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Original Research Article

Evaluation of Substrate on Production of *Calocybe indica* (Milky white mushroom) under Bihar Condition

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A B S T R A C T

Present study was carried out to evaluate the effect of three different agrocellulosic wastes (wheat straw, paddy straw and equi-proportion of wheat and paddy straw) using plastic bag technology for production of the *Calocybe indica*. Among the three different substrates, wheat straw substrate was superior which recorded minimum days for spawn run 15.3 days, pinhead formation 27 days and for first harvest 33 days with highest number of fruit bodies 24, highest pileus diameter 7.67 cm, length of stalk 7.80 cm and maximum yield 1283.6 gm/kg dry substrate. The highest biological efficiency 128.36% was also obtained in the wheat straw substrate. Wheat straw: Paddy straw (1:1) was the next best superior substrate for cultivation of milky mushroom.

Introduction

Mushrooms have been grown all over the world for many years because of their culinary, medicinal, bioremediation and biodegradation properties.

This edible mushroom has a long shelf life (5-7 days) compared to other commercially available counterparts, and grows on high temperature range (30-38^{o}C).

Mushroom fruiting bodies are basidomycetes and some ascomycetes belongs to the Kingdom Fungi and depend on the substrates on which they grow for all their nutritional requirements like carbon, water, nitrogen and minerals (Rajarathnam *et al.*, 1997).

Fleshy nature of mushroom and their nutritional value is responsible for its main attraction to human being as a source of food. Mushrooms and their products are used as delicacy and its consumption is rapidly increased as they have good taste, flavor and nutritive value.

Its products can serve to improve the nutritional status and helps in alleviating protein deficiency (Chandra, 2006).

Calocybe indica is a potentially new species to Indian mushroom growers, reported first time from West Bengal by Purkaystha and Chandra, 1974. Its edibility was confirmed later by Purkaystha, 1976.

Around 700 million people, most of them living in rural areas, are still extremely poor today. In addition, despite undeniable progress in reducing rates of undernourishment and improving levels of nutrition and health, almost 800 million people are chronically hungry and 2 billion suffer micronutrient deficiencies (Silva, 2015). Despite the numerous, nutritional, health benefits and medicinal values of mushrooms, the importance of mushrooms in food security, especially in developing nations is not appreciated. In addition, the economic importance of mushrooms is often overlooked. (Oluwalana *et al.*, 2016).

Mushroom production not only reduces environmental pollution of agricultural and industrial residues when used as substrates. It also provides an economically acceptable alternative for the production of food of superior taste and quality, as well as high value-added secondary metabolites such as enzymes or polysaccharides. Utilization of various lignocellulosic wastes for commercial cultivation and value addition of *Calocybe indica* are the demand of the hour. Since the nutritious *Calocybe indica* is studied for its yield when grown on various types of substrates.

Materials and Methods

The experiment was carried out in mushroom house and mushroom research unit, Dr. Rajendra Prasad Central Agricultural University, Pusa using CIP-13 strain fruiting body of *Calocybe indica* isolated from natural condition.

Preparation of pure culture

Each culture was separately maintained on potato dextrose agar slant at room temperature after surface sterilization of explants with 0.1% $HgCl_2$ and three subsequent washing with sterile distilled water, it was sub-cultured at monthly interval to sustain their fruiting vigor they were preserved under refrigerated conditions.

Spawn preparation

Un-infested, clean wheat grains were boiled with equal amount of water till grains become soft but were not allowed to split open. The moisture content of boiled grains was allowed to leave as such for hours by air drying so that water on surface is evaporated to obtain 50 to 55 percent moisture. Then the grains were mixed with 0.5 percent calcium carbonate and 2.0 percent calcium sulphate prevents clumps formation. First gypsum and chalk powder are mixed separately and then they are thoroughly mixed with grains. This substrate was filled into 100 ml flask or 15 × 20 cm size polypropylene bags. The mouth of container was plugged with clean, non-absorbent cotton. These bottles were sterilized for one hour at 15 lb pressure on 121ºC temperature. Upon cooling for 24 hours, the content was inoculated with bits of fresh fruiting culture of *Calocybe indica* under aseptic condition. The containers were incubated for 23-28 days for *Calocybe indica* to attain complete ramification over the substrate at 26-28ºC. These seed materials were used freshly for cultivation of mushroom.

Selection of substrate

The potential locally available lignocellulosic substrates such as wheat straw and paddy straw were selected for cultivation of *Calocybe indica*. Paddy straw and wheat straw were chopped and all substrate were cleaned with tap water separately. They were used alone and also in combinations in the ratio (1:1). All the substrates pre-soaked in cold water, and

selected substrates were filled in metallic tank separately and treated with hot water 80-85 ºC for 30 minutes. After draining excess water, substrates straw was spread on sterilized surface to evaporate extra moisture approximately to 70 percent and after cooling the spawning was done using methods of Dayaram (2009).

The substrates used for the experiment were as follows:

Wheat straw
Paddy straw
Wheat straw: Paddy straw (1:1)

Spawning and spawn run

4 kg substrates on dry weight basis in polypropylene bag were spawned with 5% spawn. Then bags were tightly closed by using plastic rings plugged with non-absorbent cotton. Bags were incubated under dark condition for spawn running. During spawn running period, temperature of 30-35 °C and relative humidity of 85-90 percent was maintained in mushroom house.

Casing

After complete mycelial formation casing was done. The method of cultivation of *Calocybe indica* was followed as recommended by Dayaram (2009). The common casing mixture (2 year old FYM + garden soil 1:1 w/w) were partially pasteurized at 80°C for 1 hour. Upon cooling, the pH of the medium was adjusted to 7 to 7.2 by addition of calcium carbonate. Then it was added over the mycelial impregnated substrate up to 3 to 3.5 cm thickness. The mouth of the bag was covered with either formalin treated or autoclaved newspaper to prevent the insect and weed molds. Light watering was done twice a day and ruffling the casing soil surface was done intermittently for good aeration.

Statistical methods

All experimental data were statistically analyzed by using completely randomized design (CRD). The significance of each data was analyzed by calculating critical difference at 5 percent level.

Results and Discussion

Effect of substrate on growth and development of mushroom were analyzed for the spawn run, days for pinhead formation, total number of fruit bodies, days for first harvest, pileus diameter, length of stalk, biological efficiency and yield (gm) have been presented in table 1.

Spawn run

Study showed that the minimum time (15.3 days) required for spawn run in case of wheat straw. This was at par with treatment equi-proportional combination of wheat and paddy straw with 18.3 days and paddy straw 19.3 days.

Days for pinhead formation

Days for pin head formation were significantly minimum 27 days for the treatment of wheat straw. Equi-proportional combination of wheat and paddy straw performed better in the next order 32 days and 33 days over paddy straw.

Days for first harvest

Significantly minimum 33 days required for the first harvest in the treatment of wheat straw, 36 days for equi-proportional combination of wheat and paddy straw and maximum for paddy straw 37 days.

Table.1 Effect of different substrates on *Calocybe indica*

Substrate	Spawn run period (d)	Pinhead formation (d)	Days for first harvest (d)	Pileus diameter (cm)	Length of stalk (cm)	Av g wt (g/button)	Yield (g)	Biological yield (%)
Wheat straw	15.3	27	33	7.67	7.80	130.6	24	128.36
Wheat straw: Paddy straw(1:1)	18.3	32	36	7.33	7.64	125.8	15	97.175
Paddy straw	19.3	33	37	7.27	6.80	123.8	20	61.9
SEm(±)	0.49	0.48	1.04	0.49	0.51	0.57	0.58	3.48
CD(0.05)	1.12	1.44	3.11	0.13	0.16	1.25	1.26	7.59
CV	4.98	4.76	6.17	1.03	1.24	4.76	6.29	2.11

Average weight of fruiting bodies

Average weight of fruit body varies from 130.6 gm to 123.8 gm. The maximum average weight of fruit body was found in the treatment of wheat straw 130.6 gm, followed by equi-proportional combination of wheat and paddy straw and paddy straw 125.8 gm and 123.8 gm respectively.

Total number of fruit bodies

The highest numbers of fruit bodies were observed significantly in the wheat straw 24 fruit bodies which were at par with equi-proportional combination of wheat and paddy straw 20 fruit bodies and paddy straw 15 fruit bodies.

Length of stalk

Data recorded from all the experiment and results about length of stalk showed that the significantly highest stalk length was recorded in the treatment wheat straw 7.80 cm, followed by combination of both substrate 7.64 cm which was at par with paddy straw substrate 6.80 cm.

Pileus diameter

Maximum diameter of pileus was found in fruiting body obtained from wheat substrate 7.67 cm. This was at par with equi-proportional combination of substrate wheat straw and paddy straw 7.33 cm and paddy straw 7.27 cm.

Yield

The fruit bodies were weighed immediately after harvest using electronic balance. Overall, the yield ranged from 2476 gm to 5134.4 gm. The considerably highest yield was recorded in substrate wheat straw 1283.6 gm/kg dry substrate. Whereas, equi-proportional mixture of wheat and paddy straw yielded 3887.0 gm/kg dry substrate. Lowest yield was recorded in the treatment of paddy straw 619 gm/kg dry substrate.

Biological efficiency

The biological efficiency of different substrate ranged from 61.9-128.36%. The highest biological efficiency 128.36% was observed in treatment of wheat straw. The next was in equi-proportional combination of wheat and paddy straw 97.175% and paddy straw performed lowest 61.9% biological efficiency.

Commercial production of *Calocybe indica* mushroom was largely determined by availability and utilization of cheap materials of which of which agricultural

lignocellulosic wastes represents the ideal and most promising substrate for cultivation. The substrate used in this study can be considered practical and economically feasible due to availability throughout the year at little or no cost in large quantity throughout the Bihar. Utilization of these agrocellulosic wastes for the production of *Calocybe indica* could be more economically and ecologically friendly. In the present investigation lignocellulosic wastes used as substrate for cultivation of *Calocybe indica* strain out of which wheat straw was found to be best substrate for cultivation, which is in agreement with the earlier reports of several scientists. Highest yield of milky mushroom was observed on wheat straw by Arora *et al.,* (2004); Tondon and Sharma (2006) and Dayaran (2009). Average yield was recorded on equi-proportional combination of wheat straw and paddy straw. Several workers had reported that paddy straw was the best substrate for cultivation of *Calocybe indica* Krishnamoorthy *et al.,* (1997); Biswas *et al.,* (2009); Pani *et al.,* (2010) and Saranya *et al.,* (2011). This varied production potential of different substrates might be due to the variations in their physical properties and nutritional composition.

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