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CULTURAL AND NUTRITIONAL FACTORS ON ENHANCING THE YIELD OF *CALOCYBE INDICA* (P&C)

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Abstract

Experiments were conducted to explore the influence of cultural and nutritional factors on enhancing the yield of *Calocybe indica* (P&C). Among the seven different media tested, PDA medium recorded maximum radial growth and biomass production. 1.0 ppm of Gibberellic acid successfully induced the fastest mycelial growth and biomass production in the medium. Among the various carbon & nitrogen sources tested, xylose followed by dextrose supported the vegetative growth and supplementation of yeast extract @ 2% , proved to be the most suitable nitrogen source.

Keywords **:** Milky mushroom, Biomass, Nutritional factors, mycelial growth

Introduction

Mushrooms belong to a group of organism called Fungi. They absorb their nutrition with the help of very fine thread like structures (Mycelium). After the mycelium has grown profusely and has absorbed sufficient food materials, it produces reproductive structures which generally comes out of the substrate and forms a fruiting body which we commonly refer to as mushrooms.

Mushrooms were considered as luxury food especially among the rich community because of their unique flavor and excitingly different taste but now they have grown to a common man's food. Mushrooms are generally termed as 'nutraceuticals' due to the presence of nutritious components and high medicinal properties.

Mushroom cultivation is an ecofriendly activity where agricultural/industrial wastes are utilized and recycled. Milky mushroom is robust, fleshy and milky white in color and hence, it has greater consumer attraction. The farmers and consumers have also developed preference to *Calocybe india* in recent years because of its advantages *viz*., its easiness of cultivation, by far the highest biological efficiency, high nutritive value, better keeping quality and easiness in postharvest handling. Hence, experiments were conducted to explore the influence of cultural and nutritional factors on enhancing the yield of milky mushrooms and reducing cost of production.

Materials and Methods

Effect of culture media on the linear growth and biomass production of *C. indica*

The surface sterilized tissue bits were placed separately on petri dishes containing Potato dextrose agar (PDA), Beetroot dextrose agar (BDA), Oat meal agar (OMA), Carrot dextrose agar (CDA), Mushroom dextrose agar (MDA), Rice leaf dextrose agar (RLDA) and Paddy straw dextrose agar (PSDA). The radial growth was recorded periodically on the 6^{th}, 9^{th} and 12^{th} day and expressed in mm. PDA Broth was inoculated with actively growing mycelium under aspectic condition and incubated at 28 ± 2°C for 15 days. Mushroom mycelial mat was harvested by filtering the contents of each flask through a previously weighed Whatman No.1 filter paper and allowed to dry at 70°C till attaining a constant weight. The dry weight of the mycelium was expressed in terms of mg.

Mycelial biomass of *C. indica* in broth supplemented with various growth regulators (Hem Lata and Sharma, 2012)

Four growth regulators *viz.,* Indole Acetic Acid (IAA), Gibberllic Acid (GA), Indole-3 Butyric Acid (IBA) and 1-Napthalene Acetic Acid (NAA) at 5 ppm and 10 ppm were added separately to PDA and BDA medium. The medium without growth regulators acted as control. The stock solution of all growth regulators except gibberllic acid were prepared in double distilled water and stored in the refrigerator. Stock solution of gibberllic acid was prepared by dissolving in 5 ml of acetone and then required concentrations were made by dilution.

Effect of different carbon and nitrogen sources on the vegetative growth of *C. indica*

In PDA medium and broth, dextrose was substituted with carbon sources *viz.,* xylose, glucose, fructose. The PA medium served as control. The linear mycelial growth of the fungus was recorded on the 6^{th}, 9^{th} and 12^{th} day and expressed in mm. Then, PDA medium was supplemented with different nitrogen sources viz., urea, peptone, yeast extract and potassium nitrate @ 2% concentration. Three replications for each treatment were maintained. In the broth, the mushroom mycelial mat was harvested on the 15^{th} day and was weighed by the standard procedure. The dry weight of the mycelium was expressed in terms of mg.

Results and Discussion

Table 1 : Effect of culture media on the linear growth and biomass production of *Calocybe indica*

Sl. No	Medium/Broth	Vegetative growth (mm)			Biomass (mg)	Colony characters
		6th day	9th day	12th day	15th day	
1	Potato Dextrose Agar (PDA)	28.45[a]	59.60[a]	90.00[a]	227.35[a]	Absolute dense white mycelial growth with regular margin
2	Carrot Dextrose Agar (CDA)	20.98[d]	42.38[d]	68.63[d]	170.63[d]	white dense mycelial growth & irregular margin
3	Oat Meal Agar (OMA)	21.25[c]	44.86[c]	76.23[c]	189.59[c]	white mycelial growth & regular margin
4	Beetroot Dextrose Agar (BDA)	26.47[b]	58.48[b]	87.88[b]	215.15[b]	White dense mycelial growth with regular margin
5	Mushroom Dextrose Agar (MDA)	17.97[e]	39.63[e]	58.62[e]	114.18[e]	White, scanty mycelial growth with strandy margin
6	Rice leaf Dextrose Agar (RLDA)	17.32[f]	38.47[f]	51.62[f]	98.74[f]	Dull white fluffy with strandy margin
7	Paddy straw Dextrose Agar (PSDA)	16.31[g]	32.49[g]	43.09[g]	95.96[g]	White scanty mycelial growth with irregular margin

*Values in the column followed by common letters do not differ significantly by DMRT (P=0.05).

Effect of culture media on the linear growth and biomass production of *Calocybe indica* (Table 2)

Among the seven different solid and liquid media tested (Table 2), potato dextrose agar (PDA) medium supported maximum linear growth of 90 mm on the 12th day and biomass production 227.35 mg on the 15th day of observation, which was followed by beet root dextrose agar (BDA) recording (87.88 mm & 215.15 mg) respectively and OMA medium. RLDA and PSDA medium were found to be inferior in supporting the vegetative growth of *C. indica*. The mycelial growth in PDA and BDA showed an absolute dense white mycelial growth with regular margin, whereas the mycelial characters of Mushroom dextrose agar (MDA) and Paddy straw dextrose Agar (PSDA) was white, scanty with strandy margins. Since PDA was found to be the most suitable media.

The medium supplies all the essential nutrients for the mycelial growth of a mushroom. The most suitable medium allows acceleration of mycelial growth; ensure quality and production all round the year (Chang and Mshigeni, 2001). The present findings show the best growth of *Calocybe indica* in potato dextrose agar (PDA) medium, suggesting that the nutrients present in the semisynthetic media *viz.*, potato dextrose agar and beetroot dextrose agar might have some specific nutrients which support the growth of the mushroom.

Table 2 : Mycelial biomass of *Calocybe indica* in broth supplemented with various growth regulators

Medium	Growth Regulators	Mycelial Dry Weight (mg)	
		5 ppm	10 ppm
Potato dextrose agar (PDA)	Napthalene Acetic Acid (NAA)	234.83[b]	239.94[d]
	Indole-3 Butyric Acid (IBA)	235.62[b]	246.98[c]
	Indole Acetic Acid (IAA)	232.62[c]	235.89[e]
	Gibberllic Acid (GA)	259.14[a]	274.28[a]
Beetroot dextrose agar (BDA)	Napthalene Acetic Acid (NAA)	226.78[d]	232.69[f]
	Indole-3 Butyric Acid (IBA)	228.28[d]	239.24[d]
	Indole Acetic Acid (IAA)	224.28[e]	224.96[g]
	Gibberllic Acid (GA)	245.12[b]	261.46[b]
Control	PDA	224.61[e]	224.61[g]
	BDA	215.51[f]	215.51[h]

*Values in the column followed by common letters do not differ significantly by DMRT (P=0.05).

Mycelial biomass of *Calocybe indica* in broth supplemented with various growth regulators (Table 2).

Efficacy of different growth regulators *viz*., NAA, IBA, IAA, GA @ 5 ppm & 10 ppm supplemented in PDA & BDA medium separately was evaluated (Table 2). Both the the media supplemented with various growth regulators showed enhanced mycelial growth of the fungus when compared to control. Among the growth regulators, both PDA & BDA media supplemented with GA @ 5 ppm & 10 ppm, showed increased mycelial dry weight recording (259.14 mg, 274.28 mg & 245.12 mg, 261.46 mg) respectively. Both IBA & NAA @ 5ppm conc. were on par with each other but varied substantially at 10 ppm. where NAA recorded an increased vegetative growth. The least dry weight was recorded by BDA supplemented with IAA which recorded on par results with the control.

Tandon *et al.* (2006) reported maximum mycelial growth in case of *Calocybe indica* in medium supplemented with 10 ppm of Gibberellic acid. Increased vegetative growth of *C. indica* was observed when the basal medium was supplemented with 1.0 ppm conc. of GA (Kaur *et al*., 2016). These observations confirm the conclusions of the present investigation.

Table 3 : Effect of different carbon and nitrogen sources on the vegetative growth and Biomass production of *C. indica*

Sl. No	Carbon sources	Vegetative growth (mm)			Mycelial dry weight (mg)
		6th day	9th day	12th day	
1	Xylose	20.45d	59.60c	90.00a	242.92b
2	Glucose	14.23f	48.39e	71.38d	212.56f
3	Dextrose	19.93d	57.55d	88.46b	233.01d
4	Fructose	13.71f	43.13f	61.40e	193.49g
5	Urea	22.28c	59.37c	89.28b	238.56c
6	Potassium nitrate	16.9e	58.77d	82.19c	220.37e
7	Peptone	23.67b	61.96b	90.00a	244.99b
8	Yeast	25.63a	65.88a	90.00a	257.08a
9	Control	11.12g	28.72g	47.24f	112.88h

*Values in the column followed by common letters do not differ significantly by DMRT (P=0.05).

Effect of different carbon and nitrogen sources on the vegetative growth of *Calocybe indica* (Table 3)

The correlation between carbon and nitrogen sources and the mycelial ramification has been theorised by many workers. Different carbon sources were tried to see their effect on vegetative growth of *C. indica*. The data (Table 3) indicated that all the media supplemented with carbon and nitrogen sources showed better results when compared to control. Among the different carbon sources xylose supported the highest vegetative growth and biomass (90 mm & 242.92 mg) followed by each of dextrose (88.46 mm & 233.01 mg) and glucose (71.38 mm & 212.56 mg) respectively. Among the treatments fructose amended medium showed the least enhancement in mycelial dry weight. PDA medium supplemented with yeast extract proved superior recording maximum vegetative growth and biomass (90 mm & 257.08 mg) followed by peptone supplementation (90 mm & 244.99 mg).

The variations in the efficiency of carbon sources indicates preferential utilization of simple sugars. According to Sharma *et al.,* (2006) organic nitrogen sources were good supporters as the maximum biomass of *C. indica* was produced by yeast and peptone.

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