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Effect of Various Substrates on Linear Mycelial Growth and Fructification of *Volvariella diplasia*

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Abstract: In the present study, effect of different substrates on mycelial growth and yield of *Volvariella diplasia* was evaluated. *Volvariella diplasia*, the paddy straw mushroom, is worldwide one of the most widely cultivated mushrooms. Cereals are the most popular basal ingredient used in synthetic substrate formulation for producing paddy straw mushroom spawn. However, the present work evaluates the best and cheap substrate for spawn culture and mushroom production. Paddy straw mushroom was cultivated on various types of cereals (Wheat, Maize, Ragi, Jowar, Bajra) and pulses (Bengal gram, Green gram). Cultivation on Bajra resulted in significantly faster mycelial growth as compared to other substrates followed by Jowar. With respect to fructification, culture on wheat shows highest production. The lowest biological and economical yields were found when the culture was on bajra. Biological efficiency and biological yield were highest with culture on wheat. The above findings reveal an opportunity for commercial implication of paddy straw mushroom especially *Volvariella diplasia* for utilization of different feasible and cheap substrates.

Key words: *Volvariella diplasia*, substrates, growth, yield

INTRODUCTION

Since, time immemorial people have developed a taste for eating mushroom collected from forest and field. The history of mushroom cultivation is traced to the Roman. In early times, cultivation failed because the biology of fungi was not understood and this led to the initial problems of mushroom cultivation (Oei, 1996; Stamets, 2000). Today, there is a better understanding of the biology, nature and development of many species of edible mushroom (Isikhuemhen *et al*., 2000; Okhuoya, 2000; Okhuoya *et al*., 2000; Kurtzman, 2000; Martinez-Carrera, 2000; Wuyep *et al*., 2002). The technology of artificial cultivation of mushroom is somewhat recent innovation, incorporation of non-conventional crops in existing agricultural system can help in improving the social as well as economical status of small farmers. Cultivation of edible mushrooms is one of the most economically viable processes for the bioconversion of lignocellulosic wastes (Bano *et al*., 1993; Cohen *et al*., 2002).

Substrate is one of the most important parameter in mushroom production, as mushrooms depend on substrates for nutrition and the substrate is normally a source of lignocellulose material which supports growth, development and fruiting of mushroom (Miles and Chang, 2004). Sawdust is the most popular basal ingredient used in substrates to produce shiitake (Miller and Jong, 1987; Palomo *et al*., 1998; Grodzinskaya *et al*., 2003). Regardless of wheat, starch-based supplements such as wheat bran, rice bran, millet, rye or corn, can be added at 10 to 40% of dry weight to the main ingredient for spawn preparation (Ivan *et al*., 2003; Royse *et al*., 1990; Royse, 1996).

Unlike other mushroom species, paddy straw mushrooms can be easily grown on varieties of natural substrates like rice husk, wheat bran, leaf litters, straw and are the easiest, fastest and cheapest to grow, require less preparation time and production technology. Scarcity and high cost of these substrates have necessitated a search for alternative substrate. Cereals and pulses offer a potential alternative substrate source for mushroom cultivation. The objective of this work was to study the effect of various substrates on the linear mycelial growth and yield of paddy straw mushroom.

MATERIALS AND METHODS

Collection and identification of *Volvoriella diplasia*: Fruiting bodies of *Volvariella diplasia* were collected from a mound of rotten paddy straw waste in Trinath Mushroom Farm at Jagadalpur during 2004. The identity of mushroom was done using the method described by

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Alofe *et al.* (1996), Oso (1975) and Zoberi (1972). The mycelium of *Volvariella diplasia* was obtained by tissue culture from the pileus of premature sporophore. A portion of the tissue from the cap was removed aseptically and was placed on sterilized Potato Dextrose Agar (PDA) and the mycelium was allowed to develop from the spores. The developed mycelium was maintained on PDA media by regular sub culturing (Three weeks) during the period of study.

Preparation of spawn and growth of *Volvariella diplasia*: Various cereals and pulses were used for determination of suitable spawn substrate. The cereal grain includes Wheat (WT), Maize (MZ), Ragi (RG), Jower (JW), Bajra (BJ) and pulses comprise Green gram (GG) and Bengal gram (BG) were washed several times to remove any suspended particles. The grains were boiled in a container with water till they soften and then spread over a polythene sheet under shade for draining excess water from grains surface. Twenty grams of Calcium carbonate were added to 1 kg of different grains each and mixed together properly in a container. About 200 g of each mixture was then transferred into a clean saline bottle. The bottles were plugged with non-absorbent cotton tightly, sterilized (22 lb psi, 2 h) and cooled, inoculated with a pure culture mycelia and incubated at (23±2°C) for 10 days. The linear mycelial growth of mushroom was measured for each treatment. The un-inoculated sterile saline bottle (15 cm length) with grains (upto 12.5 cm length) was used as control.

Preparation of bed and growth of *Volvariella diplasia*: Various agricultural wastes can be used in bed preparation. The present study was done with paddy straw. The tied paddy straw bundles were soaked in water for 12 to 16 h and then kept in an inclined manner to remove excess water. Three soaked straw bundles with their butt ends one outside were placed lengthwise very close to each other on the bamboo frame. Then another set of three bundles were placed over them in similar manner but with butt ends on the opposite side and was the first layer. After 1st layer was completed, spawn bits of thumb size was put on the top of the layer. Same process repeated for five to six times but in opposite direction in each layer.

The inoculated beds were covered with polythene sheet and incubated at 38 to 40°C temperature for ramification of the mushroom mycelia. After full ramification, polythene sheet were removed and watering on bed was done twice or thrice a day. The number of pinhead development was recorded. The harvesting was done in four flushes of one week intervals. After the 1st flush, the beds were watered and covered regularly to harvest 2nd, 3rd and 4th flushes.

RESULTS

Spawn of each mushroom mycelium was prepared using selected cereals and pulses. The average linear mycelial growth of individual mushroom mycelium was noted from each of three replicates. Table 1 indicated the ability of cereal grains to facilitate faster mycelial growth of *Volvariella diplasia* than pulses grains. The substrate (BJ) had the shortest time for full mycelial growth (14 days) followed by MZ (16 days), were as RG took nearly two and half weeks RG (17 days) for full growth of mycelium. There was no significant difference between WT and JW (18 days each) as it took 18 days for complete colonization on the substrate. BG and GG shows more than 21 days for complete ramification of the mushroom mycelia.

The effect of different grain substrates on the yield of *Volvariella diplasia* shown in Table 2 showed a little delaying response of one day more for pin head emergence in BG. In case of WT, it took only 5 days for pin head emergence. However, other substrates showed 7 days for complete pin head emergence. The biological efficiency was affected by substrate. WT spawn resulted with highest Bio-efficiency (16%) and the largest number of mushroom production. The biological efficiency on the GG, JR, RG, MZ and BG gradually decreases. BJ yielded lowest with lowest biological efficiency (6.4%). Significant yield variations were recorded on different substrates at first harvest. The highest number of mushroom (100) and

Table 1: Effect of substrates on mycelial growth (cm) per culture bottles after 7, 14 and 21 days

	Days		
Substrates	7	14	21
Wheat (WT)	6.5±0.2	11.0±0.2	12.5±0.1
Maize (MZ)	6.5±0.3	11.8±0.2	12.5±0.1
Ragi (RG)	6.2±0.2	11.5±0.3	12.5±0.3
Bajra (BJ)	6.5±0.2	12.5±0.1	12.5±0.2
Jower (JR)	4.3±0.2	10.9±0.2	12.5±0.1
Green gram (GG)	3.5±0.2	8.1±0.3	11.8±0.2
Bengal gram (BG)	4.8±0.3	9.8±0.1	11.8±0.2

Each value is mean of 3 replicates±SEM

Table 2: Effect of spawn based on different kinds of grains on yield (g) of *Volvoriella diplasia*

Spawn Substrate/yields	Days for pin head emergence	Average no. of mushroom	Yield/ bed (g)	Bio-efficiency (%)
Wheat (WT)	05	100	1600	16.00
Maize (MZ)	07	52	1150	11.50
Ragi (RG)	07	75	1300	13.00
Bajra (BJ)	07	71	1575	15.75
Jower (JR)	07	35	640	6.40
Green gram (GG)	07	65	820	8.20
Bengal gram (BG)	07	56	1580	15.80

Each value is mean of 3 replicates±SEM

economical yield was recorded with WT (1600 g). The economical yield decreases with decrease in average number of mushroom in other substrate. The lowest number of mushroom (35) and economical yield was observed in BJ (640 g).

DISCUSSION

The cultivation of edible mushroom using different substrate such as cereals and grains is a value added process as it gives base to growth of mushroom mycelium. *Volvariella diplasia* mycelia grows very well on a wide range of grains. Wheat, maize, bajra, jower, bengal gram as well as green gram supported good growth and fast mycelia extension of the mushroom. Substrate structure is an important factor for the growth of the mycelium as it should be suitable for penetration of the mycelium. Wheat is commonly used spawn for mushroom cultivation as it is cheaply and abundantly available. Present study revealed that cereal grains sustained faster growth rate of mycelium as compared to that of pulses grain. This implies that the carbohydrates present in different cereal grains were more effectively utilized by the mycelium for better vegetative multiplication whereas protein content in pulses grains as well as the thickness of seed coat (Bengal gram, Green gram) were not able to comparable with the growth rate of mycelium in cereal grains. The present study indicated that Bajra shows maximum mycelial yield. However, wheat grain alone takes nearly 18 days for complete ramification but supplemented with waste tea leaves gives faster mycelial growth and more yield than wheat alone (Bisht and Narsh, 1984). The highest mycelial growth was found in Bajra spawn but not recommended commercially since it did not support sporophore production.

The cultivation of edible mushroom using agricultural residues such as rice and wheat straw is a value added process to convert these materials, which are otherwise considered to be wastes, for mushroom production. *Volvariella diplasia* mycelia grows very well on a wide range of cellulosic wastes. Rice straw has been used for the indoor cultivation of *V. diplasia* since, the beginning of the 19th century, a practice from which the mushroom has been given the common name straw mushroom and has been cultivated under natural conditions in many countries (Quimio *et al*., 1990). Rice straw as the natural substrate on which *V. esculenta* grew and led to naming the mushroom as delicious straw mushroom was reported by Fasidi (1996). *Volvariella* sp. also grow on variety of material such as sugarcane baggage, waste tea dust, cotton wastes, oil palm pericarp wastes, oil palm bunch wastes, dry banana leaf and sawdust but their mean mycelia yields are comparably low in some of these wastes (Chen and Graham, 1973; Chua and Ho, 1973). Mostly in tropical countries agricultural waste plays a significant role in causing environmental pollution due to waste disposal problem. Wastes are either burnt or dumped nearby water bodies creating a health hazard to human life. Rice straw as substrate for mushroom bed is recommended for its cheapest quality and readily available in tropical countries. The production of mushrooms worldwide is increasing at an annual rate of about 10%. India is a agricultural country and rich in agricultural waste. The cultivation of *V. diplasia* on these agrowastes decreases the environmental problem and provides a sustainable means of adding value to the farmers. The use of these wastes in the cultivation of edible mushrooms will enhance the biological recycling of nutrients (Madan *et al*., 1987).

Volvariella diplasia, the yield potential of spawn raised on wheat grains shows highest yield. The highest yield of *V. diplasia* (1600 g) was obtained from wheat grain. These results are in line with Purkayastha *et al*. (1980) who reported that *Volvariella* sp. gave maximum growth and productivity on Wheat spawn. However the use of maize, green gram, bengal gram as a spawn substrate may not be practically feasible due to higher cost and very low productivity, when compared to wheat grain. Thus the cultivator must utilize the wheat grain for mushroom production. So, the use of wheat grain as a substrate appears to be most suitable due to improved growth and sporophore production.

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