Influence of plant growth regulators on mycelial colony proliferation and biomass production of *Calocybe indica* (P & C)

RAMANDEEP KAUR MOMI and HARPREET SINGH SODHI

Department of Microbiology, Punjab Agricultural University, Ludhiana, Punjab-141004

ABSTRACT: *Calocybe indica* strain CI-3 was investigated for mycelial growth on solid as well as on liquid medium. Three media namely Complete Yeast extract Medium (CYM), Potato Dextrose Agar (PDA) and Wheat Extract Agar medium (WEA) were supplemented with three growth regulators Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA) and Gibberellic acid (GA) @ 10, 25 and 50 ppm each separately. The maximum colony diameter (mm) and growth rate (mm/day) was observed on WEA medium supplemented with IAA (10 ppm) on 5th and 7th day, IBA (10 ppm) on 5th day, IBA (25 ppm) on 7th day, GA (50 ppm) on 7th day and CYM supplemented with GA (50 ppm) on 5th and 7th day. The biomass growth was maximum in broth supplemented with GA for all the media with maximum (24.2 g/L) in complete yeast extract broth.

Key words: Biomass, *Calocybe indica*, growth regulators, media, mycelial growth rate

Calocybe indica, also known as 'milky mushroom', is an edible tropical mushroom and was first cultivated in India by Purkayastha and Chandra in 1976. It is known by various names in different parts of India. At initial stage, cap of milky mushroom is convex and later on it expands and becomes flat like an umbrella, giving the mushroom it's most common and popular name 'DudhChatta'. The stipe of the mushroom is in the centre but sometimes eccentric, cylindrical with sub-bulbous base (Purkayastha and Chandra, 1974). It has a long shelf life of 2-3 days at 25-30°C and 10-15 days at 4°C temperature. *Calocybe indica* contains high fibre content suitable for the persons suffering from hyperacidity and constipation and is also beneficial in case of peptic ulcers and heart ailments (Doshi *et al*., 1988). It grows well at a temperature range of 25°-35°C and relative humidity more than 80%. Thus, milky mushroom can be cultivated throughout the year in the entire plains of India (Pani, 2012). However, it is being cultivated in Karnataka, Tamil Nadu and Andhra Pradesh on large scale.

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). Rerabek recorded increased fresh weight and dry matter production of mycelia with indolebutyric acid and 2, 4-dichlorophenoxyacetic acid in static culture of *Cleviceps purpurea* whereas β - indole acetic acid also augmented the fresh weight but simultaneously inhibited the dry matter production (Rerabek, 1970). Gibberellic acid at 10µg/ml increased the cell division of all strains of *H. wingei* tested by Makarem and Aldridge, 1969. IAA and KIN (Kinetin) significantly enhanced the *Mucor indicus* growth and chitosan production whereas, decreasing the ethanol production to some extent simultaneously (Safaei *et al*., 2015).

Plant growth regulators can also play an important role in *in vitro* mycelial colony proliferation of mushrooms too. Though, the effect of growth regulators attaining higher yield has been studied in few other mushroom species (Ashrafuzzaman *et al*., 2005; Alam *et al*., 2007; Sarker and Chowdhury, 2013) similar literature pertaining to *C. indica* is almost lacking. This study was aimed to investigate the mycelial colony proliferation and biomass production of *Calocybe indica* in different media with respect to three growth regulators namely Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA) and Gibberellic acid (GA).

MATERIALS AND METHODS

Culture procurement and maintenance: *C.indica* strain CI-3 was procured from the Directorate of Mushroom Research (DMR), Chambaghat, Solan, H.P. and was maintained and subcultured on potato dextrose agar slants at 30±2°C.Pure culture of *C. indica* strain CI-3 was prepared on complete yeast extract medium (CYM) by transferring the mycelial bit of the culture from the slant to petri plate containing complete yeast extract medium and was incubated at 30±2°C for ten days for mycelial growth.

Preparation of stock solutions: Stock solution of 100 ml of 1000 ppm of each growth regulator (IAA, IBA and GA) was prepared. Powdered growth regulator (100 mg) was dissolved in few drops of ethanol and then the final volume made upto 100 ml with distilled water. The stock solutions were filter sterilized and stored in refrigerator to

use as per requirement.

Linear growth measurement: Three media namely Complete Yeast extract Medium (CYM), Potato Dextrose Agar (PDA) and Wheat Extract Agar medium (WEA) were used to study the mycelial growth rate of *C. indica*. The flasks containing the sterilized agar media (autoclaved at 15 psi for 20 minutes) were supplemented with 10, 25 and 50 ppm concentrations of each growth regulator (IAA, IBA and GA) separately and a control (without any growth regulator) was maintained for each media. The sterilized agar media was poured into disposable petri plates and inoculated with 5 mm bit of *C. indica* culture from CYM agar plates and incubated at 30±2°C. The diameter of a colony was measured along the two lines perpendicular to each other from the reverse side of the plate and recorded as means of three values. The colony diameter was measured daily till the plates were fully covered.

Biomass production: Erlenmeyer flasks of 250 ml capacity were taken and in each flask, 50 ml of three broth medium (CYM, PDA and WEA) were added separately and sterilized by autoclaving at 15 psi for 20 minutes. The flasks containing sterilized broths were then supplemented with IAA, IBA and GA @ 10, 25 and 50 ppm separately. A control without any supplementation of growth regulators for each broth medium was maintained. The flasks were inoculated with mycelial bits of 5mm diameter and incubated at 30±2°C for 28 days.

Harvesting and drying of biomass: Mycelial biomass was recovered by filtration through pre-weighed Whatmann no. 1 filter paper at weekly intervals for four weeks. The biomass was dried in an oven at 50±1°C to a constant weight along with the filter paper and the dry weight of the mycelium was recorded. The biomass production was calculated by the following formula:
Biomass (g) = weight of (filter paper + biomass) – weight of filter paper

RESULTS AND DISCUSSION

Linear growth

C.indica was grown on three media (CYM, PDA and WEA) each supplemented with three growth regulators (IAA, IBA and GA @ 10, 25 and 50ppm respectively). The colony diameter (mm) and growth/day (mm/day) were observed upto 9 days of incubation at 30±2°C. The linear growth (mm/day) was maximum on WEA medium supplemented with IAA (10ppm) on 5th and 7th day, IBA (10 ppm) on 5th day, IBA (25ppm) on 7th day, GA (50ppm) on 7th day and CYM supplemented with GA (50ppm) on 5th and 7th day. The mycelium growth was very thin on WEA than the CYM and PDA although it had higher growth rate (Table 1; Fig. 1, 2, 3).

The results were in accordance with Guler and Ozkaya (2009), who reported the highest mycelial growth rate of *Morchella conica* on wheat extract agar medium supplemented with sucrose but the mycelium growth was very thin. Although in CYM supplemented with sucrose the mycelial growth rate was less than WEA but the mycelial growth was best. Growth of *C. Indica* on wheat dextrose agar, potato dextrose agar, rice straw extract agar and wheat straw extract agar medium was also investigated by Singh *et al*. (2009). Maximum linear growth was observed on wheat dextrose agar medium (7.03 cm on the 10th day) followed by potato dextrose agar (5.10cm).There are also various studies conducted by scientists that showed the positive response of growth regulators incorporated with different media. Study of effect of phytohormones indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) on mycelial growth of medicinal mushroom *Phellinus linteus* at concentration of 1.0, 1.5 and 5.0 mg/L showed maximal mycelial diameter as 8.6 ± 0.4, 7.3 ± 2.6 and 9.0 ± 1.0 mm, respectively(Gou *et al*., 2009). *Pleurotus florida*, *C. indica* and *Agaricus bisporus* when grew on PDA, YPDA and MEA (malt extract agar) @ 0, 1, 5, 10 and 20ppm with growth regulators IAA and NAA, the best mycelial growth was observed for *Pleurotus florida* in YPDA at 21 DAI. In case of growth regulators, the best mycelial growth of 5.50 cm was found at 21 DAI with 10ppm IAA; 4.03 cm at 18 DAI with 5ppm NAA and 5.38 cm at 21 DAI with control (Uddin *et al.,* 2012).

Nasr and Mahdipour (2013) evaluated the influence of two growth regulators (IAA and NAA) on the mycelium growth rate of *Agaricus bisporus* and *Pleurotus florida*. The maximum mycelium growth (6.8 cm) was observed for *Agaricus bisporus* (at hormone IAA=10 ppm & hormone NAA=5 ppm) and *Pleurotus florida* (at hormone IAA=5 ppm & hormone NAA=0 ppm) on 15th day. Hannah *et al.* (2020) tested the indole-3-acetic acid producing three bacterial strains instead of using growth regulator directly and found the best mycelium growth of white oyster mushroom with a diameter of 84.86 ± 5.45 mm with *L. boronitolerans.* Chen *et al.* (2019) recorded the highest callus proliferation index (93.15%) of organogenic cultures obtained from inflorescenced explants with 1.0 mg /L BA + 0.1 mg/L NAA under a light intensity of 10 µmol/m^2/s. The highest root length (15.57 mm) and the highest rooting frequency (17 roots per shoot) were also reported when adventitious shoots were inoculated on MS medium with 0.4 mg/L NAA + 0.4 mg/L IBA.

Biomass production

C. indica was grown in three media namely potato

Table 1: Linear growth of *C. indica* on agar media supplemented with growth regulators

Media	Days	Colony diameter in mm (Growth rate in mm/day)									Control
		Growth Regulators (conc. in ppm)									
		IAA			IBA			GA			
		10	25	50	10	25	50	10	25	50	
PDA	3	23 (7.7)	20 (6.7)	19 (6.3)	21 (7.0)	19 (6.3)	17 (5.7)	19 (6.3)	22 (7.3)	24 (8.0)	17 (5.7)
	5	44 (8.8)	43 (8.6)	36 (7.2)	41 (8.2)	42 (8.4)	40 (8.0)	37 (7.4)	41 (8.2)	46 (9.2)	31 (6.2)
	7	67 (9.6)	68 (9.7)	58 (8.3)	58 (8.3)	59 (8.4)	56 (8.0)	62 (8.9)	65 (9.3)	68 (9.7)	53 (7.6)
	9	80 (8.9)	78 (8.7)	67 (7.4)	75 (8.3)	77 (8.6)	72 (8.0)	79 (8.8)	75 (8.3)	80 (8.9)	72 (8.0)
CYM	3	27 (9.0)	26 (8.7)	17 (5.7)	20 (6.7)	22 (7.3)	21 (7.0)	29 (9.7)	31 (10.3)	34 (11.3)	19 (6.3)
	5	47 (9.4)	48 (9.6)	24 (4.8)	35 (7.0)	39 (7.8)	38 (7.6)	55 (11.0)	56 (11.2)	58 (11.6)	34 (6.8)
	7	65 (9.3)	66 (9.4)	33 (4.7)	53 (7.6)	58 (8.3)	59 (8.4)	78 (11.1)	80 (11.4)	83 (11.9)	56 (8.0)
	8	75 (9.4)	72 (9.0)	38 (4.8)	63 (7.9)	67 (8.4)	66 (8.3)	84 (10.5)	86 (10.8)	90 (11.3)	65 (8.1)
WEA	3	30 (10.0)	31 (10.3)	29 (9.7)	30 (10.0)	31 (10.3)	30 (10.0)	29 (9.7)	31 (10.3)	32 (10.7)	26 (8.7)
	5	61 (12.2)	57 (11.4)	52(10.4)	60 (12.0)	57 (11.4)	56 (11.2)	57 (11.4)	58 (11.6)	59 (11.8)	49 (9.8)
	7	81 (11.6)	78 (11.1)	74 (10.6)	80 (11.4)	81 (11.6)	80 (11.4)	76 (10.9)	80 (11.4)	81 (11.6)	70 (10.0)
	8	90 (11.3)	86 (10.8)	80 (10.0)	86 (10.8)	87 (10.9)	86 (10.8)	82 (10.3)	88 (11.0)	90 (11.3)	81 (10.1)
	CD (5%)	Media		Days		GR*			Conc.		Interaction
		0.68		0.78		0.78			0.68		4.69

*Mean of four replicates Incubation temperature: 30±2°C GR - Growth regulator, Potato Dextrose Agar Medium (PDA) Complete Yeast Extract Medium (CYM), Wheat Extract Agar Medium (WEA)

Table 2: Mycelial biomass (g/L) of *C. indica* in broth supplemented with growth regulators

Media	Day	Biomass (g/L)									Control
		Growth regulator (conc. in ppm)									
		IAA			IBA			GA			
		10	25	50	10	25	50	10	25	50	
PDB	**7**	4.2	4	1.5	3	3.6	2.6	9	9.4	10.6	1.6
	14	8	7.8	5.5	7.4	7.8	7	13.8	14.4	15.4	5.8
	21	12.2	12	9.6	11	11.6	10.6	17	17.6	19	9.2
	28	15.4	15.2	11.6	13	13.4	12.6	19.2	19.8	20	12
CYB	**7**	4.8	4.6	2.2	3.8	4.2	3.4	9.4	10.2	11.4	2.4
	14	9	8.6	6.4	7.6	8.4	7.8	14.4	15.4	16.2	6.6
	21	13	12.8	9.8	11.6	12	11.8	17.8	18.2	19.6	10
	28	16	15.8	12.5	13.8	14.2	14	20	21	24.2	12.8
WEB	**7**	3.8	3.6	2	2.6	3.2	2.2	8.6	9	10.2	1.2
	14	7.6	7.4	5.2	6.6	7.4	6.8	13.4	14	15	5.4
	21	11.6	11.4	8.6	10.4	11	10.4	16.6	18.6	18.6	8.8
	28	15	14.8	11	12.2	13	12.2	18.8	19.4	20.6	11.2
CD (5%)	Media		Days		Growth regulators		Conc.	Interaction			
	0.98		0.11		0.11		0.98	0.68			

*Mean of three replicates with three flasks each, Incubation period – 28 days, Incubation temperature – 30±2°C, Complete Yeast Extract Broth (CYB), Potato Dextrose Broth (PDB), Wheat Extract Broth (WEB)

dextrose, complete yeast extract and wheat extract broth. The media broth was supplemented with three growth regulators namely IAA, IBA and GA @ 10, 25 and 50ppm separately. The growth was measured weekly as biomass (g/L) upto four weeks. The observations made were compared with that of control (without growth regulators). The biomass obtained every week was significantly higher for IAA @ 10 and 25ppm. In case of IBA and GA the biomass was higher even at 50ppm concentration. The biomass growth was maximum in broth supplemented with GA for all the media with maximum (24.2g/L) in complete yeast extract broth. The biomass in control for three broth media ranged between 11.2-12.8 g/L (Table 2; Fig. 4, 5, 6).

This study is supported by the experiments conducted by Pani (2011). He collected maximum dry biomass of *C. indica* at 50ppm GA concentration (851.4mg). IAA also sustained appreciable dry weights (390.8-432.2 mg), however, in case of IBA the increase in mycelial harvest over control was statistically insignificant. Highest mycelial growth and yield of *Pleurotuseous* in was also obtained from gibberellic acid incorporated medium @ 2 ppm by Pal *et al.* (2014). The hormones, indole-3-acetic

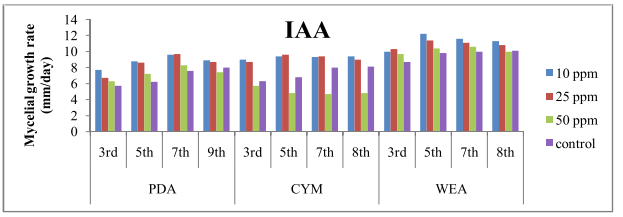

Fig. 1: Mycelial growth rate of *Calocybe indica* on agar media supplemented with IAA

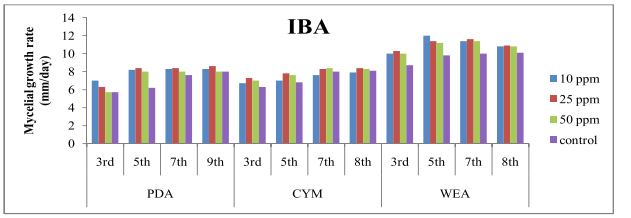

Fig. 2: Mycelial growth rate of *Calocybe indica* on agar media supplemented with IBA

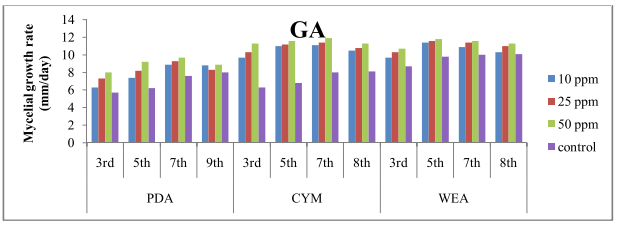

Fig. 3: Mycelial growth rate of *Calocybe indica* on agar media supplemented with GA

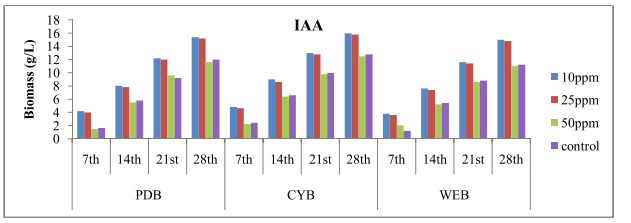

Fig. 4: Biomass production of *Calocybe indica* on broth media supplemented with IAA

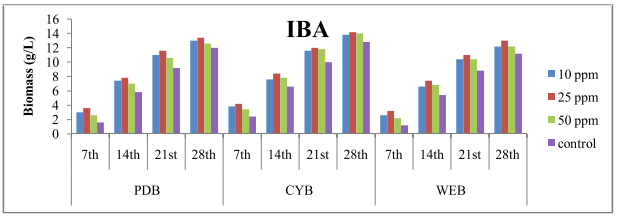

Fig. 5: Biomass production of *Calocybe indica* on broth media supplemented with IBA

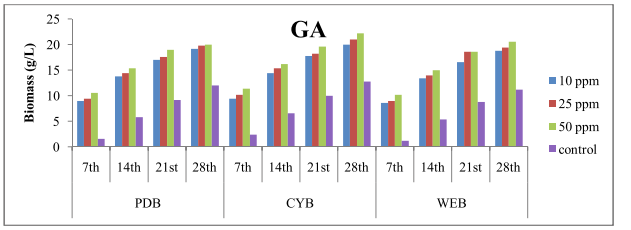

Fig. 6: Biomass production of *Calocybe indica* on broth media supplemented with GA

acid (IAA), gibberellic acid (GA3) and kinetin (KIN) increased the biomass production of *P. sajor-caju* by 15–26%, however, maximum enhancement was observed with indole-3-acetic acid (Mukhopadhyay, 2005).While, in another comparative study of effects of different growth regulators on *P. sapidus* conducted by Kaur and Atri (2016), medium supplemented with gibberellic acid @5ppm provided the maximum mycelial growth weighing 4.78mg/ml.

Du *et al.* (2020) found the NAA as the most effective inducer of microalgae biomass, which increased the biomass approximately 2-folds as compared to the control.

CONCLUSION

Among the three growth media evaluated for the mycelial growth rate of *Calocybe indica* in response to three growth regulators, it was recorded that though the mycelial growth rate on WEA was highest with IAA @10ppm and IBA @10 and 25ppm, CYM incorporated with GA at all concentrations not only produced thick colonies as compared to WEA but also higher growth rate than PDA with best @50ppm. Similarly, in the biomass production study maximum biomass was obtained from complete yeast extract broth with GA @ 50 ppm.

Therefore, knowing the effective growth regulators and their optimal concentrations can shorten the growth cycle of stock spawn. Moreover, conducting these trials in laboratory prior applying these growth regulators directly on the fruiting bodies to evaluate their effectiveness, could also prove beneficial in terms of reducing time and space that, otherwise, could be more cumbersome process.

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