IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

STANDARDIZATION OF DIFFERENT MEDIA FOR CULTIVATION OF WOOD ROTTING AND MEDICINAL FUNGUS - Lentinula edodes

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Abstract: This study was conducted to evaluate the local grain substrate, namely rice, corn, bajra, sorghum, wheat, for seed production using the small strain DMR 388. The results showed that the maximum mycelium growth on PDA medium was observed in the strain (89.4 mm) on the 20th day after inoculation, while minimal mycelium growth was observed in the wheat extract medium (72.50 mm). Among the 6 different substrates used for spawning production, the fastest mycelium growth was observed in paddy and wheat grains (31, 34.60 days, respectively), while the largest number of days was obtained in bajra (44.00 days) for the small strain DMR 388. In another experiment, various agricultural waste products such as wheat straw, paddy straw, corn straw, corn shelled cobs, sugarcane bagasse and saw dust with combination of corn shelled cobs were tested as substrates for indoor growing shiitake mushrooms using small strain DMR 388. Rapid mycelium growth was found on substrate corn shelled cobs (55.4 days). But mycelium growth was very slow on corn straw substrate (84.4 days). The yield attribute is not displayed because the fruiting body has not formed due to physical influences such as temperature, humidity, etc.

Index Terms **- - DMR 388 small, Spawn, Substrate, Fruit bodies.**

I. INTRODUCTION

Lentinus edodes (Berk.), Shiitake mushrooms, account for 17% of the world's production weight (Chang and Miles, 2004; Miles and Chang, 1997). It can grow in winter and can also grow year-round under controlled conditions. After button mushroom [*Agaricus bisporus* (J.E. Lange) Imbach], shiitake is the most widely cultivated mushroom in the world (Chang and Miles, 2004). Fungi are saprophytic and grow on dead material (Chang and Miles, 2004). Various types of trees have been used for cultivation, but most of the production is made from oak (*Quercus* spp.) (Harris, 1986; Stamets and Chilton, 1982; Przybylowicz and Donoghue, 1988). Shiitake mushrooms have a long shelf life, because the fruiting bodies are dried before sale; most other mushrooms are sold fresh. Shiitake mushrooms have been reported to strengthen the immune system, lower cholesterol levels, act as an anticoagulant, and can be used in the treatment of certain cancers (Tokuda, *et al*., 1974; Fujii, *et al*., 1978; Suzuki, *et al*., 1979; Mizuno, 1995; Wasser, 2002).

Fungi depend on the substrate for nutrition, and the substrate is usually a source of lignocellulosic material that supports the growth, development and fertilization of fungi (Chang and Miles, 2004). Sawdust is the most popular base material used in substrates for Shiitake production (Miller and Jong, 1987; Palomo, *et al*., 1998; Grodzinskaya, *et al*., 2003). Other main ingredients may include straw and corn cobs, or a mixture thereof. Regardless of the main ingredient used, starch-based additives such as wheat bran, rice bran, millet, rye, or corn can add 10 to 40% dry weight to the main ingredient (Ivan, *et al*., 2003; Royce, *et al*., 1990; Royce 1996). The abundance of sawdust freely available from various trees is a potential alternative substrate source for growing fungus in the tropics. This study was conducted to assess the suitable environment, spawning growth and yield of Shiitake mushrooms on the various available agricultural wastes.

RESEARCH METHODOLOGY

2.1. Experimental materials

The experiment was carried out at the mushroom spawn laboratory, Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, 221005, between July and December. Sawdust from Mango tree, collected from the sawmills, and different agricultural wastes collected from Agricultural Farm such as paddy straw, Wheat straw, Sugarcane bagasse, Maize shelled cobs, Maize straw, and combination of saw dust with maize shelled cobs were used as substrates. The substrates were dried for 4 to 5 days fully exposed outdoors and then stored for 15 days. The experiment was laid out in a completely randomized design (CRD) with 6 treatments and replicated 3 times.

2.2. Preparation of pure culture

The Potato dextrose agar (PDA) medium was prepared using 200 g of peeled and sliced potatoes, 20 g of dextrose and 20 g of agar in one litre of water. Clean sliced potatoes (200 g) boiled in one litre of water until the slices are soft. The boiled potato slices are mashed and filtered and the filtrate is made up to 1 litre with distilled water. The potato extract obtained was then heated and stirred after adding 20 g of dextrose and 20 g of agar. The heating was stopped when the agar was dissolved in the solution. The media was allowed to cool before 10 ml of each PDA preparation was distributed into test tubes, clogged and sterilized at a pressure of 121ºC and 15lbs. The test tube containing the sterilized PDA is kept in an oblique position. This slope is maintained to inoculate the fungus. The agar and tube are allowed to cool in an upright position for coating and insulation. To get a pure culture used the PDA culture method or tissue culture. A small piece of tissue is taken from the fruiting body of the Shiitake mushroom and placed on PDA media which is sterilized under aseptic conditions. These are incubated at 25ºC for 7-10 days for sufficient mycelium growth. Pure culture is obtained by subculture.

The pure culture of DMR 388 small, was sub-cultured 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 25 ± 2ºC in B.O.D. incubator for one to two weeks for mycelial growth. The plates with fully grown mycelium were kept in refrigerator at 5-10ºC for further inoculation to substrates.

2.3. Substrates preparation

2.3.1. Spawn Production

For spawn production, healthy cereal grains are selected, free from pests and diseases. After cleaning and draining the water, the substrate is boiled in a vessel for 20-25 minutes until the substrate softens, make sure the granules do not come off, sawdust and wood chips are soaked in water overnight with the addition of formalin. The substrate is then placed on a clean surface in the shade to dry. After drying, the substrates are mixed with chemicals such as calcium sulphate (gypsum) and calcium carbonate (lime powder). To 1 kg of substrate was added 20 g of calcium sulphate and 5 g of calcium carbonate (Sharma, *et al*., 2011). The substrate was transferred in an autoclave at 15 psi and 121 ° C for 2 hours (Pandey and Tewari, 1990).

2.3.2. Cultivation conditions

The bags are then placed tilted downward in the working space of the spawning area at 20–23 ° C in the dark and 65–70% relative humidity until the completion of the spawning grounds. After the spawning is finished, the temperature and relative humidity are changed to 19-20 ° C and 80-90% RH. The bag has already been cut and the pieces are folded back. Water is sprayed to keep humidity to the desired level in the form of a fine mist from the nozzle.

2.3.3. Fructification

To get a homogeneous product, a thermal shock is needed which consists of changing the base temperature, namely reducing the base temperature to 4 - 100 ° C. into the fruit room, and the plastic bag is moved. During fruiting, a temperature of 15 to 20ºC, 80-93% humidity is maintained almost in total darkness. Water used for humidification must contain 2 ml / L of sodium hypochlorite at a concentration of 5%. The production in each bag will gradually decrease until fertilization stops due to the decay of the mycelium.

Mycelial running rate (MRR) on each substrate was estimated on the basis of the ratio between the total distance covered by the mycelium and the time needed for growth to occur

$MRR = L/N\ (cm.day^{-1})$

where L = average length (cm) of mycelium running measured, and N = number of days.

2.4. Statistical analysis

The experiment was carried out under Completely Randomized Design (CRD) with three replications, Analysis of variance was performed and means were separated using Duncan's multiple range test (DMRT) at 5% level of probability (Gomez and Gomez, 1984).

IV. RESULTS AND DISCUSSION

4.1 Mycelial growth of shiitake mushroom on different media

Growth of mycelium on different media was observed in petri plates till 20 days after inoculation at every 4 days intervals, which showed that PDA medium, Paddy extract agar medium, least growth was seen in wheat extract agar medium in which it has taken more than 20 days for full growth in the plate.

S.No.	Culture Media	Radial growth (cm) at different interval				
		4DAI	8DAI	12DAI	16DAI	20DAI
1	Potato Dextrose Agar Medium	17.10	33.00	51.80	68.40	89.40
2	Paddy Extract Agar Medium	17.00	32.45	48.20	67.30	87.55
3	Bajra Extract Agar Medium	15.60	29.80	45.40	64.55	82.45
4	Sorghum Extract Agar Medium	14.50	28.60	43.60	60.65	80.47
5	Corn Extract Agar Medium	12.45	25.58	40.30	59.70	78.30
6	Wheat Extract Agar Medium	10.55	25.15	38.80	56.70	72.50
SEm ±		0.502	1.004	0.482	0.669	0.516
CD at 5%		1.563	3.129	1.501	2.084	1.608

Table 1: Mycelial growth of shiitake mushroom on different media

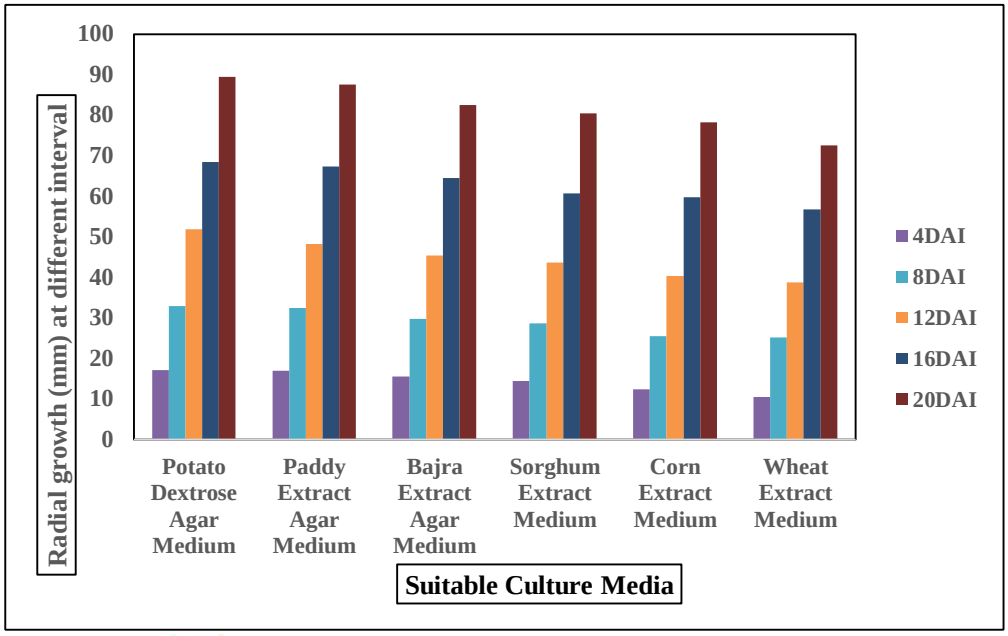


Fig 1: Mycelial growth of shiitake mushroom on different media

4.2. Number of days taken for production of spawn in different substrates

The total number of days taken by different substrates for spawn production of shiitake mushroom were evaluated under *in vitro* conditions. The different substrates used in this experiment were grains of sorghum, wheat, pearl millet, paddy, maize and the combination of maize grains and sawdust was also evaluated for spawn production. Results showed that among 6 different substrates used, the fastest growth of mycelium with lesser days was observed in paddy grains (31.00 days). It was found that highest number of days taken for full mycelium growth was observed in bajra seeds (44.00 days) for the small strain DMR 388 as shown in Fig.2, and Fig.3.

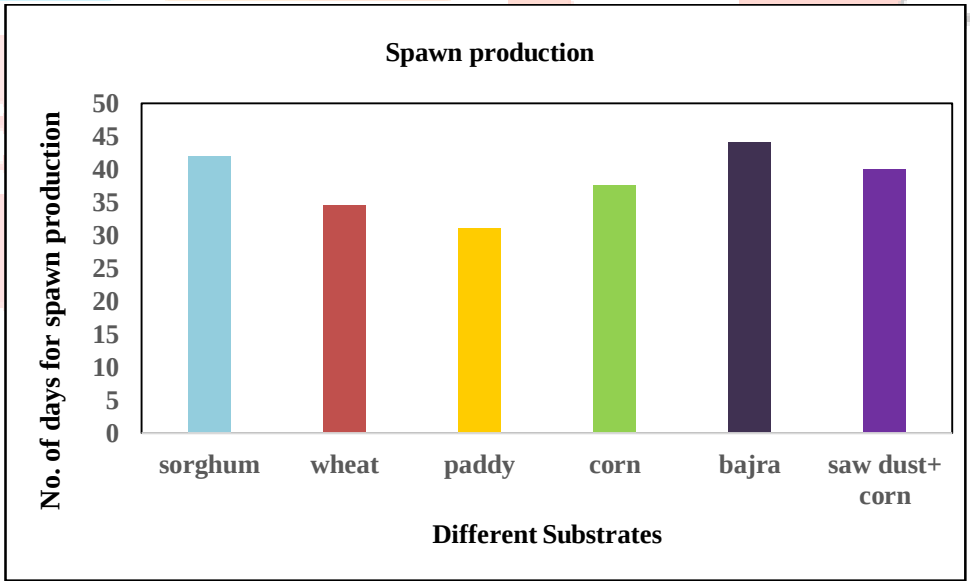


Fig 2: No. days taken for spawn production on different substrates

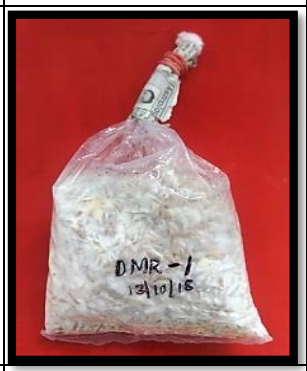
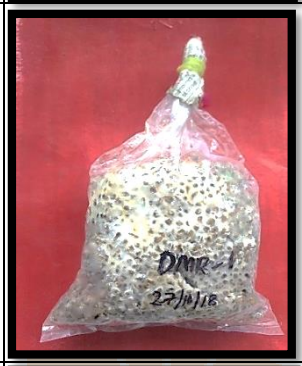
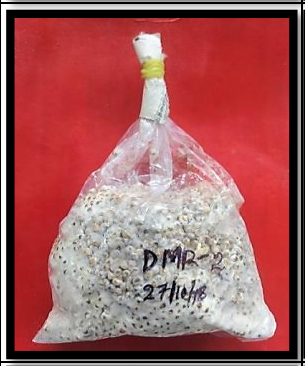
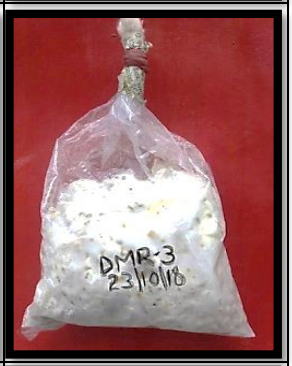
S.No	Substrates	DMR 388- R1	DMR 388-R2	DMR 388-R3
1	Paddy Grains			
2	Pearl Millet Grains			
3	Maize Grains			
4	Wheat Grains			

Fig 3: Growth of spawn in different substrates

4.3. Effect of substrates on different phases of vegetative growth of Shiitake mushroom

In case of small strain DMR 388, showed a smaller number of days for mycelial coat formation (55.4 days), in substrate maize shelled cobs, which was followed by sugarcane bagasse (61.4 days) and paddy straw (69.0 days). The highest number of days observed in maize straw substrate with (84.4.0 days), which was preceded by the combination of wheat straw for mycelial coat formation (77.0 days) and combination of saw dust with maize shelled cobs (78.3 days), whereas bump formation stage, browning,

fruiting and harvesting stages was not observed in indoor cultivation of Shiitake mushroom under *in vitro* conditions in the mushroom spawn laboratory, Institute of Agricultural Science, BHU, Varanasi.

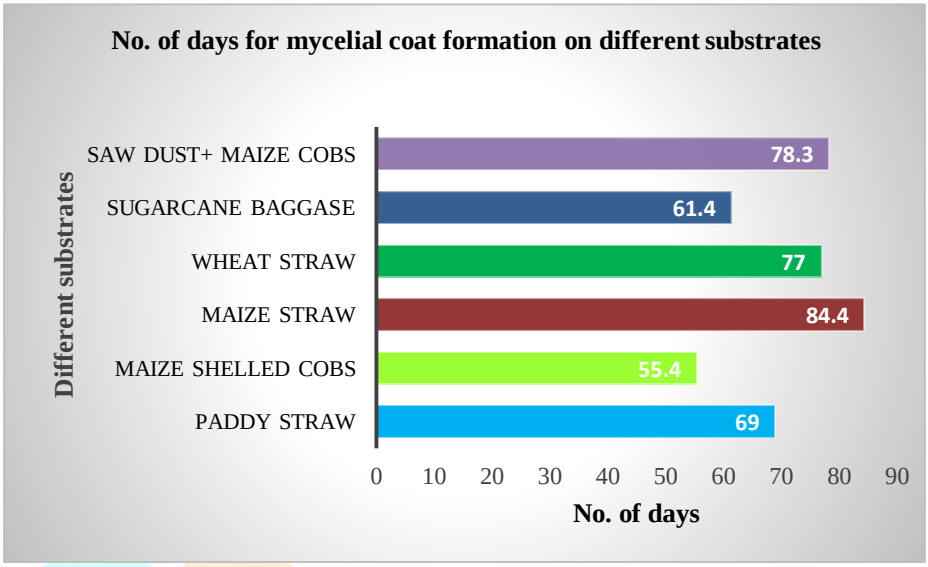


Fig.4: Effect of substrates on different phases of vegetative growth of shiitake mushroom

(a) Maize stubbles

(b) Maize shelled cobs

(c) Wheat straw

(d) Paddy straw

Fig 5: No. of days for mycelial coat formation on different substrates

II. ACKNOWLEDGMENT

My deepest love and thanks to my family for instilling the right values of hard work, dedication, commitment and social justice and for all the moral support.

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