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Effect of different concentrations of growth regulators, trace elements and vitamins on the vegetative growth of local indigenous strain of *Pleurotus cystidiosus*

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ABSTRACT

The physiological studies are important for understanding the requirement of different factors in the growth of mushroom mycelium and subsequently on the fruiting process. During the present study, the experiments were carried out to observe the effect of different growth regulators, trace elements and vitamins on the vegetative growth of *Pleurotus cystidiosus* O. K. Miller at variable concentrations. Amongst these three parameters evaluated maximum dry weight of mycelium was obtained at 5 ppm concentration of Kinetin (6.36 mg/mL), 1 ppm of Manganese (6.51 mg/mL) and 45 ppm of Riboflavin (6.93 mg/mL). The results obtained during the study can be used to supplement media for better mycelial growth for different purposes such as development of nutritional and food supplements, non-food uses of the mycelial, etc.

Key words: Vegetative growth, growth regulators, trace elements, vitamins

Multiplication of mushroom mycelium is an important aspect which is now gaining importance because of its use as mycelial powder, mycelia extract, as food supplement and in the preparation of eco-friendly packaging material (Moharram *et al*., 2008; Kim and Ruedy, 2019). It is also reported that mushroom mycelium is easily digestible in comparison to mature fruit bodies thereby serving to boost overall health of our gut. In modern medicinal technology edible mushrooms have been treated as important tools due to their medicinal values. Food and Health Organization (FAO) recommended edible mushrooms as food to meet protein requirements of developing countries where the maximum population depends mainly on cereals (World Bank, 2004). It is a well-established fact that the vegetative growth of mushroom mycelia is primarily affected by the quality of the media used (Fasidi and Olorunmaiye, 1994; Eswaran and Ramabadran, 2000), variable concentrations of growth regulators, vitamins and nutrient elements (Chodchoi, 1986; Sukla, 1995; Maniruzzaman, 2004; Mukhopadhyay *et al*., 2005). For raising the higher mass of the vegetative mycelium of different mushroom species requirement of suitable liquid medium is an obligatory requirement (Litchfield, 1967).

The mushroom mycelium used in the present investigations was obtained by raising the pure culture of the indigenous strain of *P. cystidiosus* O. K. Miller collected from the living stem of *Lagerstromia speciosa* (L.) Pers. growing at Punjabi University

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campus, Patiala. This mushroom is a member of Phylum *Basidiomycota*, Class *Agaricomycetes*, Order *Agaricales* and Family *Pleurotaceae* (Kirk *et al*., 2008). Just like other oyster mushrooms, *P. cystidiosus* is also an edible and an equally equipped mushroom with multifarious culinary advantages like presence of high proportion of proteins, low fats, no cholesterol, high fibre content and presence of quality minerals, vitamins and other components of nutraceutical relevance (Rathore *et al*., 2019). Present study is an attempt to investigate the effect of various growth regulators, trace elements and vitamins at different concentrations in the identified basal medium (Yeast Glucose Medium) for the mycelia growth using culture of indigenous strain of *P. cystidiosus* raised through tissue culture technique under laboratory conditions.

MATERIAL AND METHODS

Germplasm used

The germplasm used in the present study was collected from the living stem of *Lagerstroemia speciosa* (L.) Pers. growing along road sides on the Punjabi University Campus, Patiala having an altitude of 350 m. The sample was worked out taxonomically using traditional and molecular taxonomic techniques. Dried material of the investigated specimen was deposited in the Herbarium of Botany Department, Punjabi University, Patiala under PUN 11054 and molecular sequence in NCBI GenBank under accession number MT705887. Viable mycelial culture of the collected germplasm was raised through tissue culture technique by taking a portion of the actively growing tissues from the point of confluence of the stipe and pileus of the freshly collected mushroom. The mushroom mycelium was repeatedly sub-cultured on sterile Potato Dextrose Agar (PDA) medium slants to purify it. The inoculated slants were incubated at 28 ± 2ÚC. The whole procedure of culturing was done aseptically under laminar air flow. The purified culture was deposited in the Mushroom Culture Bank of ICAR-Directorate of Mushroom Research, Chambaghat Solan under accession number DMRP-394.

Effect of different growth regulators, trace elements and vitamins on the vegetative growth of *P. cystidiosus*

Mushrooms require variable amount of growth regulators, trace elements and vitamins in small quantities. To evaluate their role in the vegetative growth of test fungus, liquid Yeast Glucose Medium was used, which was selected as suitable medium for the growth of the mushroom culture. This medium was prepared according to the composition given by Tuite (1969). The methodology followed for performing the experiments to evaluate the role of growth regulators, trace elements and vitamins is given below.

Effect of different growth regulators

Four different growth regulators, namely Indole-3-acetic acid (IAA), Gibberelic acid (GA), Indole-3-butryic acid (IBA) and Kinetin (K) were used in variable concentrations of 5 ppm, 10 ppm, 15 ppm and 20 ppm. A combination of all these four growth regulators in equal proportion was also used. A control was kept without addition of growth regulators. The basal medium was purified using 5 g/L of activated charcoal as per the methodology given by Madan and Thind (1998).

Effect of different trace elements

Different trace elements, namely Boron (H_3BO_3), Ferrous sulphate ($FeSO_4.7H_2O$), Zinc sulphate ($ZnSO_4.7H_2O$) and Manganese sulphate ($MnSO_4.7H_2O$), along with their mixture in equal proportion at variable concentrations, *i.e*. 1 ppm, 2 ppm and 5 ppm were used during present study. A control was also kept without any trace element. All the stock solutions of the trace elements were prepared in deionized water and sterilized separately through Millipore (0.22 µm pore size) filters before use. For removing trace element impurities, basal medium was purified using 15 g/L $CaCO_3$ following the methodology of Madan and Thind (1998).

Evaluation of different vitamins

Three vitamins, namely Riboflavin, Thiamine, and Ascorbic acid along with their mixture in equal proportion at three different concentrations of 15 ppm, 30 ppm and 45 ppm were used to study their effect on the vegetative growth of *P. cystidiosus*. Basal medium without vitamins was kept as control during the experimentation. The vitamin impurities were removed from the basal medium by using 5 g/L of activated charcoal following the methodology given by Madan and Thind (1998).

Experimental design and Statistical Analysis

All the experiments were conducted in Mycology and Plant Pathology laboratory, Department of Botany, Punjabi University, Patiala. The experiments were arranged in a complete randomized design with three replicates per treatment. In different experiments performed, the differences exhibited by the treatments were tested for their significance through analysis of variance followed by t-test. The standard deviation and standard error were also calculated.

RESULTS AND DISCUSSION

Evaluation of growth regulators for vegetative growth of *P. cystidiosus*

Amongst all the growth regulators used during the study, maximum mycelium dry weight was obtained when the basal medium was supplemented with 5 ppm concentration of Kinetin (6.36 mg/mL). On the other hand, least growth of the mycelium was obtained in Control (4.73 mg/mL). Higher concentration of growth regulator didn't result in higher biomass production. The results of the experiment depicting mean dry weight of the mycelium along with standard deviation is presented in Table 1 and Fig 1. The results obtained have also been compared through t-test for significance of differences and calculated t-values are presented in Table 2. The results clearly indicated that the supplementation of the basal medium with lower concentration of individual growth regulator or even their mixture is quite helpful in increasing the biomass of the mushroom mycelium under consideration.

As per the t-values calculated at 5 ppm concentration vegetative growth of the mycelium in

Table 1. Effect of supplementation of basal medium with different concentrations of growth regulators for mycelia growth ± Standard Deviation (SD)

S.No.	Growth Regulator	Mean dry weight of mycelium (mg/mL) ±SD at variable concentrations			
		5 ppm	10 ppm	15 ppm	20 ppm
1	Indole-3-acetic acid (IAA)	6.0 ± 0.74	4.38 ± 0.42	3.98 ± 0.28	3.7 ± 0.54
2	Gibberellic acid (GA)	5.91 ± 0.15	5.85 ± 0.08	5.7 ± 0.12	5.06 ± 0.71
3	Indole-3-butyric acid (IBA)	5.43 ± 0.67	5.18 ± 0.27	4.98 ± 0.56	4.58 ± 0.55
4	Kinetin	6.36 ± 0.65	4.85 ± 0.19	4.60 ± 1.03	4.0 ± 1.04
5	Mixture	5.36 ± 0.49	4.28 ± 0.83	4.15 ± 0.30	4.05 ± 0.37
6	Control	4.73 ± 0.20	4.73 ± 0.20	4.73 ± 0.20	4.73 ± 0.20

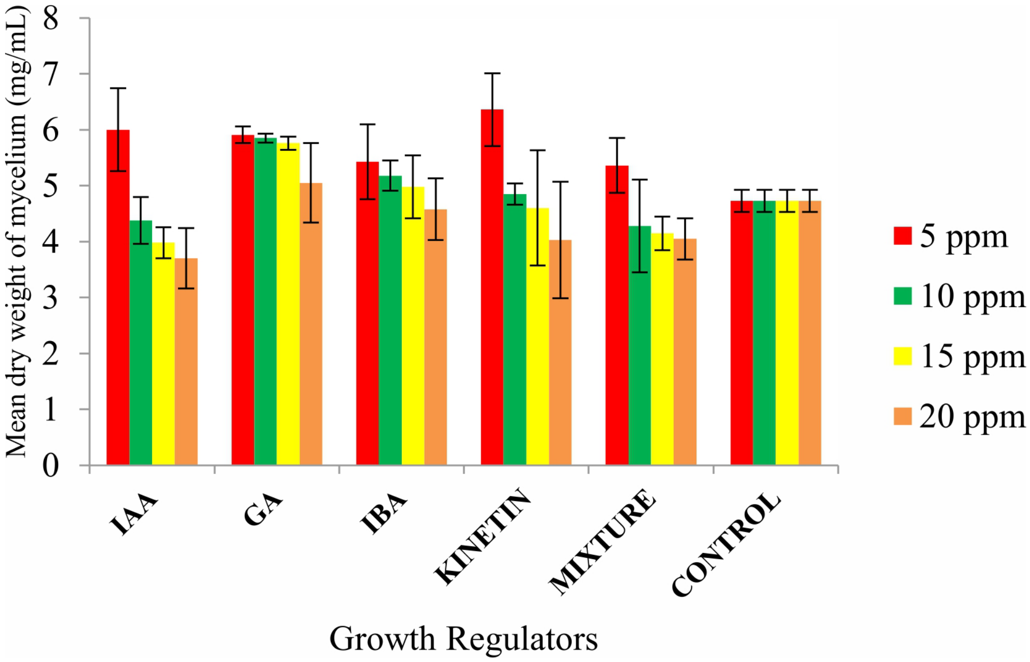

Fig. 1. Histogram showing the effect of different concentrations of growth regulators on mycelia growth

Table 2. Matrix table of t-values showing the effect of supplementation of basal medium with 5 ppm concentration of different growth regulators on mycelia growth

S.No.			1	2	3	4	5	6
			IAA	**GA**	**IBA**	**Kinetin**	**Mixture**	**Control**
	Growth Regulator	**Dry wt. (mg/mL)**	6.0	5.91	5.43	6.36	5.36	4.73
1	Indole-3-acetic acid (IAA)	6.0	—	0.20	1.0	0.0	1.25	2.88**
2	Gibberellic acid (GA)	5.91		—	1.23	0.0	1.89	8.42**
3	Indole-3-butyric acid (IBA)	5.43			—	0.0	0.14	1.75
4	Kinetin	6.36				—	2.17**	4.17**
5	Mixture	5.36					—	2.10**
6	Control	4.73						—

**Significant at level 0.01

Kinetin (6.36 mg/mL) was found to be highly significant as compared to vegetative growth obtained in Mixture (t=2.17, df=5, p>0.05) and Control (t=4.17, df=5, p>0.01). Vegetative growth obtained in Indole-3-acetic acid (6.0 mg/mL) was also significantly higher than growth obtained in Control (t=2.88, df=5, p>0.05), while in comparison growth obtained in Gibberellic acid, Indole-3-Butyric acid and mixture of growth regulators was found to be insignificant.

There are reports in the literature about the positive impact of variable concentrations of growth regulators, vitamins and trace elements on the vegetative growth as well as on the production of sporophore of higher fungi (Szabo *et al*., 1972; Aleksandrov, 1964; Atri and Guleria, 2013; Atri *et al*., 2013). The results of the present study on the mycelial growth performance of *P. cystidiosus* are in conformity with the above observation. Addition of

Indole-3-acetic acid has been reported to increase the growth of mycelium in *P. ostreatus* (Hong, 1978; Vinklarkova and Sladky, 1978; Reddy *et al*., 2002). Higher growth obtained in lower concentration of IAA during the present experiment is also in conformity of earlier reported results. Hong (1978) reported increased growth of *P. cystidiosus* mycelium with the addition of Gibberellic acid @ 10 ppm concentration. Mehta (1985) also reported the enhancement in the growth of *P. sapidus* mycelium with the addition of Gibberellic acid and Indole-3-butyric-acid in the basal medium. The lower concentrations of Gibberellic acid have been reported to enhance the growth of various *Pleurotus* species, while higher concentrations have been reported to inhibit the growth (Hong, 1978; Vinklarkova and Sladky, 1978). Similar results are reported by Fasidi and Olorunmaye (1994) and Eswaran and Ramabadran (2000) in *P. eous* with the addition of Gibberellic acid (0.50 ppm). Kaur and Atri (2016) also reported increased growth of *P. sapidus* with 5 ppm concentration of Gibberellic acid. Contrary to the presently achieved results while working with *P. cystidiosus*, use of Indole-3-acetic acid at variable concentrations has been reported to completely inhibit the growth of *P. ostreatus* (Kikon, 1979). Dabargainya *et al*. (2022) reported an increase in total yield of *P. ostreatus* with the application of GA compared to control. Ambhure *et al*. (2021) reported significant enhanced yield of *P. sajor-caju* with spraying of Gibberellic acid on substrate beds.

Evaluation of effect of trace elements on the vegetative growth of *P. cystidiosus*

Trace elements are essential for the growth of mushroom mycelium in small quantities. The results obtained during the present study are presented in Table 3 and Fig 2. Maximum biomass production could be recorded in 1 ppm supplementation of Manganese in basal medium (6.51 mg/mL), whereas least mycelia growth of 4.66 mg/mL was recorded when basal medium was supplemented with Iron. However, higher biomass could be observed in all the treatments than control (4.55 mg/mL). The mean differences in dry weight of the mycelium at variable concentrations of trace elements as supplements in the basal medium have also been compared through t-test and calculated t-values are presented in Table 4.

Maximum dry weight of mycelium was recorded in 1 ppm supplementation of Manganese (6.51 mg/mL) which was highly significant in comparison to the dry weight of the mycelium obtained in Boron ($t=15$, $df=5$, $p>0.01$), Zinc ($t=4.73$, $df=5$, $p>0.01$), Iron ($t=23.12$, $df=5$, $p>0.01$), Mixture ($t=18$, $df=5$, $p>0.01$) and Control ($t=21.77$, $df=5$, $p>0.01$). Insignificant difference was observed in biomass production in Iron supplementation.

Table 3. Effect of supplementation of basal medium with different concentrations of trace elements on the mycelia growth ± Standard Deviation (SD)

S.No.	Trace Element	Mean dry weight (mg/mL) ±SD at variable concentrations		
		1 ppm	2 ppm	5 ppm
1	Manganese	6.51 ± 0.15	4.6 ± 0.26	3.0 ± 0.20
2	Boron	5.31 ± 0.05	5.0 ± 0.08	4.66 ± 0.21
3	Zinc	4.71 ± 0.65	4.36 ± 0.16	3.48 ± 1.4
4	Iron	4.66 ± 0.07	3.26 ± 0.17	3.08 ± 0.64
5	Mixture	4.70 ± 0.12	3.13 ± 0.25	2.66 ± 0.25
6	Control	4.55 ± 0.08	4.55 ± 0.08	4.55 ± 0.08

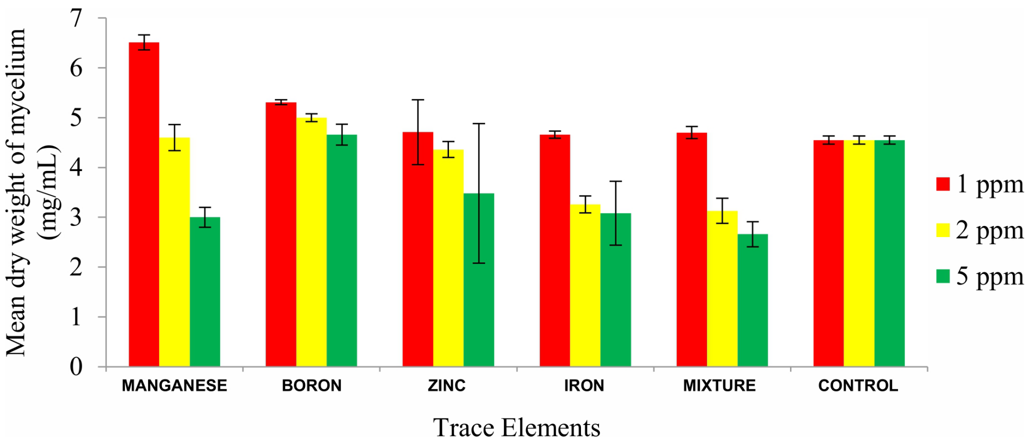

Fig. 2. Histogram showing the effect of different concentrations of trace elements on the mycelia growth

Table 4. Matrix table of t-values showing the effect of supplementation of the basal medium with 1 ppm concentration of different trace elements on mycelia growth

S.No.			1 Mn	2 B	3 Zn	4 Fe	5 Mixture	6 Control
	Growth Regulator	**Dry wt. (mg/mL)**	6.51	5.31	4.71	4.66	4.70	4.55
1	Manganese	6.51	—	15.0**	4.73**	23.12**	18.0**	21.77**
2	Boron	5.31		—	2.85*	0.0	10.0**	19.0**
3	Zinc	4.71			—	0.13	0.0	0.43
4	Iron	4.66				—	0.0	2.2*
5	Mixture	4.70					—	2.28*
6	Control	4.55						—

*Significant at level 0.05; **Significant at level 0.01

Somewhat similar observations regarding the effect of different trace elements on the vegetative growth of fungi was documented by number of investigators. Fasidi and Olorunmaye (1994) reported significant enhancement in the growth of *P. tuber-regium* when basal medium was supplemented with traces of Cu, Fe, Mn and Zn. Present findings agree with the results of Eswaran and Ramabadran (2000) who reported $CuSO_4$ at concentration of 3 ppm supporting the maximum mycelia yield in case of *P. eous*. They also reported that the growth of *P. eous* was influenced by addition of Boric acid (5 ppm) and $MgSO_4$ (0.5 ppm). During the present study also 1 ppm concentration of Manganese and Boron in the basal medium resulted in better mycelia yield as to higher concentration of these trace elements. Adejoye *et al*. (2006) while working with *P. florida* reported the requirement of Fe and Zn in very low concentration for its propagation. Adenipekum and Gbolagade (2006) investigated Zn and Fe as the best micronutrient for the growth of *P. florida*. As compared, better mycelia growth was documented when 1 ppm concentration of Mn and B were used as trace elements in comparison to growth in 1 ppm concentration of Zn and Fe. Kaur and Atri (2016) reported maximum mycelia growth of *P. sapidus* with of 5 ppm concentration of Boron (3.78 mg/mL). However, during the present study, the mycelium of

P. cystidiosus showed better growth when basal medium was supplemented with 1ppm and 2 ppm concentration of Boron in comparison to 5 ppm concentration.

Evaluation of the effect of vitamins on the vegetative growth

Three vitamins, namely Riboflavin, Thiamine, and Ascorbic acid along with their mixture in equal proportion at three different concentrations of 15 ppm, 30 ppm and 45 ppm were used to study their effect on the vegetative growth of *P. cystidiosus*. Maximum biomass production was recorded in in riboflavin (6.93 mg/mL) while least dry weight of the mycelium was recorded when basal medium was supplemented with 45 ppm of thiamine (3.9 mg/mL). however, biomass production was found statistically at par on 30 and 45 ppm concentrations of riboflavin. Results obtained are presented in Table 5 and Fig 3.

Mean differences in dry weight of the mycelium at variable concentrations of vitamins as supplements in the basal medium have also been compared through t-test and calculated t-values are presented in Tables 6. At 45 ppm concentration the differences in the mean was found to be significant in riboflavin and thiamine ($t=6.88$, $df=4$, $p>0.01$) and in Control ($t=6.80$, $df=4$, $p>0.01$) while the vegetative growth of the mycelium obtained in the medium with Riboflavin as supplement was found to be insignificant when compared with the growth obtained in the basal medium having Ascorbic acid and Mixture as supplements. The mean dry weight of mycelium

Table 5. Evaluation of impact of supplementation of the basal medium with variable concentrations of vitamins on the vegetative growth ± Standard Deviation (SD)

S.No.	Vitamin	Mean dry weight (mg/mL) ±SD at variable concentrations		
		15 ppm	30 ppm	45 ppm
1	Riboflavin	5.26 ± 0.12	6.85 ± 0.21	6.93 ± 0.25
2	Thiamine	5.61 ± 0.38	5.26 ± 0.51	3.90 ± 0.73
3	Ascorbic Acid	5.15 ± 0.55	5.78 ± 2.0	5.91 ± 1.23
4	Mixture	5.68 ± 0.34	5.71 ± 0.53	6.70 ± 0.50
5	Control	4.48 ± 0.58	4.48 ± 0.58	4.48 ± 0.58

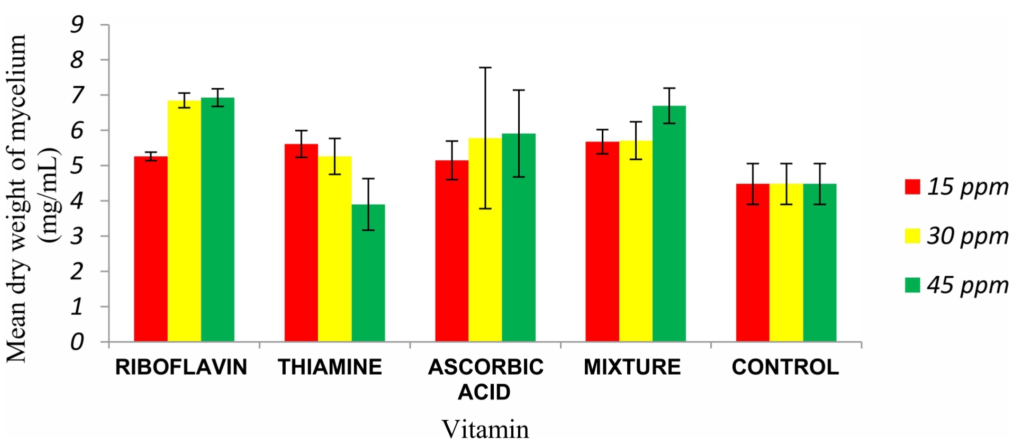

Fig. 3. Histogram showing the effect of supplementation of the basal medium with different concentrations of vitamins on the mycelia growth

Table 6. Matrix table of t-values showing the effect of supplementation of the basal medium with 45 ppm concentration of different vitamins on the mycelia growth

S.No.			1	2	3	4	5
			Riboflavin	**Thiamine**	**Ascorbic Acid**	**Mixture**	**Control**
	Vitamin	**Dry wt. (mg/mL)**	6.93	3.9	5.91	6.70	4.48
1	Riboflavin	6.93	—	6.88**	1.41	0.71	6.80**
2	Thiamine	3.9		—	0.0	0.0	0.0
3	Ascorbic Acid	5.91			—	0.0	1.83
4	Mixture	6.70				—	5.04**
5	Control	4.48					—

**Significant at level 0.01

obtained in the basal medium having Mixture (6.70 mg/mL) as supplement was found to be significant when compared with the growth obtained in Control (t=5.04, df=4, p>0.01).

Amongst the different vitamins used, best mycelia yield of *P. cystidiosus* was obtained when the basal medium was supplemented with 30 ppm and 45 ppm concentration of Riboflavin. The present observations are in complete agreement with the results of Adejoye *et al*. (2007), who also reported Riboflavin as the best vitamin for mycelia run in case of *Schizophyllum commune* (85 mg/mL). For the mycelia growth of *Pleurotus florida* and *P. tuber-regium*, Thiamine followed by Pyridoxine was reported as the best vitamins by Adenipekum and Gbolagade (2006) and Eswaran and Ramabadran (2000). Manjunathan and Kaviyarasan (2010) also reported Thiamine as the best vitamin for mycelia growth of *Lentinus tuber-regium*. Atri and Guleria (2013) reported thiamine at 10 ppm, Indole-3-Butyric Acid at 15 ppm and Iron at 1 ppm concentration supporting the maximum vegetative growth of *Lentinus cladopus*. Similarly, in case of *L. squarrosulus*, Atri *et al*. (2013) reported maximum mycelia growth of 17.55 mg/ml in 20 ppm concentration of gibberellic acid and least mycelia growth of 12.45 mg/ml in the same concentration of Indole-3-butyric acid, while vegetative growth of *L. squarrosulus* was maximum with nicotinic acid @ 15.5 mg/ml. It is apparent from the above discussion that every mushroom has its own specific requirement of growth regulators, trace elements and vitamins as for vegetative growth is concerned.

CONCLUSION

From the above observations it can be concluded that *P. cystidiosus* required different concentration of each respective growth regulator, trace element and vitamins for the vegetative growth in the basal medium. The results from the present observations revealed that Kinetin at 5 ppm, Manganese at 1 ppm and Riboflavin at 45 ppm concentration in the basal medium supported maximum mycelia growth of *P. cystidiosus*.

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