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Effect of Methods of Substrates Pre-Treatment on Growth and Yield of White Milky Mushroom (*Calocybeindica)*

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Abstract: ***An experiment was carried out during summer season of 2012 to study the effect of substratespre-treatment on growth and production of Calocybeindica. The results indicated that the substratespre-treated by autoclaving (T_1) recorded significantly shortest spawn run (20.25 days), pin head initiation after casing (6.50 days), maximum number of pin head(23.50) number of sporophores (15.00) and yield (1546.35 g per bed) in comparison to other treatments. Maximum Biological Efficiency (103.09%) was also recorded by treatment T_1. The substrates pre- treated by autoclaving recorded maximum length of stipe (14.76 cm), diameter of stipe (7.90 cm) & diameter of pileus (11.74 cm), weight of sporophore (103.09g) followed by substrates pre-treated by hot water and by Formaldehyde @100 ml/100 liters water plus Bavistin (Carbendazim) @10 gm/100 litre of water. Although autoclaving was the best method for substrates pre-treatment, howeversubstrates pre-treatedby hot water and substrates pre-treated by formaldehyde @100 ml/100 litre of water plus Bavistin(Carbendazim) @10 g/100 litreof water provided to be a viable and promising methods of substrates pre-treatment which can be adopted to produce a good yield of milky mushroom(Calocybeindica) especially in rural areas where autoclave sterilization is not feasible.***

Keywords: *Calocybeindica*, substrates pre-treatment, growth parameters, yield, biological efficiency

1. Introduction

Mushrooms are the eukaryotic, spore bearing organisms under macro-fungi, lacking chlorophyll and grow on dead decomposed matter as saprophytes. They derive nutrients through their mycelia. Mushrooms provide a rich addition to the diet in the form of protein, carbohydrates, valuable salts, minerals and vitamins. As food, the nutritional value of mushrooms lies between meat and vegetables. Like other vegetables it contains about 90 per cent moisture (Crisian and Sands, 1978) and are basically low calorie food (25-30 calorie / 100 gm fresh weight). Carbohydrates are present in the form of chitin and glycogen while starch is absent. Mushrooms are low fat food with 2 to 8% fat on dry weight basis (Crisian and Sands, 1978). It contains high proportion of unsaturated fatty acids especially linoleic acid with no cholesterol. Among sterols, ergosterol is abundant and cholesterol is absent. On dry weight basis mushrooms contains 19-35 per cent protein having 70-90 per cent digestibility. Mushrooms due to high quality and quantity of protein have been recognized by FAO as the food contributing to protein nutrition of the country depending largely on cereals. They are also good source of vitamins and minerals, especially those of B complex group but are relatively poor in fat soluble vitamins, A, D, E and K. Among B complex vitamins, mushrooms are especially rich in thiamine (B1), riboflavin (B2), niacin and biotin (Chang and Miles, 1989). Folic acid and vitamin B12 which are generally absent in plant food but present in mushrooms. Mushrooms also contain vitamin C and minerals like potassium, phosphorus, magnesium, sodium, calcium, zinc and iron in significant quantities.

Milky mushroom (*Calocybeindica*) has become the third commercially grown in India after button and oyster mushrooms.This mushroom is also called White summer mushroom and DudhChhatta. This mushroom is gaining popularity due to its attractive robust, white sporocarps long shelf life and taste (Chadha and Sharma, 1995). Mature sporocarp of *Calocybeindica*contains 4% soluble sugars, 2.9% starch and 7.4% ash (Doshi*et al*.,1988). Milky mushroom grew well on uncomposted substrates under artificial indoor condition. Wide ranges of diverse cellulosic substrates are used for cultivation of milky mushroom. Paddy straw , wheat straw, soybean straw, sugarcane baggase, cotton waste & coconut coir pith are the commonligno cellulosic substrates used for cultivation of the mushroom (Chakravarthy*et al*., 1981; Doshi*et al*., 1989). Sanchez (2010) reported that substrates used for the oyster mushroom cultivation do not require sterilization, but only pasteurization, which is less expensive to diminish the damages produced by different pathogen (bacteria, moulds or insect pests) on mushroom development yield. Diana *et al* (2006) recommended disinfection of the substratum before spawning which should only destroy the competitive fungi and not the useful micro-organism. Sterilization of substrates is not an easy job for the cultivation of mushroom and the right sterilization time and temperature depend on the possible pathogens in a given substrates material (Kwon and Sik Kim, 2004).Potentiality of white milky mushroom production prevails in Jharkhand, but unfortunately people of this region are not aware about the benefits of mushroom consumption and production, although, the tribals consume mushrooms grown naturally.Unfortunately, a very little research work has done in Jharkhand for improvement in the production technology of milky mushroom. The objective of this study was to compare the effectiveness of different substrate pre-treatment methods for growth and yield of *Calocybeindica*.

2. Materials and Methods

To determine the best method for softening and elimination of competitive microflora, substrate (chopped paddy straw)

was treated in various ways. The experiment was conducted during the year2012 in mushroom production unit, Department of Plant Pathology, Faculty of Agriculture, Birsa Agricultural University, Ranchi, Jharkhand. The temperature and relative humidity of the cropping room ranged from 30-35°C and 80-85 per cent, respectively. 18-20 days old spawn raised on wheat grain through standard technique was used during experimentation. The various substratespre-treatment used as treatment were as follows:

T_1 -Substrates pre-treatment by autoclaving,T_2 - Substratespre-treatment with Formaldehyde @ 100 ml/100 litreof water, T_3 -Substratespre-treatment with Bavistin@ 10 g/100 litre of water, T_4 - Substratespre-treatment with Formaldehyde @ 100 ml/100 litre of water plus Bavistin (Carbendazim) @ 10 g/100 litre of water, T_5 - Substratespre-treatment with hot water (by boiling), T_6 – Untreated.

There were six treatments and four replications, The experiment was carried out in Completely Randomized Design (CRD) under room condition.

Preparation of substrates

Well dried, clean paddy straw werechopped into 3-4 cm pieces (Gogoi*et al.,* 2016). For autoclaving pre-treatment chopped paddy straw were pre-soaked in water for 12 hours, cleaned by repeated washing and filled in polythene bags and it was autoclaved at 20 pound pressure for two hours. In hot water treatment, chopping pre-wetted paddy straw were exposed to boiling water and kept for two hours.For chemical treatment chopped paddy straw were immersed in water containing solutions of the following chemicals. Formaldehyde @ 100 ml/100 litre of water, Bavistin @ 10 g/100 litre of water and Formaldehyde @ 100 ml/100 litre of water plus Bavistin @ 10 g/100 litre of water. Plain water soaked straw were used as control. After 12 hours soaking, the water was drained off and one to two washing was done by clean water in case of Formaldehyde treatment to ensure that there was no smell of formalin left in the straw. Sterilized straw was taken out and drained off excess water and spread over on polythene sheet to dry for 1 to 1.5 hours depending on the prevailing weather condition. The moisture content of the straw was kept at 65-70 percent (Biswas*et al*., 2012).

Milky mushroom cultivation using chopped pre-treated paddy straw in polypropylene bags (60cm×40cm of 100 gauges) (Pani,2012) with layer spawning was followed(Pani and Das, 1998).The bottom of the bag was tied with a rubber band to make a cylindrical shape to the bed. Then the bag is sterilized with spirit dipped cotton by swapping and then the bag was turned over so that the tied portion comes inside. Bottom of the bag was slightly widened. The bag was filled with alternate layers of straw (approximately 1.5 kg sterilized dry straw per bag) and spawn (300 g/kg of dry straw). Press it with palm to let the air go out. The bag was then tied with a rubber band along with a label of the species and date of spawning. About 10-15 holes were made into the polythene bags for the exchange of air and gases. Spawned bag was stacked in racks which were arranged in spawn running room. During spawn running period, temperature of 26 ± 4°C was maintained. These partially controlled conditions were maintained for 20 to 25 days for complete spawn running period when whitish cottony mycelia growth completely covered the straw in polythene bags. The polythene bags were cut into two halves with a hacksaw blade. After cutting of bags, casing soil were applied to a height of 2 cm above the newly exposed surface of the bags.

Ten days after spawning, casing mixture were prepared in the ratio of 1:1 by using Garden soil (pH 5.3) and two years old farmyard manure (FYM, pH 6.45). The casing mixturewas chemically sterilized by spraying with two per cent formalin and then covered with polythene sheet for three days. The mixture were turned on alternate days for four days to remove the fumes of formalin from the casing mixture. Beds were kept on racks in cropping room for fruitingafter casing. During this period the temperature and humidity for fruiting were maintained 30-35°C and 80-85 per cent, respectively. Ventilation was reducedafter casing of beds. Watering was done two times a day by a hand sprayer and it was withheld a day before harvesting. Observations regarding time taken (days) for spawn run, pinhead initiation after casing, first picking, days for number of pinhead/bed, number of sporophore, biological efficiency and yield were recorded.Fully matured sporophores of white milky mushroom were harvested from each bed and fresh weight was determined immediately andrecorded the length and diameter of stipe, and pileus& weight of sporophore.

Biological efficiency of mushroom was calculated by using the following recommended by Chang and Miles (1989).

Per cent biological efficiency = $\frac{\text{Fresh weight of mushroom (g)}}{\text{Dry weight of substrate (g)}} \times 100$

Statistical analysis of data was done by using appropriate programme. In order to compare the effect of methods of substrate pre-treatment simple completely randomize design (CRD) was used. Critical difference (CD) calculated at 5 percent level of significance were used for comparison of difference between the treatment means.

3. Results and Discussions

The experiment was carried out to know the effect of methods of substrate pre-treatment on growth and yield of *Calocybeindica*.

Growth and yield parameters such as spawn run (days), pinhead initiation (days), number of pinhead per bed, number of sporophore per bed, yield per bed and biological efficiency were studied **(Table-1, Plate-1).**The results showed that the time taken (days)in spawn run in different treatments ranged from 20.25 days to 27.75 days. Shortest period (20.25 days) was observed in T_1 (autoclaving) which was found to be significantly superior to other treatments. This was followed by 21 days in T_4(formaldehyde @ 100 ml/100 litre of water + bavistin (Carbendazin) @ 10 g/100 litre of water) and T_5 (hot water). Longest period (27.75 days) was observed in T_6 (control) followed by 22.75 days in T_2 (formaldehyde @ 100 ml/100 litre of water). The time taken (days) for pinhead initiation after casing in different treatment ranged from 6.5 days to 9 days. Shortest period (6.50 days) was observed in T_1 (autoclaving) which was

found statistically at par (7 days) with T_4(formaldehyde @ 100 ml/100 litres of water + bavistin(Carbendazim) @ 10 g/100 litres of water) and T_5 (hot water).Longest period (9 days) was observed in T_6 (control) followed by T_2 (formaldehyde @ 100 ml/100 litres of water) (7.25 days).The maximum number of pinhead (23.50) per bed was observed in T_1 (autoclaving) which was found statistically at par with T_5 (hot water) andT_4 (formaldehyde @ 100 ml/100 litre of water + Bavistin (Carbendazim) @ 10 g/100 litre of water)and minimum number of pinhead (11.75) per bed was observed in T_6 (control).

The number of sporophores harvested in different treatments ranged from 8.50 to 15.Maximum number of sporophores (15.00) was harvested in T_1 (autoclaving) which was found to be significantly superior to other treatments. This treatment was followed by T_4 (formaldehyde @ 100 ml/100 litres of water + bavistin @ 10 g/100 litres of water) 14.25 and T_5 (hot water). Minimum number of sporophores (8.50) was observed in T_6 (control).The yield per bed in different treatments varied from 659.77 g to 1546.35 g. Maximum yield (1546.35 g) per bed was recorded in T_1 (autoclaving) which was found to be significantly superior to other treatments. Minimum yield (659.77 g) per bedwas recorded in T_6 (control) followed by T_2 (formaldehyde @ 100 ml/100 litre of water) (1123.72 g) and 1215.55 g in T_3 (Bavistin @ 10 gm/100 litres of water). Various methods of substrates treatment to check and manage various pest and pathogen have been tried by various workers in different mushroom (Zadrazil and Scheiderit, 1972; Bano*et al.*, 1979; Vijay and Sohi, 1987) with varying success but overall steam pasteurization has proved to be best. The better colonization and highest yield on steam pasteurization substrate may be attributed to change in quality of substrate on heating coupled with release of soluble sugars and phenotic compounds as observed by Zadrazil(1976) poor yield in control signifies the importance of substrate treatment.

Maximum biological efficiency (103.09%) was recorded inT_1 (autoclaving) followed by 95 per cent in T_5 (hot water) and 93.87 per cent in T_4 (formaldehyde @ 100 ml/100 litre of water plusBavistin @ 10 g/100 litre of water). Minimum biological efficiency (43.98%) was recorded in T_6 (control). Similar results were obtained by several earlier workers. There were significant effects of substrate pre-treatment methods on the average yield of *Calocybeindica*and highest growth vigour, yield and biological efficiency were obtained byautoclaving pre-treated substrates.

Size and weight of sporophore

The results pertaining to size and weight of sporophore are presented in **Table 2**.The resultsshowed that the length of stipe, diameter of stipe and pileusin different treatments ranged from 8.83 cm to 14.76 cm, 5.38cm to 7.90 cm and 7.86cm to 11.74 cm respectively. Maximum length of stipe (14.76 cm), diameter of stipe (7.90 cm) and diameter of pileus (11.74 cm) were recorded in T_1 (autoclaving) which was found statistically at par with T_5 (hot water) and T_4(formaldehyde @ 100 ml/100 litre of water plus Bavistin (Carbendazim) @ 10 g/100 litre of water). The weight of sporophore in different treatments varied from 77.62 g to 103.09 g. Maximum weight of sporophore (103.09 g) was recorded inT_1 (autoclaving) which was found statistically at par with T_5 (hot water) and T_4(formaldehyde @ 100 ml/100 litres of water + Bavistin (Carbendazim) @ 10 g/100 litre of water). The minimum weight of sporophore (77.62 g) was recorded in T_6 (control).

Substrates pre-treatment is essential for elimination of micro-organisms during mushroom cultivation. Autoclaving, hot water and chemical treatment eliminated all the weed moulds (Nallathambi and Marimuthu, 1994). The results of present investigation clearly showed that autoclaved paddy straw gave early spawn run and produced maximum number of sporophores, yield and biological efficiency. Autoclaved paddy straw also gave maximum size (length of stipe and diameter of pileus) and weight of sporophores followed by hot water and chemical treatment with formaldehyde @ 100 ml/100 litre of water plus Bavistin(Carbendazim) @ 10 gm/100 litre of water. It is clear from the results that autoclaving is the best methods for substrate treatment due to the elimination or inactivation of all microorganisms. This was in conformity with Sharma *et al.* (2006). Bahukhandi (1990) also reported that autoclaved paddy straw gave highest yield followed by hot water and further observed that chemical and tap water treatment was less effective. The performance of Bavistin(Carbendazim) (@ 10 gm/100 litre water) in sterilization of paddy straw for good yield of *Pleurotus spp*. was observed by other workers (Krishnamoorthy*et al.*, 1991; Nallathambi and Marimuthu, 1994; Chitale and Singh, 1995). Formaldehyde @ 100 ml/100 litres water treated substrate yielded less as compared toBavistin(Carbendazim) (@ 10 gm/100 litre of water. This was may be due to incomplete sterilization of substrate by the chemical used. Sohi (1988) and Jadhav and Jagtap (1991) recorded the poor performance of formaldehyde treated substrate than Bavistin. This slight difference in the concentration of chemicals used and nature of contamination prevailing in substrates. Hot water treatment at 80°C for 90 minutes was found significantly better as compared to the treatment with formaldehyde (500 ppm) and Bavistin (75 ppm) separately (Singh and Dwivedi, 1991). Chemical sterilization with formaldehyde plus Bavistin gave the highest yield of *P. sajor-caju* (Upadhyaya*et al.*, 1987). In addition Vijay and Sohi (1987) treated the substrate either by boiling water or steeping in chemical solution of Formalin (500 ppm) + Bavistin (75 ppm) for one hour. Wajeed and Shetty (1995) also showed that treatment of substrate with Carbendazim (50 ppm) + formalin (500 ppm) followed by steam pasteurization has enhanced the sporophore, yield and thereby biological efficiency of *Calocybeindica* to maximum extent. From the present study, it can be concluded that pre-treated paddy straw substrates by formaldehyde @100ml/100lts of water plus Bavistin (Carbendazim)@ 10g/100lt of water provided to be a viable and promising methods of substrate pre-treatment which can be adopted to produce a good yield of Milky mushroom especially in rural areas where autoclave sterilization is not feasible.

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Table 1: Effect of substrate treatment on the linear growth and productivity of *Calocybeindica*

Treatments	Average spawn run days	Average pinhead initiation (days) after casing	Average No. of pinhead/ bed	Average No. of sporophore/ bed	Average yield/ bed (gm)	Biological efficiency (%)
T_1 - Autoclaving	20.25	7.00	23.50	15.00	1546.35	103.09
T_2 - Formaldehyde @ 100 ml/100 litres of water	22.75	7.75	17.75	13.00	1123.72	74.91
T_3 -Bavistin @ 10 gm/100 litres of water	21.50	7.50	19.25	13.25	1215.55	81.03
T_4 - Formaldehyde @ 100 ml/100 litres of water + Bavistin @ 10 gm/100 litres of water	21.00	7.25	23.00	14.25	1408.18	93.87
T_5 - Hot water	21.00	7.25	23.25	14.25	1425.14	95
T_6 - Untreated (control)	27.75	9.00	11.75	8.50	659.77	43.98
SEm ±	0.24	0.21	0.24	0.22	28.99	
CD at 5%	0.72	0.63	0.70	0.64	86.80	

Table 2: Effect of substrate treatment on size and weight of sporophore of *Calocybeindica*

Treatments	Average length of stipe (cm)	Average diameter of stipe (cm)	Average diameter of pileus (cm)	Average weight of sporophore (gm)
T_1 - Autoclaving	14.76	7.90	11.74	103.09
T_2 - Formaldehyde @ 100 ml/100 litres water	11.37	6.42	9.69	86.44
T_3 - Bavistin @ 10 gm/100 litres water	12.23	6.52	9.85	91.74
T_4 - Formaldehyde @ 100 ml/100 litres of water + Bavistin @ 10 gm/100 litres of water	13.48	7.09	10.40	98.82
T_5 - Hot water	13.57	7.21	10.57	100.01
T_6 – Untreated (control)	8.83	5.38	7.86	77.62
SEm ±	0.42	0.33	0.44	2.39
CD at 5%	1.28	1.02	1.34	7.22
CV%	5.84	4.83	6.70	4.46

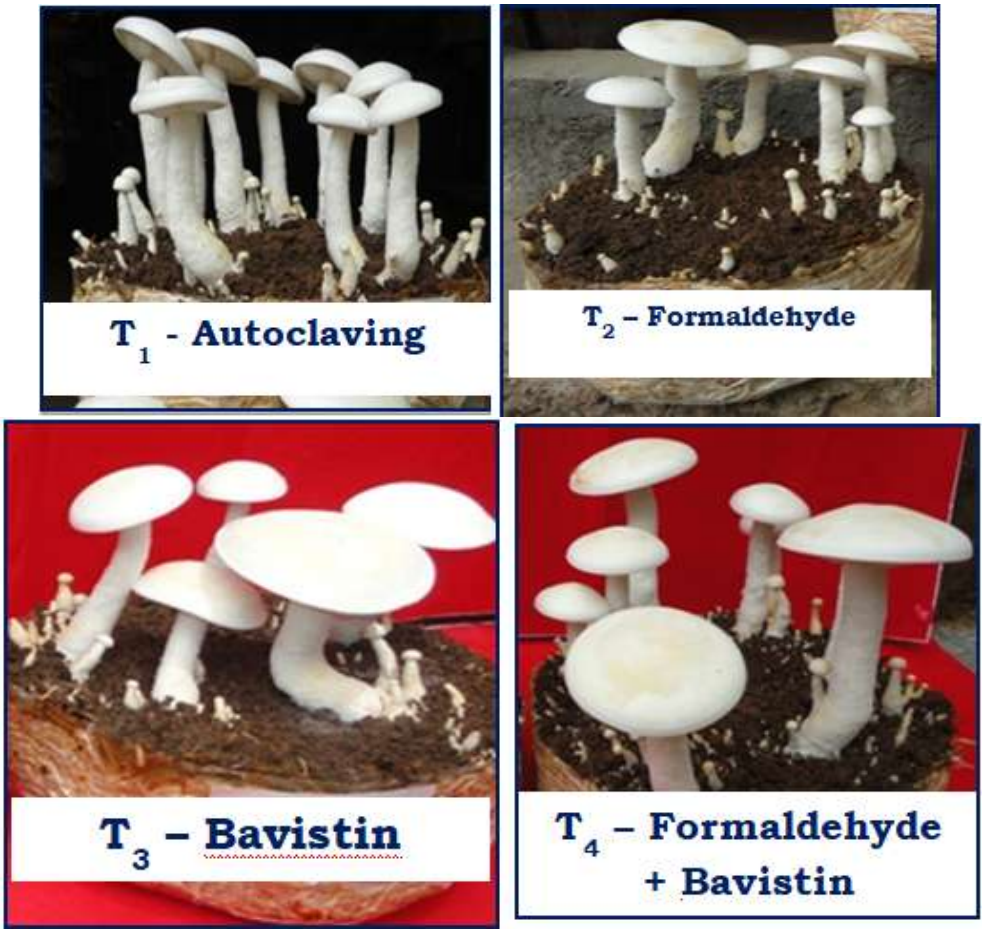

Plate 1: Fruiting bodies of *Calocybeindica* grown on different treated substrate