

Determination of Optimum Spawn Quantity for Maximum Yield of *Pleurotussajorcaju* cultivated on Agro Waste

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ABSTRACT

Mushroom cultivation is one of the promising areas of food and agricultural microbiology. Mushrooms are an excellent food source and addition to any diet or menu. Beyond tasting great, mushrooms are also a very nutritious addition to any cuisine. Mushrooms are by and large high in protein and dietary fiber while being low in fat, cholesterol, and carbohydrates. In the present study the optimum spawn quantity of mushroom *Pleurotussajorcaju* is investigated. The auxiliary objective of the present research is to explore the potential to convert agricultural waste into nutritionally and pharmaceutically useful metabolites. Optimum spawn requirement for 3 kilograms of agricultural and office waste substrate was determined. Different types of substrates were tested for obtaining the yield of *Pleurotussajorcaju*. Carton & paper was found to be a better substrate as compared to wheat straw in terms of time of mushroom pinhead body appearance. It was found that ten per cent or more spawn quantity was optimum for better yield of mushroom. It is also concluded the and standardized that ten per cent spawn on the basis of the dry weight of substrate was required for maximum yield of mushroom *P. sajorcaju*.

Keyword: Mushroom, *Pleurotussajorcaju*, Nutraceuticals, Spawn, Antioxidant activities, Agricultural wastes, Office waste, Wheat Straw

INTRODUCTION

Growing mushrooms is a popular activity all over the world since it produces nutraceuticals and is utilised as a wholesome food source. India is a country with a large agricultural sector, and this sector forms the backbone of the Indian economy. A million tonnes of agricultural trash are produced in India together with important crops. India generates over 600 million tonnes of agricultural waste each year, most of which is burned on-site or allowed to organically decay (Manjit Singh, ICAR, 2011). Mushroom growing is a

bioconversion strategy that is suitable for managing agricultural and agro-industrial leftovers (Chang and Miles, 1992). Therefore, if we use trash in mushroom culture, we may turn agricultural waste into a useful item and also solve the landfill problem. India can create 3 million tonnes of mushrooms and roughly 15 million tonnes of compost by diverting just 1% of agro-waste towards mushroom growing (Manjit Singh, ICAR 2011). Therefore, mushroom farming benefits both the local economy and the economy of India. India raises four to five important mushroom species: Button mushroom (*Agaricus spp*), Oyster

mushroom (*Pleurotus*), Paddy straw mushroom (*Volvariella*), Milky (*Calocybe indica*) and Shiitake (*Lentinula edodes*).

Only the kinds Button and Oyster, which account for about 9 per cent of all Indian production, are grown in Gujarat. As a result, India's mushroom production is gradually but steadily rising. Here is a graph showing the increase in production.

Unique characters of *Pleurotus* spp are:

(1) The Wide Range of Substrates: Unlike other species, oyster mushrooms may easily grow on any cellulose or lignin substrate.

(2) Do not need elaborate controlled conditions: Oyster mushrooms are subtropical mushrooms and may grow at temperatures between 20 and 30 °C and humidity levels between 80 and 90%. By retaining humidity, we can grow it while the temperature rises. As a result, oysters grow best in the winter, and the climate in India is better for oyster mushrooms.

(3) Species Varieties: Oyster mushrooms come in a variety of hues, including white, pink, yellow, light brown, and others. The species of mushroom and the surrounding environment have a significant impact on the colour of the mushroom.

(4) Higher Biological efficiency : In general, productivity is higher than that of other mushrooms, and one can obtain the highest yield.

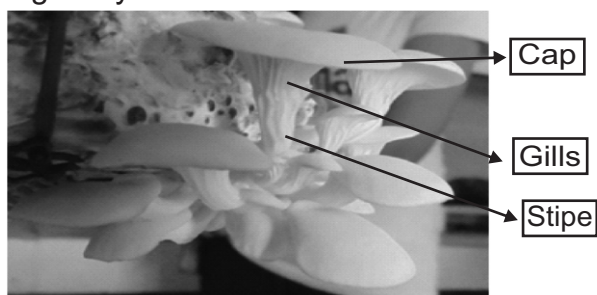


Fig 1: Mushroom *Pleurotus sajor-caju*

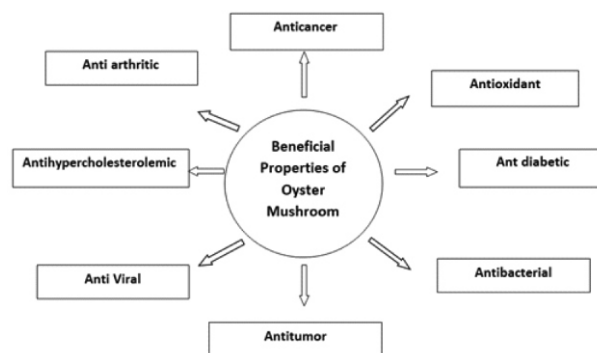


Fig 2: Applications of Oyster Mushroom

Objective :

Determination of optimum spawn quantity for maximum yield of *P. sajorcaju* cultivated on agro waste.

MATERIALS AND METHODS

(1) Experimental site :

The Department of Life Science at Bhakta Kavi Narsinh Mehta University and the Department of Plant Pathology at Junagadh Agricultural University carried out the cultivation and laboratory experiments.

(2) Experimental duration:

The experimental work has been done for total 6 months in winter-early summer season.

(3) Experimental materials:

The experiments were conducted on *Pleurotus sajorcaju*. Wheat straw and coconut husk used as substrates and sorghum seed were used as spawn.

A . Preparation of pure culture of *P. sajorcaju* :

Because it has a good source of carbohydrates and has an acidic pH, which both support fungal growth, Potato Dextrose Agar was chosen as the medium. In the laminar air flow, inoculation was performed by slicing tiny sections of the

mushroom tissues from cap to base using a sterile blade. The inner area of the junction between the stalk and the pileus was sampled for tissues. A small piece was inserted into petri dish. Plates were incubated at 25° C or room temperature. After 5 to 6 days, the white mycelia fully covered the plate. Microscopic structure of fungi was observed with the help of lactophenol blue stain in 10x and 45x. Fungal growth was transferred into PDA slant and preserved in the refrigerator.

B. Preparation of master and mother spawn bags :

In simple words, spawn is grains covered with mushroom mycelium & used as propagating material by a grower. A pure culture of *P sajorcaju*, sorghum seeds, Chloramphenicol antibiotic capsules, CaCO₃ powder, plastics bags, cotton plugs etc were utilized for the said preparation.

Good quality of sorghum seeds (around 5 kg) were taken and washed it with water thoroughly. Seeds were taken in the vessel and sufficient amount of water was added and the antibiotic capsule was added (1 capsule was added per 1 kg of grain) Then, grains were boiled until they became soften but did not split apart. After it cooled down, excess water was removed and seeds were spread over the cotton cloth. Again antibiotic was added to prevent contamination. CaCO₃ was mixed with grains for maintaining the pH. After proper mixing, plastic bags were filled with grains and mouths of bags were plugged with non absorbent cotton and pipe. All bags were autoclaved at 121° C, 15 psi. A pure culture of fungi was inoculated inside the laminar air flow, and bags were incubated in walking chamber.

C. Preparation of mother spawn bags:

The mother culture underwent the same process. Grain that had been boiled was combined with an antibiotic and CaCO₃. Following the preparation and autoclaving of the bags, inoculation was created by transferring grains from the master bag to the new bag. With the aid of the sterile spatula, approximately 5gm of master spawn grains were injected in mother spawn bags inside the laminar air flow. In the walking chamber, all of the bags were incubated. The experiment was conducted using bags that were completely coated in mycelia after a few days of growth.

D. Cultivation of Mushroom *P. sajorcaju*:

3 steps were followed for cultivating the mushroom on different substrate and determination of optimum spawn quantity for its maximum yield.

- i. Substrate preparation
- ii. Preparation of mushroom bags
- iii. Harvesting

i. Substrate preparation:

Separately, 8 to 9 kg each of wheat straw and coconut husk were placed in a tub and left to soak for 24 hours in water containing 50 ml of formaldehyde and 5 g of bavistin-fungicide. The saturated substrate was retrieved after 24 hours and given two more washes with plain water. After washing, any extra water was wiped away. When dealing with office garbage, small pieces of paper and carton were placed inside a plastic bag before the bag was autoclave sterilised at 121°C and 15 psi pressure. Papers and carton pieces were employed as substrates following sterilisation.

iii. Preparation of mushroom bags

In order to give the plastic bag a pleasing oval shape, the bag's end was tied with

plastic thread after being inverted. After then, bags were changed back. The substrate was then taken, the extra water was squeezed out, and the first layer of substrate was created in the bag. Hands were cleaned with alcohol before harvesting spawn. Around the edge of the bag, a layer of spawn was created. The substrate was then created in a second thick layer in the same manner. A new spawn layer was created. The substrate and spawn were formed in layers of 4 to 5, with the last layer of the substrate being made thinner than the others. To ensure there is no air within the bag, the thread was tightly tied to the bags. The bag's perforations for aeration were cut with sterilised scissors. Bags were stored in a room that had a 20 to 25 °C temperature and an 80 to 90% humidity level. Three bags of 9 to 10 kg wet substrate each weighed around 3 kg each were manufactured. Typically, 250 to 300 gm of spawn were utilised per 2.5 to 3 kg of substrate for 1 bag. 50gm, 100gm, 150gm, 200gm, and 250gm of spawn were utilised per 2 kg of dry substrate to find the ideal spawn rate. 3 replicas were made for 1 spawn treatment; meaning that total 3 bags were prepared those contain 50 gm spawn per 2.5 kg substrate. Same procedure was repeated for paper and carton in the bag and in between the layer water spraying was carried out for maintaining the moisture.

iv. Harvesting:

After 8 to 9 days, when mycelia had colonised the entire bag, the bag was opened, and the plastic was removed. To keep the substrate moist when the bag was taken off, water was sprayed twice daily. After the fruiting body had emerged, water was sprayed only on the substrate, avoiding

the fruiting body itself. A mushroom is harvested when it reaches maturity by removing it from the substrate, and the fresh weight of the mushroom is recorded in a weigh scale. Mushrooms were stored in plastic bags in the refrigerator for later analysis.

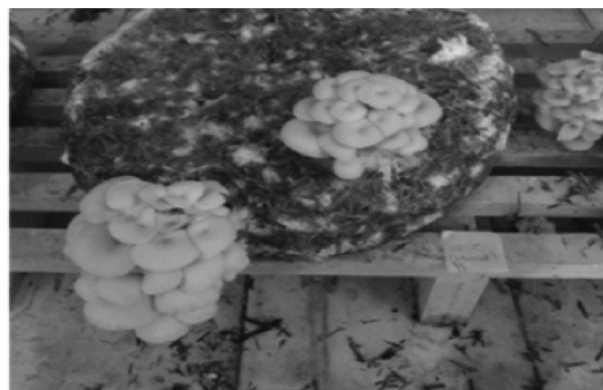
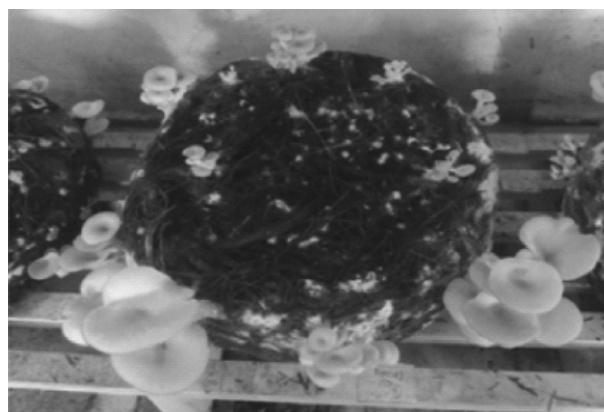


Fig 3: Mushroom harvested after maturation



Biological Efficiency (BE):

Biological efficiency means the substrate's efficiency to produce a maximum product. It is calculated by the given formula:

$$BE = \frac{\text{Total yield or total weight of mushrooms}}{\text{Total weight of dry substrate}} \times 100$$

Period between flushes:

The period between 1st harvest to 2nd time harvest is essential because it gives an idea about fungal colonization and capacity of

substrate to give best propagating medium to fungi. It can be calculated by,

$$\text{Period between flushes} = \frac{\text{Days taken for first harvest to last}}{\text{Total number of harvesting.}}$$

RESULTS AND DISCUSSION

PDA plates were used to cultivate the fungus *P. sajorcaju*. With the use of lacto phenol cotton blue stain, the microscopic structure of fungus and fungal spores was examined under 10X and 45X magnification. The outcomes were as follows:

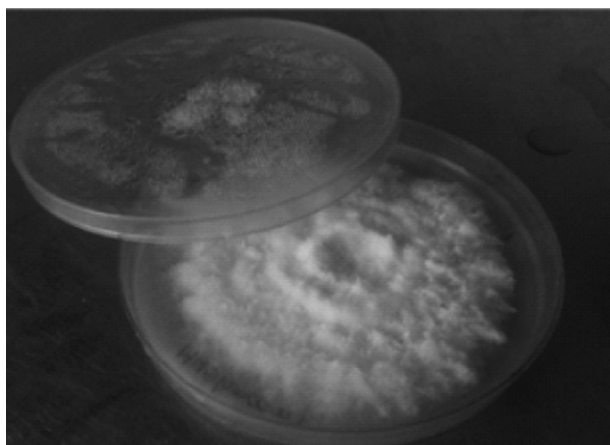


Fig 4 : Fungi *P. sajorcaju* on PDA plate

Cultivation of mushroom on different agro waste substrates :

It has been noted that mushrooms may grow readily on both coconut fibres and wheat straw. Additionally, *P. sajorcaju* can grow on coconut husk, according to reports by Nurudeen T.A. et al. (2013) and Shubhra Shukla and A. K. Jaitly (2011).

Effect of spawn quantity on spawn run days, formation of pinhead body as well as 1st maturation.

It was shown that spawn quantity had a

P. sajorcaju mushroom cultivation and biochemical characterization were completed. Wheat straw and coconut husk (fibre) were used as the two different substrates for growing *P. sajorcaju*. To establish the ideal spawn quantity for the highest possible mushroom yield, multiple amounts of sorghum spawn were utilised. 50gm, 100gm, 150gm, 200gm, and 250gm of spawn used per 2 kg dry substrates. It was observed that quantity of spawn, quality of spawn, quality substrates, highly affected the yield of mushrooms. Temperature, humidity and water spraying have affected the color of mushroom as well as the yield of mushroom.

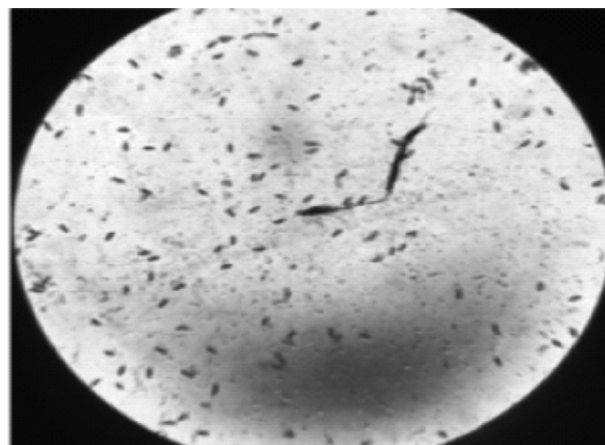


Fig 5: Basidiospores of fungi *P. sajorcaju*

significant impact on mycelial growth, the fungi's time to colonise an area, the number of pinhead bodies produced per day, the days it took for pinhead bodies to become apparent, the maturity period, and the number of fruiting bodies. On wheat straw, the 250 gm spawn rate had the fastest spawn run (9 days), which was followed by the 200 gm spawn rate (14 days). The 150gm spawn took a moderate amount of time, and the 100 and 50gm spawn rates ran the slowest. Pinhead bodies first

showed up in 250gm spawn (over the course of 11 days), then in 200, 150, 100, and 50 gm spawn. Mature fruiting bodies developed in 250 and 200 gm spawn in less

time (17,19 days respectively) whereas in 150, 100, 50 gm time was increased (22,26,37 days respectively) with a decreased quantity of spawn (Table 1)

Table 1: Effect of Spawn Rate on Spawn run days, Pinhead body Formation & Maturation

Substrate	Spawn Rate (Gm)	Spawn Run Days	Pinhead body Formation (Days)	Days taken for 1 st Harvest
Wheat Straw	50	25	27	37
	100	20	21	26
	150	17	18	22
	200	14	15	19
	250	9	11	17
Coconut	50	14	15	19
	100	12	13	18
	150	10	12	17
	200	8	11	16
	250	7	9	14

Effect of different spawn quantity on total yield of mushrooms and biological efficiency of the substrates:

On a 2 kg dry weight basis, it was shown that substrates with 250 and 200gm spawn produced the maximum yield. 250 gm of spawn produced 1048 gm of 52.2% BE on wheat straw and 1037.8 gm of 51.89% BE

on coconut. With less spawn available, the yield reduced. In both situations, spawn rates of 150, 100, and 50 gm resulted in subpar yield and BE. (Table 2). Additionally, it was noted that, in comparison to 150, 100, and 50, the time between 2 flushes was significantly shorter in the higher spawn rates of 250 and 200.

Table 2: Effect of Spawn Rate on Yield of mushroom and Biological Efficiency of Substrates

Substrate	Spawn Rate	Period between Flushes	Yield (gm) per 2 kg dry substrate				Biological Efficiency (%)
			1st harvest	2nd harvest	3rd harvest	Total yield	
Wheat Straw	50	12	44	32	30	106	5.3
	100	8.66	64	50	40	154	7.7
	150	7.33	90	75	55	220	11.0
	200	6.66	395	355	265.2	1015.2	50.76
	250	5.66	410	367	263	1042	52.1
		C.D.	15.73	10.82	9.25		3.91
		C.V.%	4.31	3.38	3.89		8.46
Coconut	50	6.33	44	32.5	22.5	99	4.9
	100	6	62	43	31.09	136.09	6.80

150	5.60	71	53.5	35.2	159.7	7.98
200	5.33	370	310	298.66	978.6	48.93
250	5	395	353.2	289.66	1037.8	51.89
	C.D.	7.05	7.40	5.34		4.20
	C.V.%	2.06	2.57	2.17		9.59

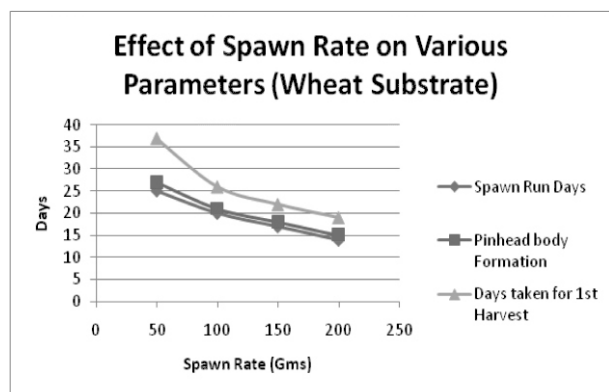


Fig 6 : Effect of Spawn Rate on Spawn Run days, Pinhead body Formation & Maturation (Wheat Straw Substrate)

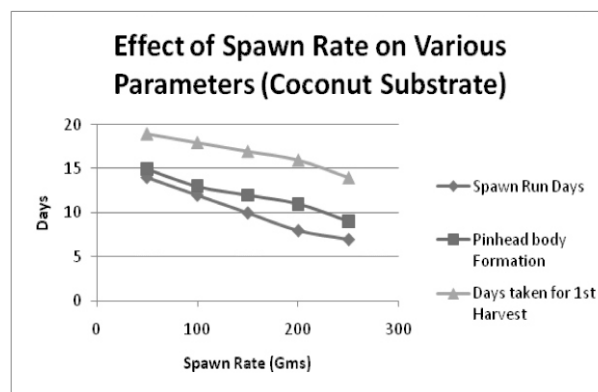


Fig 7 : Effect of Spawn Rate on Spawn Run days, Pinhead body Formation & Maturation (Coconut Substrate)

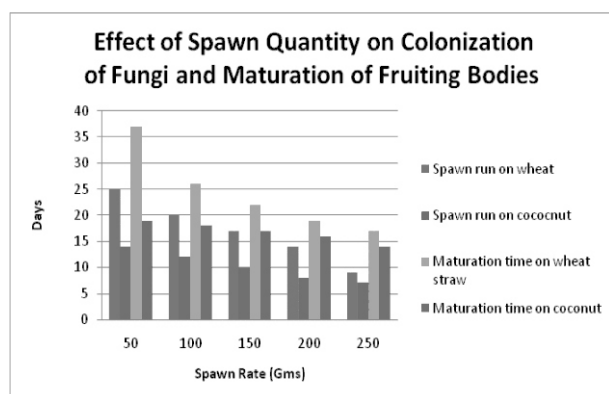


Fig 8: Effect of Spawn Quantity on Colonization of Fungi and Maturation of Fruiting Bodies

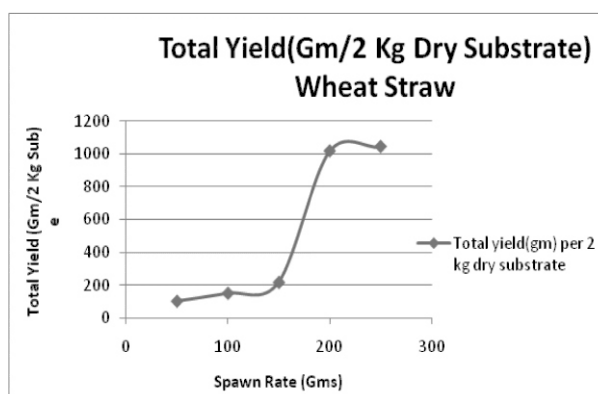


Fig 9 : Effect of Spawn Quantity on Yield of Mushroom (Wheat Straw)

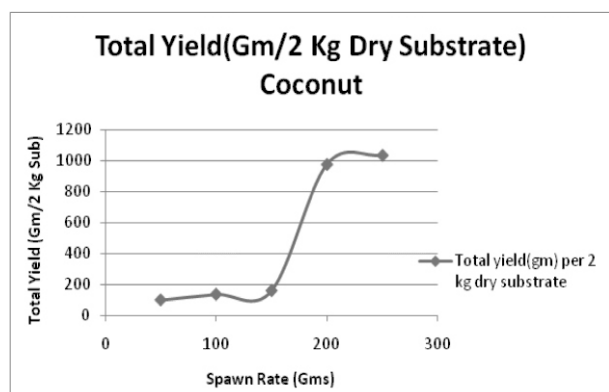


Fig 10 : Effect of Spawn Quantity on Yield of Mushroom (Coconut)

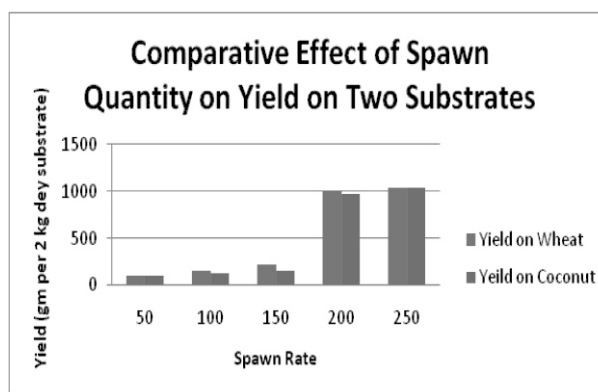


Fig 11: Effect of Spawn Quantity on Yield of Mushroom



Fig 12: mature fruiting bodies in 150 gm spawn on wheat straw.



Fig 13: mature fruiting bodies in 150 gm spawn on wheat straw.

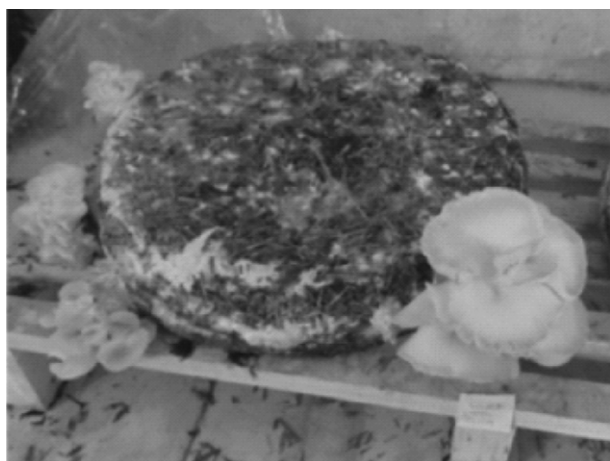


Fig 14: Pinhead bodies in 200 gm spawn on wheat straw

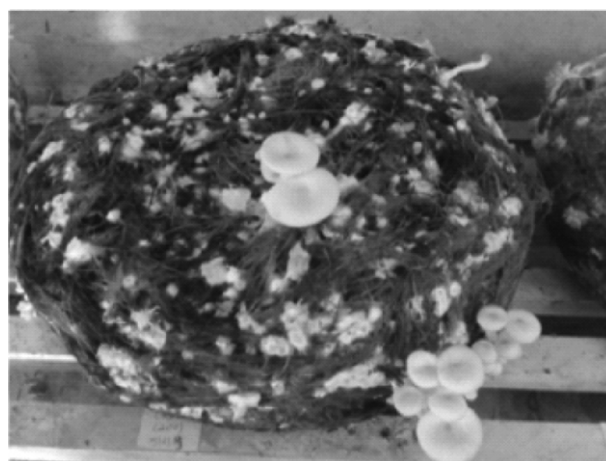


Fig 15: Pinhead bodies in 200 gm spawn on coconut husk



Fig 16: Mature fruiting bodies in 200 gm spawn on wheat straw



Fig 17 : Mature fruiting bodies in 200 gm spawn on coconut

It was found that when spawn number rose, maximum yield and BE (200 and 250 gm spawn) were attained. In increased spawn quantities (200 & 250 gm spawn), spawn run time, pinhead body appearance, and maturation were all significantly reduced. It was discovered that 10% or more spawn quantity was ideal for greater mushroom yield. The same experiment also showed that 10% spawn, based on the dry weight of the substrate, was necessary for the mushroom *P. sajorcaju* to produce its highest production. 10% spawn was required for oyster mushroom production, according to R.C. Upadhyay (ICAR, 2011). According to M.I.Bhatti et al. (2007), 1kg of substrate required 70gm of spawn. According to Pradeep et al. (2018), the 6% of the dry substrate spawn of bajra seed produced the best yield. They also noted that the six per cent spawn had the shortest spawn run, lasting just 15 days, and the six per cent spawn produced pinhead bodies in just 17 days. The maximum yield (540gm/1kg) with 54 per cent BE came from 6% of the spawn. *P. pulmonarius* was grown on wheat straw by Jogindar Pal et al. (2017), who reported that 8% spawn produced the maximum yield (168.7 per 200 g dry substrate) and had an 84.33 per cent BE. With a drop in spawn rate, yield and BE fell. They discovered that the 8 per cent spawn had the fastest spawn run (10.50 days) and took much less time than the 6 per cent, 4 per cent, 2 per cent, 1 per cent, and 0.5 per cent spawns to form pinhead bodies and mature. The physiological needs for oyster mushroom growing, such as steady temperature, humidity, and lighting arrangements, may vary under controlled or semi-controlled settings, explaining the discrepancy between the results published

by other study workers. The response of various substrates varies in terms of how long it takes for pinheads to form, how long it takes for fruiting bodies to mature, how long it takes between flushes, how many flushes there are, and how much yield is produced. Results are much closer to those of other researchers, nevertheless.

CONCLUSION

With the addition of 10 per cent spawn on a dry weight basis of the substrate, oyster mushroom *P. sajorcaju* can grow readily on agricultural wastes such wheat straw and coconut husk at low temperature and high humidity. So, by growing mushrooms, we contribute to the local economy as well as the Indian economy by turning garbage into a useful good. Due to its excellent nutritional value (high protein, low fat, and fibre content), mushrooms are utilised as food supplements and are also beneficial for diabetic and cardiac patients. The market is flooded with natural organic items that work as antioxidants, but many of them are prohibitively expensive or difficult for everyone to access. However, the mushroom is the only product that is both an antioxidant and a meal. One of the promising fields in food microbiology, agricultural microbiology, cancer biology, gerontological sciences, and bioconversion technology is mushroom farming. Therefore, by using office and agricultural waste to produce mushrooms, we can boost the economy, reduce trash, recycle waste, and, most significantly, enhance health and make a step towards curing cancer.

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