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Research Article

EVALUATION OF VITAMIN, PHYTOHORMONE AND TRACE ELEMENT REQUIREMENTS OF LENTINUS CLADOPUS LÉV

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ABSTRACT

Lentinus cladopus Lév is an edible and wood decaying mushroom. It was collected from the stem of *Albizia chinensis* and pure culture was raised on Potato Dextrose Agar.

Objective: The objective of present study was to investigate the effect of biochemical sources viz. vitamins, phytohormones and trace elements on the vegetative growth of *L. cladopus* Lév.

Material and Methods: The culture of *L. cladopus* Lév was raised in Malt Broth liquid medium at 30±1° C for investigating the role of vitamins (Ascorbic Acid, Biotin, Nicotinic Acid, Thiamine), phytohormones (Gibberellic Acid, Indole-3-Acetic Acid, Indole-3-Butyric Acid, Kinetin) and trace elements (Iron, Boron, Manganese, Molybdenum, Zinc).

Results: Thiamine at 10ppm, Indole-3-Butyric Acid at 15ppm and Iron at 1ppm were found to be supporting the maximum mycelial growth of *L. cladopus* Lév.

Conclusion: The vegetative growth of *L. cladopus* Lév can be enhanced by adding Thiamine, Indole-3-Butyric Acid and Iron in basal medium.

Keywords: *Lentinus cladopus* Lév, Vegetative growth, Vitamins, Phytohormones, Trace elements.

INTRODUCTION

Edible mushrooms are rich sources of nutraceuticals [1, 2, 3, 4]. One such edible mushroom is *Lentinus cladopus* Lév, which belongs to Class–Agaricomycetes, Order–Polyporales and Family–Polyporaceae [5]. It is reported growing in nature on variety of substrates including wooden logs in caespitose clusters and is characterized by membranous whitish smooth convex, depressed to subinfundibuliform pileus and cylindrical concolorous stipe [6]. In the present work, biochemical studies on *L. cladopus* Lév was carried out specifically to understand the role of different vitamins, growth regulators and trace elements on the vegetative growth of this mushroom.

MATERIAL AND METHODS

The fruit body of *L. cladopus* Lév was collected from the stem of *Albizia chinensis* Palampur (Himachal Pradesh) in North-West India. It was taxonomically investigated and identified [7]. Molecular sequence has been deposited in NCBI Gene Bank (accession number JQ868754). The specimen (PUN–3948) has been deposited in the Herbarium of Department of Botany, Punjabi University Patiala (Punjab) India. The pure culture of the fungus was raised on Potato Dextrose Agar (PDA) medium and maintained through periodic transfer onto PDA medium test tube slants at 27±1° C for further maintenance [8]. The culture has been deposited at Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology (IMTECH) Chandigarh, India under MTCC number 109481.

The Malt Broth liquid medium [9] was used as the basal medium for the study. The basal medium was prepared in deionized water and purified for removing impurities by using the standard methods [10]. As much as 20ml of sterilized medium was dispensed in each sterilized 100ml flask which was subsequently sealed with cotton wool and four replicates were prepared for each concentration.

Effect of vitamins

Five vitamins, namely Ascorbic Acid (AA), Biotin (B), Nicotinic Acid (NA), Riboflavin (R) and Thiamine (T) were selected to study the effect of variable concentrations on the vegetative growth of the fungus. For this purpose different concentrations (10ppm, 20ppm, 30ppm, 40ppm and 50ppm) of each vitamin were prepared and amended in the basal medium. Two controls were kept, one with the mixture of all the five vitamins and the second was basal medium without any vitamin.

Effect of growth regulators

Four growth regulators, namely Gibberellic Acid (GA), Indole-3-Acetic Acid (IAA), Indole-3-Butyric Acid (IBA) and Kinetin (K) were selected and in each case four concentrations (5ppm, 10ppm, 15ppm and 20ppm) were prepared to study the effect on the mycelial growth of *L. cladopus* Lév. Variable concentration of each growth regulator so prepared was amended in the basal medium. Basal medium devoid of any growth regulator and with the mixture of all four growth regulators served as checks.

Effect of trace elements

Five trace elements used at 1ppm, 2ppm and 5ppm concentration included Iron (Fe), Boron (B), Manganese (Mn), Molybdenum (Mo) and Zinc (Zn). Different salts consisting of these trace elements namely $FeSO_4.7H_2O$, H_3BO_3, $MnSO_4.7H_2O$, NH_4Mo and $ZnSO_4.7H_2O$ were used for the experiment. Stock solution of respective trace element of each concentration was prepared and then amended into the basal medium. The mixture of all the five trace elements and the basal medium devoid of any trace elements were used as controls.

Later on all the flasks were inoculated with 1ml homogenate containing mycelial bits of *L. cladopus* Lév. Complete procedure was performed aseptically under laminar air flow. Subsequently these inoculated flasks were incubated at 30±1° C for 10 days. To measure the mycelial growth rate, the mycelial mat from each flask was harvested, washed and dried at 65° C for 24 hours. The dry weight of mycelium was recorded for two subsequent days and an average of the two was taken as the actual weight.

RESULTS

The result obtained revealed that out of all the vitamins evaluated at variable concentrations, maximum mycelial growth of 6.30mg/ml was recorded at 10ppm concentration of Thiamine. Next best vegetative growth was supported by Ascorbic Acid (5.83mg/ml) at 20ppm concentration followed by vegetative growth of 5.69mg/ml in the Mixture at 10ppm concentration. The results achieved are presented in the table 1.

From amongst the different concentrations of growth regulators used, maximum vegetative growth of 4.17mg/ml was recorded in 15ppm concentration of Indole-3-Butyric Acid. Next best vegetative

growth was supported by Kinetin (4.08mg/ml) in 15ppm concentration followed by vegetative growth of 3.60mg/ml in Gibberellic Acid in 20ppm concentration. The mean dry weight of the fungus in mg/ml with ± standard deviation (SD) has been presented in table 2.

While working with variable concentrations of trace elements, mycelium was observed growing at different rates and maximum vegetative growth (7.46mg/ml) was obtained in 1ppm concentration of Iron followed by growth in 5ppm Control devoid of any trace elements. The results achieved are presented in table 3.

Table 1: Effect of variable concentrations of vitamins on mycelial growth of L. *cladopus* Lév

Vitamin	Mean dry weight of mycelium (mg/ml) ± standard deviation (SD) at variable concentrations				
	10ppm	20ppm	30ppm	40ppm	50ppm
Ascorbic Acid (AA)	5.49 ± 1.55	5.83 ± 0.37	5.49 ± 0.49	1.16 ± 0.25	0.68 ± 0.39
Biotin (B)	2.89 ± 1.66	1.34 ± 0.26	1.82 ± 0.67	1.40 ± 0.00	1.88 ± 0.07
Nicotinic Acid (NA)	4.93 ± 1.04	2.05 ± 0.49	1.78 ± 0.53	1.55 ± 0.10	0.57 ± 0.14
Riboflavin (R)	2.05 ± 0.22	4.67 ± 2.83	2.52 ± 0.64	1.97 ± 0.34	2.34 ± 0.70
Thiamine (T)	6.30 ± 0.96	5.31 ± 0.20	5.33 ± 0.93	3.61 ± 0.72	3.78 ± 0.82
Mixture	5.69 ± 0.71	3.88 ± 0.14	5.49 ± 0.96	3.40 ± 0.19	3.73 ± 0.29
Control	1.77 ± 0.14	1.77 ± 0.14	1.77 ± 0.14	1.77 ± 0.14	1.77 ± 0.14

Table 2: Effect of variable concentrations of growth regulators on mycelial growth of *L. cladopus* Lév

Growth Regulator	Mean dry weight of mycelium (mg/ml) ± standard deviation (SD) at variable concentration			
	5ppm	10ppm	15ppm	20ppm
Gibberellic Acid (GA)	2.84 ± 0.22	2.91 ± 0.53	1.93 ± 0.10	3.60 ± 1.62
Indole-3-Acetic Acid (I-3-AA)	1.74 ± 0.36	2.21 ± 0.23	2.28 ± 0.87	1.48 ± 0.20
Indole-3-Butyric Acid (I-3 BA)	2.23 ± 0.16	2.63 ± 0.16	4.17 ± 0.76	3.24 ± 0.25
Kinetin (K)	3.14 ± 0.79	2.54 ± 0.17	4.08 ± 0.22	2.34 ± 0.51
Mixture	1.95 ± 0.10	2.14 ± 0.48	1.62 ± 0.22	2.04 ± 0.28
Control	1.66 ± 0.48	1.66 ± 0.48	1.66 ± 0.48	1.66 ± 0.48

Table 3: Effect of variable concentration of trace elements on mycelial growth of *L. cladopus* Lév

Trace Element	Mean dry weight of mycelium (mg/ml) ± standard mycelium (SD) at variable concentrations		
	1ppm	2ppm	5ppm
Manganese (M)	6.92 ± 0.56	7.21 ± 0.22	6.83 ± 0.20
Iron (Fe)	7.46 ± 0.22	6.89 ± 0.17	6.79 ± 0.59
Molybdenum (Mo)	5.70 ± 1.23	7.07 ± 0.22	5.60 ± 0.41
Boron (B)	4.64 ± 0.50	4.60 ± 0.55	4.05 ± 0.36
Zinc (Zn)	4.55 ± 0.24	5.27 ± 1.16	3.99 ± 0.30
Mixture	5.88 ± 0.35	5.40 ± 0.81	6.28 ± 0.55
Control	7.38 ± 1.38	7.38 ± 1.38	7.38 ± 1.38

DISCUSSION

Mushrooms require vitamins, growth regulators and trace elements in small concentration for vegetative growth. [11] and [12] documented that Thiamine stimulated the maximum mycelial growth in *L. edodes* (Berk.) Singer. [13] have found Thiamine at 20ppm concentration to be stimulating for vegetative growth of *L. edodes* (Berk.) Singer. Biotin was evaluated as the most suitable vitamin for the vegetative growth of *L. subnudus* Berk. by [14]. The best mycelial growth of *L. connatus* Berk. was recorded in 0.01mg/ml of Thiamine and in comparison least vegetative growth was recorded in 0.01mg/ml of Ascorbic Acid [15]. [16] evaluated Thiamine as the best amongst the vitamins for mycelial growth of *L. tuberregium* (Fr.) Fr. The maximum vegetative growth of *L. squarrosulus* (Mont.) Singer was recorded in Nicotinic Acid which ranged from 15.45mg/ml in 10ppm to 15.95mg/ml in 50ppm concentration [17]. In the present study, the maximum vegetative growth of 6.30mg/ml was recorded at 10ppm concentration of Thiamine.

[11] and [12] reported that Gibberellic Acid enhanced the mycelial growth of *L. edodes* (Berk.) Singer. [13] documented the best mycelial growth of *L. edodes* (Berk.) Singer in Gibberellic Acid which was significantly higher in comparison to growth obtained in the other growth regulators. [14] evaluated 1ppm of 2, 4-D as the most favourable condition for the optimum mycelial growth of *L. subnudus* Berk. followed by 0.01ppm of Gibberellic Acid. Substantial vegetative growth of *L. connatus* Berk. was obtained in 5ppm Indole-3-Butyric Acid followed by 5ppm Naphthalene Acetic Acid and least mycelial growth was recorded in 40ppm of Naphthalene Acetic Acid [15]. Gibberellic Acid gave maximum vegetative growth of *L. squarrosulus* (Mont.) Singer which ranged from 16.50mg/ml in 5ppm to 17.55mg/ml in 20ppm concentration [17]. In this study, maximum growth of 4.17mg/ml was recorded at 15ppm concentration of Indole-3-Butyric Acid.

[12] documented the role of trace elements Mg, Mn, Cu and Zn in stimulating the mycelial growth in *L. edodes* (Berk.) Singer. [14] observed that Mg, K and Ca stimulated the vegetative growth in *L. subnudus* Berk. In case of *L. squarrosulus* (Mont.) Singer, [18] documented the importance of Ca^{2+} and Mn^{2+} ions in stimulating the vegetative growth. In the same vein, [17] reported that best vegetative growth of *L. squarrosulus* (Mont.) Singer was supported by Manganese which ranged from 14.95mg/ml in 1ppm to 15.25mg/ml in 5ppm concentration. According to [19] $CuSO_4.7H_2O$ showed the highest biomass of *Agaricus heterocystis*. In the present study, the maximum vegetative growth of 7.46mg/ml was obtained at 1ppm concentration of Iron.

CONCLUSION

The results obtained in the present study revealed that Thiamine at 10ppm concentration, Indole-3-Butyric Acid at 15ppm concentration and Iron at 1ppm concentration supported the maximum vegetative growth of *L. cladopus* Lév. The present findings have helped us to understand the biochemical requirements of *L. cladopus* Lév for enhancing the vegetative growth of this mushroom in the basal medium.

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