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STUDIES ON TISSUE CULTURES AS A BIOTECHNOLOGICAL TOOL FOR IMPROVING PRODUCTION OF MILKY MUSHROOM (*CALOCYBE INDICA)*

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ABSTRACT: Tissue cultures were raised separately from different growth stages (button, stipe with small pileus, stipe with well differentiated pileus and mature fruiting body) and regions (posterior of stalk, middle of stalk, pileus, gills and, joint of stalk and pileus) of milky mushroom (*Calocybe indica*) and their effects on growth and yield were investigated. Highest yield (70.3% BE), quickest substrate colonization(15 days), earliest primordial initiation(29 days), highest pileus diameter(15.1mm), maximum stipe length(17.2 mm) and highest linear growth(87.0 mm) were recorded from the fruiting body consisting of stipe with a well developed pileus. Tissues taken from the joint of stalk and pileus induced significantly higher mushroom yield (71.1 % BE), earliest substrate colonization (15 days), highest number of fruiting bodies (6) , quickest primordial initiation (30 days), higher pileus : stipe ratio and maximum radial growth(91.1mm).Hence, tissue cultures taken from the joint of stalk and pileus from a mushroom consisting of stipe with a well differentiated pileus were ideal for achieving optimum growth and reproduction of the fungus.
Key words: Tissue culture, milky mushroom, sporophore, yield, biological efficiency

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INTRODUCTION

As a new introduction to the Indian mushroom world, the milky mushroom (*Calocybe indica*) has great prospects of commercial cultivation in the country. The simple production technique, substantial and sustainable yield, increased shelf- life, ability to thrive on a variety of substrates in a wide range of temperature and humidity conditions as well as its attractive colour and shape have caught the imagination of the growers at large and this mushroom is gradually gaining acceptance by the consumers. Among various critical requirements, healthy and productive microbial culture plays a crucial role in achieving high yield and quality mushroom crop. Strainal purity in mushrooms is maintained by tissue culture [1]. As frequent sub- culturing of fungal cultures tend to cause a loss in their vigour and yield potential [2], rejuvenation of strains is often attempted adopting tissue cultures from cultivated crop of sporophores [3]. However, not much work has been done in this regard especially pertaining to *C. indica* .Therefore, an attempt was made to study the relative yield potential of spawn prepared by tissue cultures from different stages and regions of sporophores of *C. indica.*

MATERIALS AND METHODS

Four different growth stages such as button, stipe with small pileus, stipe with well differentiated pileus and mature fruiting body were selected for the experiment. Five different regions of the mushroom included in the study were posterior of stalk, middle of stalk, pileus, gills and, joint of stalk and pileus. All the specimens were selected from the first flush of mushroom crop.

Tissue cultures were made from individual growth stages and regions on PDA slants as per standard techniques [4]. Large number of tissue cultures were isolated and the most fast growing ones were maintained by periodical mycelia transfer for further use. While taking tissues from different growth stages of mushroom, care was taken to collect the tissues from the joint of stalk and pileus. For determination of radial growth, mycelial disc (5 mm) from two weeks old pure culture were inoculated in petriplates and incubated for 15 days. The radial growth was measured in two cross wise directions and the average was taken. Three replications were maintained for each treatment. Data were subjected to statistical analysis.

Three weeks old pure mycelial cultures were used as inoculums for preparation of wheat grain spawn as per standard procedure [5] with slight modifications. Mushroom cultivation in high density polythene bags using twenty-one days old spawn was followed [6]. Three replications were maintained for each treatment. Mushrooms were harvested 7-8 days after primordial initiation from two economic flushes. Observations were recorded immediately after harvest. Data pertaining to yield were analyzed statistically.

RESULTS AND DISCUSSION

It was observed that highest linear growth (87.0 mm) of the fungus was achieved in response to the tissue culture obtained from the mushroom which consisted of a stipe with a well differentiated pileus (Table-1). It was statistically *at par* with the mycelial growth (85.0 mm) obtained from a matured fruiting body. Significantly poor growth (23.0 mm) was supported when young buttons were utilized for tissue culture. It was also observed (Table-2) that tissue culture made from the joint of the stalk and pileus produced the maximum linear growth (91.0 mm) followed by pileus (84.0 mm). Significantly lower radial growth (44.2 - 59.3 mm) of the fungus was recorded from tissue cultures raised from other parts of sporophore.

The comparative performances of spawn raised by tissue culture from different stages of sporophore were presented in Table 3.It was revealed that the spawn raised from the button stage could not colonize the substrate and produce any mushroom though some growth was noticed on petriplates. Maximum production (703.3 g) with biological efficiency of 70.3 % was recorded from the spawn raised from the fruiting body consisting of stipe with a well differentiated pileus followed by matured stage (672.6 g, 67.2 % BE). Young fruiting body having stipe with small pileus sustained significantly lower yield of mushroom (414.6 g, 41.4 % BE). Cultures obtained from mushrooms (stipe and well differentiated pileus) produced quickest substrate colonization (15 days), earliest primordial initiation (29 days), highest pileus diameter (15.1 cm) and maximum stipe length (17.2 cm).

Spawn raised from different regions of sporophore showed variable productivity (Table-4). The joint of stalk and pileus induced significantly higher mushroom yield (711.3 g, 71.1 % BE), earliest substrate colonization (15 days), highest number of fruiting bodies (6) , quickest primordial initiation (30 days) and higher pileus : stipe ratio. Though the pileus was the next suitable region, the yield (54% BE) from such cultures was significantly lower *vis- a- vis* joint of stalk and pileus but higher than other regions of sporophore. Mehta [7] also reported that pileus cultures exhibited minimum variability and significantly out yielded the stipe and gill cultures in *Agaricus bisporus*. Least number of sporophores (2) and minimum harvest (276.6 g) were obtained with the gill portion. Posterior end of stalk however supported highest average weight of sporophers (140.0 g). It was also revealed that maximum pileus diameter (14.6 cm) and stalk length (15.7 cm) were also recorded in the spawn prepared from the joint of stalk and pileus. Bhandal and Mehta [8] also reported variations of yield among tissue culture isolates from different parts of fruiting bodies of *A. bisporus*. It was revealed from the investigation that the tissue cultures made from the joint of stalk and pileus from a sporophore consisting of stipe with well differentiated pileus sustained the maximum colony diameter, increased mushroom size, quicker substrate colonization as well as higher number and weight of sporophores. Pani and Das [9] have also reported similar findings in case of paddy straw mushroom.

Table 1: Linear mycelial growth of *Calocybe indica* from different stages of sporophore

Stage of Mushroom	Colony diameter (mm)[a]
Button	23.0
Stipe with small pileus	74.2
Stipe with well differentiated pileus	87.0
Mature fruiting body	85.0
CD (0.05)	16.72

a = average of three replications

Table 2: Linear mycelial growth of *Calocybe indica* from different regions of sporophore

Regions of sporophore	Colony diameter (mm)[a]
Posterior end of stalk	48.0
Middle of stalk	59.3
Pileus	84.0
Gills	44.2
Joint of stalk and pileus	91.0
CD (0.05)	17.59

a = average of three replications.

Table 3: Effect of spawn prepared from different stages of sporophore on the production of *Calocybe indica*

Stage of mushroom	Substrate colonizaton (days)	Primordial initiation (days)	No. of mature fruiting body	Size of mushroom		Yield (g)	Average weight of sporophore (g)	Biological efficiency (%)
				Pileus diameter (cm)	Stalk length (cm)			
Button	-	-	-	-	-	-	-	-
Stipe with small pileus	21	42	3	9.8	12.8	414.6	138.2	41.4
Stipe with well differentiated pileus	15	29	6	15.1	17.2	703.3	117.2	70.3
Matured fruit body	17	31	5	14.6	16.2	672.6	134.5	67.2
CD (0.05)	40.25							

Each observation was the average of three replications.

Table 4: Effect of spawn prepared from different regions of sporophore on production of *Calocybe indica*

Regions of sporophore	Substrate colonizaton (days)	Primordial initiation (days)	No. of mature fruiting body	Size of mushroom		Yield (g)	Average weight of sporophore (g)	Biological efficiency (%)
				Pileus diameter (cm)	Stalk length (cm)			
Posterior end of stalk	22	39	3	5.4	12.2	420.0	140.0	42.0
Middle of stalk	19	37	4	7.2	10.6	465.3	116.3	46.5
Pileus	16	33	4	6.8	10.4	540.0	135.0	54.0
Gills	17	38	2	5.0	9.6	276.6	138.3	27.6
Joint of stalk and pileus	15	30	6	14.6	15.7	711.3	118.5	71.1
CD (0.05)	56.50							

Each observation was the average of three determinations.

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