pieces of willow tree and sawdust of willow tree took 33.80, 36.00, 37.00 and 38.00 days respectively (Plate 11). Combination of maize grains and saw dust of jack fruit tree took 39.80 days for full growth, followed by wood pieces of jack fruit tree (41.00 days), paddy grains (42.00 days), sawdust of jack fruit tree (44.00 days) and wood pieces of pine tree (58.00 days) whereas sawdust of pine tree took (60.00 days) for full growth on substrate in strain DMR 388.

In case of the strain DMR 32, sorghum grains took lesser days for complete mycelium growth (22.20 days), followed by wheat grains (23.20 days). It was followed pearl millet grains took (32.00 days), maize grains (34.00 days), wood pieces of willow tree (35.00 days), saw dust of willow tree (36.00 days), combination of maize grains and saw dust of jack fruit tree (37.00 days), wood pieces of jack fruit tree (38.60 days), paddy grains (39.00 days), saw dust of jack fruit tree (41.00 days) and wood pieces of pine tree (54.60 days) whereas the maximum number of days taken for complete covering of mycelium was observed on saw dust of pine tree (59.60 days) respectively.

Strain Meghalaya Local (ML) showed its full growth in sorghum grains with 14.40 days, which was followed by wheat grains (17.80 days), followed by pearl millet grains took (24.20 days), maize grains took (26.60 days), wood pieces of willow tree (29.20 days) sawdust of willow tree (30.80 days), combination of maize grains and saw dust of jack fruit tree (34.80 days), wood pieces of jack fruit tree (35.40 days), paddy grains (37.40 days), sawdust of jack fruit tree (40.00 days) and wood pieces of pine tree (50.00 days). The highest number of days taken for complete covering of mycelium was observed on sawdust of pine tree (56.60 days). In all the three different strains of shiitake mushroom, sorghum grains (14-31 days) showed significantly lesser number of days for complete mycelium growth, followed by wheat grains (17-32 days), whereas maximum number of days were taken in the sawdust of pine tree (56-60 days) for production of spawn as shown in Fig. 2 and Plate 11.

Table. 2: Number of days taken for production of spawn of shiitake mushroom strains in different substrates

STRAINS / SUBSTRATES	Growth (Days)*			
	DMR 388	DMR 32	Meghalaya Local	Substrate Mean
Paddy Grains	42.00	39.00	37.40	39.70
Pearl Millet Grains	33.80	32.00	24.20	30.00
Maize Grains	36.00	34.00	26.60	32.20
Wheat Grains	32.20	23.20	17.80	24.40
Sorghum Grains	31.00	22.20	14.40	22.50
Maize Grains + JFT (SD)	39.80	37.00	34.80	37.20
Willow Tree (WP)	37.00	35.00	29.20	33.70
Willow Tree (SD)	38.00	36.00	30.80	34.90
Jack Fruit Tree (WP)	41.00	38.60	35.40	38.30
Jack Fruit Tree (SD)	44.00	41.00	40.00	41.60
Pine Tree (WP)	58.00	54.60	50.00	54.20
Pine Tree (SD)	60.00	59.60	56.60	58.70
Mean	41.07	37.68	33.10	
SE(m) ±	Strains = 1.30	Substrates =5.23	Interaction = 0.62	
CD @ 5%	Strains = 3.65	Substrates =14.63	Interaction = 1.75	

Note: *Values are the mean of five replications.

SD - Saw Dust, WP - Wood Pieces, JFT - Jack Fruit Tree.

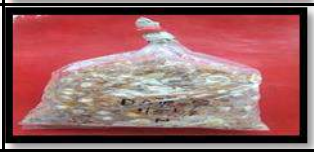
Sl. No	Substrates	DMR 388	DMR 32	Meghalaya Local
1	Paddy Grains			
2	Pearl Millet Grains			
3	Maize Grains			
4	Wheat Grains			
5	Sorghum Grains			
6	Maize Grains +JFT (SD)			
7	Willow Tree (SD)			
8	Jack Fruit Tree (WP)			
9	Willow Tree (WP)			
10	Jack Fruit Tree (SD)			
11	Pine Tree (WP)			
12	Pine Tree (SD)			

Plate 11 Full growth of spawn in bags of three strains of shiitake mushroom in different substrates

Alemu (2014) used only sorghum grains for spawn production of *Pleurotus ostreatus* and found lesser days (25) for complete covering of mycelium. Present study results were similar to the findings of Puri (2011) who evaluated different locally available poplar and teak sawdust, sorghum and wheat grains in combination with chalk powder and gypsum for the spawn production of shiitake mushroom. The fast and maximum covering of spawn in test tubes was noticed by the sorghum grains without any contamination whereas, poplar sawdust took greater time for mycelium coverage. Hence, sorghum used as best substrate for spawn production of shiitake mushroom.

Kumar (2015) evaluated three different mushrooms *viz., Agaricus bisporus, Pleurotus florida* and *Calocybe indica* by using different substrates such as sorghum, maize, wheat, barley, oat and bajra for production of spawn. He found that sorghum grains took lesser days for full growth of mycelium (12.66 days) followed by maize, wheat, barley, oat and bajra took (15.66, 18.66, 19.66 and 25.33 days) for spawn production of *Agaricus bisporus*. Similarly, he got results for *Pleurotus florida* (10.33, 12.33, 14.66, 16.66 17.00 and 22.00 days) and *Calocybe indica* showed (10.66, 13.66, 17.00, 17.66, 18.66 and 23.33 days) for full mycelial growth. Further, he reported that the substrates sorghum, maize and wheat were the best grain substrates for spawn production of different mushrooms. These results supporting the present experiment that sorghum, bajra and wheat grains as the superior substrates which took lesser number of days for mycelium growth.

Similar experiment was conducted by Chittaragi *et al.*, 2018 using different grains such as wheat, sorghum and pearl millet grains alone or in combination with grain powder at 9:1 ratio as substrate for spawn production of shiitake production.

The lesser number of days for spawn production was taken by the sorghum grains (20 days) and the more number of days taken by the pearl millet grains (45 days), combination of pearl millet grain + pearl millet grain powder showed (44 days). The grains took 20, 34 and 45 days growth by sorghum, wheat and pearl millet grains respectively for spawn production. They observed that with the addition of grain powder to the grains took 26, 30 and 44 days on sorghum, wheat and pearl millet grains respectively.

Sorghum and wheat grains took same number of days (12 and 13 days) and the rice grains recorded minimum number of days (10 days) for spawn development of *Pleurotus florida* mushroom (Kalaiselvam *et al.*, 2017). These earlier findings support the current results that, sorghum grains as the best substrates, followed by the wheat and other grains.

Dhiman (2009) stated that wheat + bajra grains showed the highest linear mycelium growth on 6, 12 and 18th day of inoculation (20.7, 58.3 and 97.3 mm respectively), which was followed by bajra grains (18.3, 54.3 and 91.7 mm) and wheat grains (15.8, 50.0 and 88.3 mm). The lowest linear mycelium growth on 6, 12 and 18th day of inoculation was obtained for the wheat straw (9.33, 27.3 and 49.7 mm). The results present work are in agreement with the earlier work.

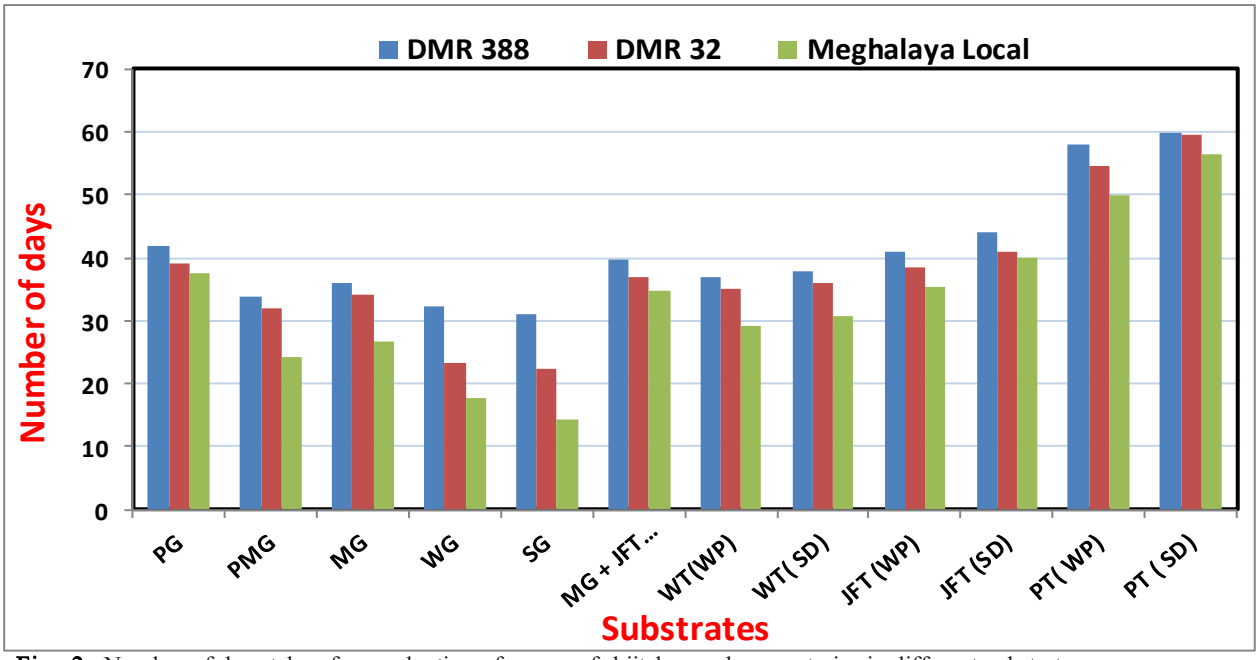


Fig. 2: Number of days taken for production of spawn of shiitake mushroom strains in different substrates

Note: PG - Paddy Grains, PMG - Pearl Millet Grains, MG - Maize Grains, WG - Wheat Grains, SG - Sorghum Grains, WT - Willow Tree, PT - Pine Tree, SD - Saw Dust, WP - Wood Pieces, JFT - Jack Fruit Tree.

The current results for mycelium growth was depicting similar results of Mishra *et al.* (2018), maximum mycelium growth for three strains of mushroom *viz.*, *Pleurotus florida, P. flabellatus* and *P. sapidus* was obtained by maize grains 89.25, 86.50 and 90 mm respectively and sorghum grains 66, 64.5 and 89 mm compared to oat, barley, pearl millet and minimum mycelium growth was found in wheat grain substrate 54.5, 50.75 and 77 mm and maize grain would be recommended as most suitable substrate for spawn growth and cultivation of all three *Pleurotus* spp.

The results of Singh *et al.* (2016) showing that highest mycelium growth was obtained for chickpea grains (98.33 mm) and less growth on barley grains (50.33 mm), followed by wheat (52.33 mm) for spawn growth on 4, 6, 8, 10 and 12th day for different grains which were identical to current investigation for the linear mycelium growth was maximum (89.74 mm) at 20th day and minimum mycelium growth (17.10 mm) at 4th day of inoculation.

6.3 *Economics of Mushroom spawn production*

The economics involved for spawn production from 10 kg of grains/substrates, the required materials were grains/substrates cost is approximately Rs.400, polypropylene bags (100 pcs) is Rs.50, calcium sulphate (gypsum) for 200 g is Rs.40, calcium carbonate (chalk powder) for 50 g is Rs.40, non- absorbent cotton (400 g Roll) is Rs.100 rubber band is Rs.60 and other miscellaneous (autoclave, power supply, boiling drum and BOD incubator instruments for one time use for 10 kg production of spawn) cost is Rs.310. The total expenditure incurred for production of 10 Kg of spawn is Rs.1000 and the total outcome by the sale of 100 spawn @ Rs.30 per bag is Rs.3000. The cost benefit ratio is 1:3 for the production of mushroom spawn.

4. Conclusion

From the research work carried out on shiitake mushroom spawn production, it can be concluded that sorghum grains were found to be the ideal substrate for spawn production of shiitake mushroom, followed by wheat grains compared to other different substrates. Among three strains, Meghalaya Local was the best strain for spawn production of shiitake mushroom. The economics involved for spawn production from 10 kg of grains/substrates, the cost benefit ratio is 1:3 for the production of mushroom spawn.

5. Acknowledgements

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