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Growth Regulators Impact on Mycelial Growth and Yield Performance of Milky Mushroom (*Calocybe indica*)

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ABSTRACT

Calocybe indica grows in hot humid climate hence this mushroom is highly suitable for cultivation in most of the plains of India almost throughout the year (except in cold climatic conditions). The aim of the present study was to investigate the radial growth and yield performance of *C. indica* in different concentrations with respect to two growth regulators, namely, Indole-3-acetic acid (IAA) and Gibberellic acid (GA). Incorporation of the growth regulators increased the yield and number of sporophores of both strains APK-2 and CI-6. Wheat straw substrate supplemented with different concentrations of growth regulators took less period for pinhead formation, first harvesting, higher yield and number of sporophore in case of both strains as compared to control (without supplemented).

Key words: *Calocybe indica*, fruiting bodies, growth regulators, gibberellic acid, indole-3-acetic acid

INTRODUCTION

Mushrooms are traced as special kind of food, since ancient times because mushrooms are almost fat free proteinaceous edible fungi popular for their pleasant aroma, vitamins of B group and minerals (Singh *et al.*, 2018). *Calocybe indica* also known as "milky mushroom" is an edible tropical mushroom originated from India. It is also known as "Dudh Chhatta/Milky Mushroom" because of its milky white appearance and large-sized sporophores or as "white summer mushroom" because of its tropical nature. It is cultivated indoor on wheat/paddy straw during the summer season (30±2°C; Singh *et al.*, 2015). Mushrooms are low in calories but rich in protein, and the nutritionally mushrooms can be placed between meat and vegetable. The mushroom also has anti-cancerous (carcinostatic), hypolipidemic, hypocholesterolemic, hypoglycemic, hypotensive, immunomodulatory, haemato-protective properties including properties of lowering blood sugars and blood pressure, antibacterial, antiviral and antifungal activities (Rijal *et al.*, 2021). They are also useful in bioremediation (Das *et al.*, 2021). More than 15000 fleshy fungi have been identified, among them 2000 species are considered as edible throughout the world and more than 300 species have been reported from India (Ambhure *et al.*, 2021). Milky mushroom has become the third commercially grown mushroom in India after Button and Oyster (Kumar *et al.*, 2018; Shashikant *et al.*, 2022). The mycelial growth of different mushroom species is greatly influenced by media (Rezal *et al.,* 2019).

Plant growth regulators or phytohormones are organic chemical substances, other than nutrients and vitamins, which regulate the growth of plants when applied in small quantities (Prajapati *et al.*, 2015). Plant growth regulators viz., IAA, GA3 and Kin at different concentrations increased the biomass production of *P. sajor-caju* by 15-26% and also increased the protein content of the mycelia (Kumar *et al.*, 2018). Though, the effect of growth regulators attaining higher yield has been studied in few other mushroom species (Godse *et al.*, 2021) similar literature pertaining to *C. indica* is almost lacking. The

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present study was aimed at investigating the radial growth and yield performance of *C. indica* in different concentrations with respect to two growth regulators, namely, Indole-3-acetic acid (IAA) and Gibberellic acid (GA).

MATERIALS AND METHODS

The experiments were conducted in Mushroom Laboratory of Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh for the investigation of effect of growth regulators on radial growth and effect of different growth regulators in the substrate on yield performance of *Calocybe indica*.

For this investigation, two growth regulators viz., Gibbrellic Acid (GA) and Indole Acetic Acid (IAA) were carried out at different concentrations i.e. 10, 15, 20 and 25 ppm on potato dextrose agar (PDA) medium for the observation of radial growth. Each sterilized Petri plate containing PDA subsequently inoculated with 9 mm disc on seven days old culture of strains APK-2 and CI-6 and were incubated at 28±1°C.

The second experiment was laid out with wheat straw substrate to find out the effect of similar concentrations of two growth regulators (IAA and GA) i.e. 10, 15, 20 and 25 ppm. For this experiment, the wheat straw substrate after spawning @ 6% was filled in polythene bags and kept in spawn running room at 28-30°C. These growth regulators were then sprayed separately on bags with fully impregnated mycelium just before casing. In control (treatment devoid of growth regulators) equal volume of water was sprayed instead of growth regulators. The experiment was conducted on two strains of *C. indica,* namely, APK-2 and CI-6 and observations regarding growth parameters and sporophore yield were recorded as total yield (g/kg dry straw), days for pinhead formation (DFPF), days for first harvesting (DFFH), number of pinhead initiation (NOPI) and average weight of fruiting body (g/FB).

Data with appropriate transformations were analyzed with the help of one-way analysis of variance table wherever required. The F-value was tested and critical difference (CD) was calculated at 5% of significance for comparing treatment means. In order to compare the means of various entries, critical difference (CD) was calculated as:

$$\text{Critical difference (CD)} = SE \times \text{'t'}$$

where, SE was standard error of the difference of the treatment means to be compared and

$$SE = (2_{Mse}/r)^{1/2}$$

RESULTS AND DISCUSSION

The application of different concentrations of growth regulators significantly increased the radial growth of two strains of *C. indica* as compared to control (Table 1). In case of gibberellic acid, maximum radial growth of strain APK-2 was recorded on 9th day of observation at 15 ppm concentration (9.00 cm) followed by 10 ppm concentration (8.30 cm) and in case of IAA at 15 ppm concentration (8.90 cm) followed by 25 ppm concentration (8.40 cm). Similarly, in case of gibberellic acid, maximum radial growth of strain CI-6 on 9th day was observed at 15 ppm concentration (8.95 cm) followed by 10 ppm concentration (8.10 cm) and in case of IAA at 15 ppm concentration (8.85 cm) followed by 20 ppm concentration (8.12 cm). The poorest growth was recorded in strains APK-2 (7.28 cm) and CI-6 (7.63 cm) in control (without growth regulators). These results are in accordance with Kannaujia *et al.* (2020) where three different growth regulators viz., Gibberellic acid (GA), Indole Butyric acid (IBA) and Indole Acetic acid (IAA) were tried at different concentration (10, 20 and 30 ppm) to see their effect on the mycelial weight of *C. indica* and observed that mycelial weight was similar at 10 and 20 ppm concentrations. At concentration of 30 ppm, mean mycelial weight was found to be lowest. Maximum mean mycelial weight was recorded with GA followed by IBA. Mean mycelial weight of GA and IBA were statistically similar but differed significantly from that of IAA and control (Subbiah and Balan, 2015). Similarly, the experimental results carried out to study the effect of IAA and NAA on mycelia, colony proliferation of milky mushroom found that the MEA medium supplemented with 5 mg/l IAA gave highest mycelial growth (8.20 cm) and lowest (6.50 cm) with 20 mg/l at 21 DAI; NAA at 10 mg/l gave the best mycelial growth (8.10 cm) and poor (6.90 cm) with 5 mg/l at 21 DAI. Wheat straw substrate supplemented with different concentrations of growth regulators took less period for pinhead formation, first

Table 1. Effect of growth regulators at different concentration for the radial growth of *Calocybe indica* strains APK-2 and CI-6 on potato dextrose agar medium (PDA)

S. No.	Concentration	3rd day		5th day		7th day		9th day			
		APK-2	CI-6	APK-2	CI-6	APK-2	CI-6	APK-2	% increase/ decrease	CI-6	% increase decrease
1.	GA @ 10 ppm	2.70	2.30	5.15	4.53	7.22	7.07	8.30	14.01	8.10	6.16
2.	GA @ 15 ppm	2.98	2.73	5.30	5.23	7.94	7.67	9.00	23.62	8.95	17.30
3.	GA @ 20 ppm	2.92	2.47	4.63	4.73	7.20	6.80	8.23	13.05	7.98	4.59
4.	GA @ 25 ppm	2.78	2.35	4.67	4.45	6.77	6.65	7.83	7.55	7.82	2.49
5.	IAA@ 10 ppm	2.52	2.42	4.93	4.57	7.07	7.00	8.22	12.91	8.00	4.85
6.	IAA@ 15 ppm	3.17	2.90	5.43	5.12	7.72	7.43	8.90	22.25	8.85	15.99
7.	IAA @ 20 ppm	3.28	2.77	5.02	4.93	7.23	7.05	8.38	15.11	8.12	6.42
8.	IAA @ 25 ppm	2.83	2.57	4.98	4.85	7.23	6.95	8.40	15.38	8.07	5.77
9.	Control	2.50	1.93	4.10	4.05	5.92	6.45	7.28	-	7.63	-
C. D. (P=0.05)		Growth regulators = 0.1489 Strains = 0.0702 Growth regulators x Strains = 0.211									

Average of three replications.

harvesting, higher yield and number of sporophore in case of both strains APK-2 and CI-6 as compared to control (without supplemented). With reference to strain APK-2 and CI-6, Gibbrellic Acid (GA) supplemented treatment with 25 ppm concentration recorded significantly less time period for pinhead formation (9.67 and 10.00 days) and first harvesting (14.67 and 17.00 days) followed by 20 ppm concentration (10.00 and 11.00 days and 15.67 and 18.00 days), respectively, as compared to control (Tables 2 and 3). Similarly, for both strains Indole Acetic Acid (IAA) supplemented treatment with 25 ppm concentration recorded significantly less time period for pinhead formation (10.00 and 10.33 days) and first harvesting (15.33 and 17.33 days) followed by 20 ppm concentration (10.33 and 11.33 days and 16.33 and 18.33 days), respectively, as compared to control. In case of APK-2, GA and IAA both showed their maximum numbers of pinhead initiation at 25 ppm concentration (39.67 and 36/bag), while CI-6 resulted in maximum numbers of pinhead initiation at 15 ppm concentration (41.67/bag) in case of GA and at 10 ppm concentration (42.00/bag) in case of IAA. In case of APK-2 and CI-6 maximum number of fruiting bodies were harvested at 20 ppm concentration in both the cases of GA (16.33 and 16.67/bag) and IAA (both 15.33/bag), respectively. Strain APK-2 produced maximum yield at 20 ppm concentration with both growth regulators (GR) GA and IAA (610.00 and 600.00

Table 2. Effect of different concentrations of growth regulators on cropping period and yield of *Calocybe indica* strain APK-2

S. No.	Concentration	DFPF	DFFH	NOPI	NOFB	Yield (g/kg dry substrate)	Average weight (g/FB)	Stipe length	Pileus breadth	Biological efficiency
1.	GA @ 10 ppm	12.33	17.00	36.67	14.67	548.33	37.64	13.17	7.50	54.83
2.	GA @ 15 ppm	11.33	16.33	38.67	15.00	575.00	38.74	13.33	7.67	57.50
3.	GA @ 20 ppm	10.00	15.67	37.33	16.33	610.00	37.48	13.00	7.17	61.00
4.	GA @ 25 ppm	9.67	14.67	39.67	15.00	555.00	37.10	11.33	6.50	55.50
5.	IAA @ 10 ppm	12.67	17.67	33.67	13.33	536.67	40.31	15.00	10.67	53.66
6.	IAA @ 15 ppm	12.33	16.67	34.67	13.67	565.00	41.41	15.83	12.13	56.50
7.	IAA @ 20 ppm	10.33	16.33	30.67	15.33	600.00	39.17	14.33	9.17	60.00
8.	IAA @ 25 ppm	10.00	15.33	36.00	15.00	551.67	36.84	9.83	4.50	55.16
9.	Control	13.33	18.67	30.67	12.33	520.00	42.23	9.00	6.50	52.00
C. D. (P=0.05)		1.052	1.882	3.343	1.996	24.447	NS	2.495	1.816	-
S. E.(d)		0.497	0.889	1.579	0.943	11.547	2.244	1.179	0.858	-

DFPF – Days for pinhead formation, DFFH – Days for first harvesting, NOPI – Number of pinhead initiation and NOFB – Number of fruiting body.
Average of three replications.

Table 3. Effect of different concentrations of growth regulators on cropping period and yield of *C. indica* strain CI-6

S. No.	Concentration	DFPF	DFFH	NOPI	NOFB	Yield (g/kg dry substrate)	Average weight (g/FB)	Stipe length	Pileus breadth	Biological efficiency (%)
1.	GA @ 10 ppm	13.33	20.00	33.33	14.33	536.67	37.45	12.33	9.17	53.66
2.	GA @ 15 ppm	12.67	19.67	41.67	15.00	558.33	37.37	11.83	8.83	55.83
3.	GA @ 20 ppm	11.00	18.00	38.33	16.67	603.33	36.23	11.17	9.13	60.33
4.	GA @ 25 ppm	10.00	17.00	35.33	14.33	571.67	39.91	16.17	11.93	57.16
5.	IAA @ 10 ppm	13.67	20.33	42.00	12.33	490.00	40.19	17.00	10.87	49.00
6.	IAA @ 15 ppm	12.33	19.67	38.33	14.00	525.00	37.83	13.00	8.77	52.50
7.	IAA @ 20 ppm	11.33	18.33	35.33	15.33	588.33	39.02	14.67	10.50	58.83
8.	IAA @ 25 ppm	10.33	17.33	38.67	13.33	485.00	36.71	9.67	8.70	48.50
9.	Control	15.33	21.67	32.67	12.00	468.33	39.26	13.17	9.07	46.83
C. D. (P=0.05)		1.052	1.152	4.102	2.329	25.117	NS	3.094	1.119	-
S. Ed		0.497	0.544	1.937	1.100	11.863	2.992	1.461	0.529	-

Average of three replications. Details of abbreviations are given in Table 2.

g/kg of dry substrate with 61.00 and 60.00% B.E.), respectively. In the same way, strain CI-6 produced maximum yield at 20 ppm concentration with both growth regulators (GR) GA and IAA (603.33 and 588.33 g/kg of dry substrate with 60.33 and 58.83% B. E.), respectively. However, maximum average weight per fruit body was recorded in control treatment (42.23 g) which was found non-significantly superior to other treatment in strain APK-2, while in case of CI-6 maximum average weight per fruit body was recorded at 10 ppm concentration of IAA (40.19 g) which was found non-significantly superior to other treatment. Stipe length and pileus breadth were also recorded and it was found that 15 ppm concentrations of GA and IAA were most suitable for the sporophore development of strain APK-2 (13.33 and 7.67 cm and 15.83 and 12.13 cm) (Kaur and Atri, 2016). In case of CI-6, 25 ppm concentration of GA showed maximum stipe length and pileus breadth (16.17 and 11.93 cm), while 10 ppm concentration of IAA was recorded better for stipe length and pileus breadth (17.00 and 10.87 cm).

The outcome of this experiment was in accordance to Chaurasia *et al.* (2020), who saw the effect of growth regulators on the quality and the productivity of *C. indica.* Three growth regulators viz., Gibbrellic Acid (GA_3), Indole Butyric Acid (IBA) and Indole Acetic Acid (IAA) were tried at different concentrations to see their effect on the mycelial weight, stipe length, stipe diameter and total sporophore yield. The maximum mycelial weight was recorded with GA_3. Addition of 10 ppm IBA resulted in maximum pileus diameter and stipe length. IBA resulted in significantly higher yield as compared to control. In the same way, Shashikant *et al.* (2022) studied the effect of growth regulators on milky mushroom (*C. indica*) both *in vitro* and *in vivo*. Field trials revealed that spraying 50 ppm GA on the emerging primordia increased the number as well as size of mushrooms and produced significantly higher yield (82.2% B. E.) compared to control (67.8% B. E.; Zhu *et al.*, 2021). Increase in sporophore yield, though not statistically significant than control, was also recorded with 10 ppm Kinetin (76.0% B. E.), 25 ppm IAA (73.1% B. E.) and 25 ppm NAA (71.6% B. E.).

CONCLUSION

It was observed that incorporation of the growth regulator (GR) increased the yield and number of sporophores of both strains of *C. indica*. Wheat straw substrate supplemented with different concentrations of growth regulators took less period for pinhead formation, first harvesting, higher yield and number of sporophore in case of both strains APK-2 and CI-6 as compared to control (without supplemented). In case of APK-2 and CI-6, maximum number of fruiting bodies was harvested at 20 ppm concentration in both cases of GA (16.33 and 16.67/bag) and IAA (both 15.33/bag), respectively. Strain APK-2 produced maximum yield at 20 ppm concentration with both growth regulators (GR) GA and IAA (610.00 and 600.00 g/kg of dry substrate with 61.00 and 60.00% B. E.), respectively. In the same way, strain CI-6 produced maximum yield at 20 ppm

concentration with both growth regulators (GR) GA and IAA (603.33 and 588.33 g/kg of dry substrate with 60.33 and 58.83% B. E.), respectively. Stipe length and pileus breadth were also recorded and it was found that 15 ppm concentration of GA and IAA were most suitable for the sporophore development of strain APK-2 (13.33 and 7.67 cm, and 15.83 and 12.13 cm), respectively. In case of CI-6, 25 ppm concentration of GA showed maximum stipe length and pileus breadth (16.17 and 11.93 cm), while 10 ppm concentration of IAA was recorded better for stipe length and pileus breadth (17.00 and 10.87 cm).

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