Mycosphere 1080–1090 (2013)
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Article

ISSN 2077 7019
Mycosphere
Online Edition

Doi 10.5943/mycosphere/4/6/6

Effect of vitamins and growth regulators on the vegetative growth of *Lentinus squarrosulus*

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Atri NS, Kumari B, Singh R, Upadhyay RC 2013 – Effect of vitamins and growth regulators on the vegetative growth of *Lentinus squarrosulus*. Mycosphere 4(6), 1080–1090, Doi 10.5943/mycosphere/4/6/6

Abstract

The experiments were carried out to observe the effect of vitamins and growth regulators on the vegetative growth of *Lentinus squarrosulus* (Mont.) Singer. Out of the vitamins and growth regulators employed, nicotinic acid and gibberellic acid, respectively supported the maximum vegetative growth for this mushroom.

Key words – basidiomycetes **–** edible – *Lentinus squarrosulus* **–** mushroom

Introduction

Lentinus squarrosulus belongs to the *Basideomycotina* sub-division and to *Polyporaceae* family, recognized by as many as 40 species the world over (Kirk et al. 2008). It is an edible species of the genus *Lentinus* that is highly appreciated and has a commercial potential in many countries especially China, Japan and United States, collected from rotten stumps of *Juglens regia* from Kotla Barog (Himachal Pradesh). The present paper documented the details of *in-vitro* studies undertaken to investigate the suitability of different vitamins and growth regulators for the mycelial growth of indigenous strain of *L. squarrosulus* cultured on Potato Dextrose Agar from the wild sample.

Materials and methods

The culture of *L. squarrosulus* (Mont.) Singer that was used in present experiment was raised from the indigenous collection made from the dry stem of *Juglens regia* from Kotla Barog of Sirmour District in Himachal Pradesh during July, 2006 (Fig. No. 1). Morphological observations in the field were made according to the field key to mushroom collector (Atri et al. 2005) and taxonomically identified.

Effect of vitamins

From the basal media prepared for trace element studies, vitamin impurities were removed by

Submitted 11 October 2013, Accepted 16 October 2013, Published online 24 November 2013
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adding 5 g/l of activated charcoal and autoclaving at 15 psi pressure for 15 minutes and subsequently filtering through Micropore filters (0.22 µm pore size). Stock solution of the vitamins was stored in amber colored bottles so as to avoid their oxidation. Five vitamins with five different concentrations were tried to study the effect on mycelial growth (Table 1). In addition to the mixture of all five vitamins, one check with no vitamin was also maintained. The stock solutions of all vitamins except biotin, were prepared in double glass distilled water and stored at 2 - 5^0 C in refrigerator. The stock solution of biotin was prepared in 5 ml of 50 per cent ethanol and volume made up with double glass distilled water. Vitamins were sterilized by filtration through Micropore filters (0.22 µm pore size).

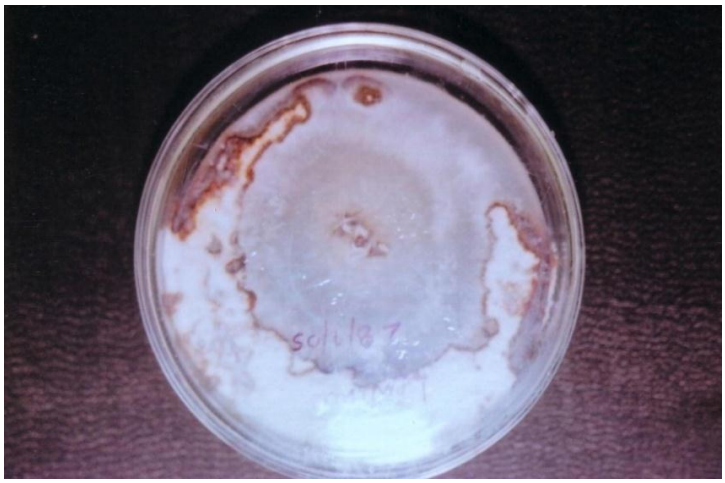

Fig. 1 – Pure culture of *Lentinus squarrosulus* on Potato dextrose agar

Table 1 The vitamins along with their concentrations used

Vitamins	**Concentrations (ppm)**
Riboflavin	10, 20, 30, 40, 50
Nicotinic Acid	10, 20, 30, 40, 50
Biotin	10, 20, 30, 40, 50
Ascorbic Acid	10, 20, 30, 40, 50
Folic Acid	10, 20, 30, 40, 50

(ii) **Effect of growth regulators:** The stock solutions of all growth regulators except gibberellic acid (GA) were prepared in double glass distilled water and stored at 5^0C in refrigerator (Table 2). GA was first dissolved in 10 ml of acetone and then required dilutions were prepared. Different concentrations of growth regulators used were as follows:-

Table 2 The growth regulators along with their concentrations used are given below:-

Growth Regulators	**Concentrations (ppm)**
Gibberellic Acid (GA)	5, 10,15, 20
Indole Acetic Acid (IAA)	5, 10, 15, 20
Indole Butyric Acid (IBA)	5, 10, 15, 20
Kinetin (K)	5, 10, 15, 20
Nephthyl Acetic Acid (NAA)	5, 10, 15, 20

Results and Discussion (Figs 2 & 3)

(i) Evaluation of different vitamins for mycelial growth

At 10 ppm concentration, maximum mycelial growth of 15.45 mg/ml was recorded in nicotinic acid followed by 15.25 mg/ml mycelial growth in the mixture medium where least mycelial growth of 13.75 mg/ml was supported by folic acid. At 20 ppm concentration maximum mycelial growth of 15.50 mg/ml was again recorded in nicotinic acid followed by 15.35 mg/ml mycelial growth in the mixture medium where least mycelial growth of 14.15 mg/ml was supported by biotin. At 30 ppm concentration maximum mycelial growth of 15.50 mg/ml was recorded in nicotinic acid followed by 15.45 mg/ml mycelial growth in the mixture medium where least mycelial growth of 14.20 mg/ml was supported by biotin. At 40 ppm concentration maximum mycelial growth of 15.75 mg/ml was recorded in nicotinic acid followed by 15.50 mg/ml mycelial growth in the mixture medium where least mycelial growth of 15.00 mg/ml was supported by biotin. At 50 ppm concentration maximum mycelial growth of 15.95 mg/ml was recorded in nicotinic acid followed by 15.50 mg/ml mycelial growth in the mixture medium where least mycelial growth of 15.15 mg/ml was supported by biotin and only 13.70 mg/ml mycelial dry weight was recorded in control. Mean dry weight of mycelium in different vitamins along with ± S.D. are depicted in Table-3 and histogram (Fig-2).

Table 3 Evaluation of impact of variable concentrations of vitamins in the basal medium on the mycelial growth of *L. squarrosulus.*

Sr. No.	Vitamin	Mean Dry Weight (mg/ml) ± S.D. at variable concentrations				
		10 ppm	20 ppm	30 ppm	40 ppm	50 ppm
1	Riboflavin	14.30 ± 1.73	14.75± 2.12	14.80 ± 1.00	15.20± 1.53	15.25±1.41
2	Nicotinic acid	15.45 ± 0.71	15.50± 2.00	15.50 ± 1.15	15.75± 1.00	15.95±1.00
3	Biotin	14.75 ± 0.58	14.15± 1.15	14.20 ± 2.83	15.00± 2.83	15.15±1.53
4	Ascorbic acid	14.75 ± 1.41	15.00± 2.83	15.30 ± 1.15	15.30± 1.53	15.30±1.53
5	Folic acid	13.75 ± 0.71	14.30± 1.53	14.55 ± 2.12	15.15± 1.41	15.20±1.73
6	Mixture	15.25 ± 1.41	15.35± 2.12	15.45 ± 1.73	15.50± 1.15	15.50±1.15
7	Control	12.70 ± 1.00	12.80± 2.83	13.05 ± 2.00	13.15± 1.73	13.70±1.41

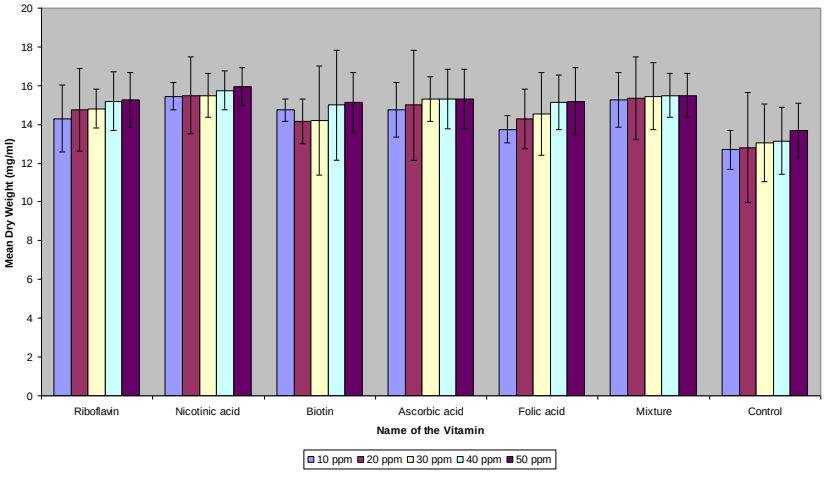

Fig. 2 – Histogram showing effect of different concentration of vitamins for mycelial growth of *L. squarrosulus*.

At 10 ppm concentration, as per t-values calculated, maximum vegetative growth was supported by nicotinic acid which was calculated significantly higher than growth with biotin ($t=0.0678$, $df=6$, $p<0.05$), ascorbic acid ($t=0.0125$, $df=6$, $p<0.05$), folic acid ($t=0.0478$, $df=6$, $p<0.05$), and control ($t=0.0634$, $df=6$, $p<0.05$) whereas in comparison the growth in the mixture was found to be non significant (Table-4).

Table 4 t-value showing the effect of 10 ppm concentration of different vitamins on mycelial growth of *L. squarrosulus*.

Sr. No.	**Vitamin**		**1 Riboflavin**	**2 Nicotinic acid**	**3 Biotin**	**4 Ascorbic acid**	**5 Folic acid**	**6 Mixture**	**7 Control**
		Dry wt. (mg/ml)	14.30	15.45	14.75	14.75	13.75	15.25	12.70
1	Riboflavin	14.30	--	0.0173*	0.0162*	0.0057	0.0083	0.0120*	0.0226*
2	Nicotinic acid	15.45		--	0.0678*	0.0125*	0.0478*	0.0035	0.0634*
3	Biotin	14.75			--	0.0027	0.0015	0.0037	0.0134*
4	Ascorbic acid	14.75				--	0.0179*	0.0070	0.0033
5	Folic acid	13.75					--	0.0026	0.0024
6	Mixture	15.25						--	0.0041
7	Control	12.70							--

* Significant at 0.05 level

Table 5 t-values showing the effect of 20 ppm concentration of different vitamins on mycelial growth of *L. squarrosulus*.

Sr. No.	**Vitamin**		**1 Riboflavin**	**2 Nicotinic acid**	**3 Biotin**	**4 Ascorbic acid**	**5 Folic acid**	**6 Mixture**	**7 Control**
		Dry wt. (mg/ml)	14.75	15.50	14.15	15.00	14.30	15.35	12.80
1	Riboflavin	14.75	--	0.0072	0.0070	0.0019	0.0048	0.0056	0.0155*
2	Nicotinic acid	15.50		--	0.0165*	0.0040	0.0134*	0.0014	0.0220*
3	Biotin	14.15			--	0.0078	0.0022	0.0140*	0.0124*
4	Ascorbic acid	15.00				--	0.0061	0.0027	0.0155*
5	Folic acid	14.30					--	0.0113*	0.0131*
6	Mixture	15.35						--	0.0203*
7	Control	12.80							--

* Significant at 0.05 level

Next best vegetative growth was supported by mixture of all the five vitamins which was significantly higher than growth in riboflavin ($t=0.0120$, $df=6$, $p<0.05$). In comparison, the growth with nicotinic acid, ascorbic acid, folic acid and biotin was found to be non significant (Table-4).

Maximum vegetative growth at 20 ppm was supported by nicotinic acid which was significantly higher than growth with biotin ($t=0.0165$, $df=6$, $p<0.05$), folic acid ($t=0.0134$, $df=6$, $p<0.05$), and control ($t=0.0220$, $df=6$, $p<0.05$) whereas in comparison the growth with ascorbic acid and mixture was found to be non significant (Table-5).

Next best results with respect to vegetative growth was supported by mixture of all the five vitamins which was significantly higher than growth in control ($t=0.0203$, $df=6$, $p<0.05$). Least vegetative growth was recorded in biotin (Table-5).

At 30 ppm concentration also maximum vegetative growth was supported by nicotinic acid which was significantly higher than growth achieved in folic acid ($t=0.0111$, $df=6$, $p<0.05$), and control ($t=0.0300$, $df=6$, $p<0.05$) whereas in comparison the growth with biotin, ascorbic acid and mixture was found to be non significant (Table-6).

Table 6 t-values showing the effect of 30 ppm concentration of different vitamins on mycelial growth of *L. squarrosulus*

Sr. No.	Vitamin		1 Riboflavin	2 Nicotinic acid	3 Biotin	4 Ascorbic acid	5 Folic acid	6 Mixture	7 Control
		Dry wt. (mg/ml)	14.80	15.50	14.20	15.30	14.55	15.45	13.05
1	Riboflavin	14.80	--	0.0129*	0.0565*	0.0092	0.0030	0.0092	0.0221*
2	Nicotinic acid	15.50		--	0.0069	0.0034	0.0111*	0.0006	0.0300*
3	Biotin	14.20			--	0.0101*	0.0027	0.0106*	0.0093
4	Ascorbic acid	15.30				--	0.0087	0.0020	0.0275*
5	Folic acid	14.55					--	0.0093	0.0145*
6	Mixture	15.45						--	0.0256*
7	Control	13.05							--

* Significant at 0.05 level

At this concentration the next best vegetative growth was supported by mixture of all the five vitamins which was significantly higher than growth in control ($t=0.0256$, $df=6$, $p<0.05$). Least vegetative growth was recorded in biotin (Table-6).

As per the t-values obtained, the maximum vegetative growth at 40 ppm concentration was supported by nicotinic acid in the basal medium which was significantly higher than growth in control ($t=0.0368$, $df=6$, $p<0.05$). In comparison the growth with biotin, ascorbic acid, folic acid and mixture was found to be non significant (Table-7).

Second maximum vegetative growth was supported by mixture of all the five vitamins which was significantly higher than growth in control ($t=0.0319$, $df=6$, $p<0.05$). Least vegetative growth was recorded in biotin (Table-7).

Table 7 t-values showing the effect of 40 ppm concentration of different vitamins on mycelial growth of *L. squarrosulus*.

Sr. No.	Vitamin		1 Riboflavin	2 Nicotinic acid	3 Biotin	4 Ascorbic acid	5 Folic acid	6 Mixture	7 Control
		Dry wt (mg/ml)	15.20	15.75	15.00	15.30	15.15	15.50	13.15
1	Riboflavin	15.20	--	0.0085	0.0017	0.0013	0.0006	0.0044	0.0251*
2	Nicotinic acid	15.75		--	0.0070	0.0069	0.0098	0.0046	0.0368*
3	Biotin	15.00			--	0.0026	0.0013	0.0046	0.0157*
4	Ascorbic acid	15.30				--	0.0020	0.0029	0.0263*
5	Folic acid	15.15					--	0.0544*	0.0253*
6	Mixture	15.50						--	0.0319*
7	Control	13.15							--

* Significant at 0.05 level

At 50 ppm concentration again maximum vegetative growth was supported by nicotinic acid which was significantly higher than growth in biotin ($t=0.0123$, $df=6$, $p<0.05$), mixture ($t=0.0835$, $df=6$, $p<0.05$) and control ($t=0.0368$, $df=6$, $p<0.05$) whereas in comparison the growth with ascorbic acid and folic acid was found to be non significant (Table-8).

Next best vegetative growth was supported by mixture of all the five vitamins which was significantly higher than growth in control ($t=0.0279$, $df=6$, $p<0.05$). Least vegetative growth was recorded in biotin (Table-8).

Table 8 t-values showing the effect of 50 ppm concentration of different vitamins on mycelial growth of *L. squarrosulus*.

Sr. No.	Vitamin		1 Riboflavin	2 Nicotinic acid	3 Biotin	4 Ascorbic acid	5 Folic acid	6 Mixture	7 Control
		Dry wt.(mg/ml)	15.25	15.95	15.15	15.30	15.20	15.50	13.70
1	Riboflavin	15.25	--	0.0114*	0.0013	0.0006	0.0006	0.0038	0.0219*
2	Nicotinic acid	15.95		--	0.0123*	0.0010	0.0010	0.0835*	0.0368*
3	Biotin	15.15			--	0.0019	0.0006	0.0517*	0.0917*
4	Ascorbic acid	15.30				--	0.0122*	0.0029	0.0217*
5	Folic acid	15.20					--	0.0040	0.0190*
6	Mixture	15.50						--	0.0279*
7	Control	13.70							--

* Significant at 0.05 level

(ii) ***Evaluation of different growth regulators for mycelial growth***

So as to evaluate the effect of variable concentrations of growth regulators in the basal medium, five different growth regulators namely 1-napthyl acetic acid (NAA), indole 3-acetic acid (IAA), gibberellic acid (GA), kinetin (K) and indole-3-butyric acid (IBA) with concentrations of 5 ppm, 10 ppm, 15 ppm, and 20 ppm along with the mixture of all the five growth regulators were selected. Yeast glucose medium with pH 4.0 was taken as the basal medium. The medium flasks were inoculated with freshly homogenized inoculum and then respective growth regulators were added through micropore filters. Then these inoculated flasks were incubated at 30 ± 1^0C for 13 days and mycelial dry weight was recorded in milligrams. At 5 ppm concentration, maximum mycelial growth of 16.05 mg/ml was recorded in gibberellic acid followed by 15.25 mg/ml mycelial growth in NAA where least mycelial growth of 11.75 mg/ml was supported by IBA. At 10 ppm concentration maximum mycelial growth of 16.50 mg/ml was recorded in gibberellic acid followed by 15.50 mg/ml mycelial growth in NAA where least mycelial growth of 11.85 mg/ml was supported by IBA. At 15 ppm concentration maximum mycelial growth of 16.75 mg/ml was recorded in gibberellic acid followed by 15.90 mg/ml mycelial growth in NAA where least mycelial growth of 12.20 mg/ml was supported by IBA. At 20 ppm concentration maximum mycelial growth of 17.55 mg/ml was recorded in gibberellic acid followed by 16.20 mg/ml mycelial growth in NAA where least mycelia growth of 12.45 mg/ml was supported by IBA and only 12.30 mg/ml mycelial dry weight was recorded in control. Mean dry weight of the mycelium in different growth regulators along with ± S.D. are depicted in Table-9 and histogram (Fig – 3).

Table 9 Effect of variable concentration of growth regulators on mycelial growth of *L. squarrosulus.*

Sr. No.	Growth Regulator	Mean Dry Weight (mg/ml) ± S.D. at variable concentrations			
		5 ppm	10 ppm	15 ppm	20 ppm
1	1-Nepthyl Acetic Acid	15.25± 1.53	15.50 ± 1.00	15.9± 1.41	16.20± 1.41
2	Indole Acetic Acid	12.65± 0.71	12.80 ± 2.52	13.25± 0.58	13.55 ± 1.15
3	Gibberellic Acid	16.05± 1.73	16.50 ± 2.83	16.75 ± 1.15	17.55 ± 2.31
4	Kinetin	14.20± 0.71	14.65 ± 1.15	15.30 ± 1.41	15.55 ± 1.00
5	Indole Butyric Acid	11.75± 1.41	11.85 ± 0.58	12.20 ± 1.71	12.45 ± 2.65
6	Mixture	15.15± 1.21	15.25 ± 1.00	15.60 ± 0.63	15.90 ± 1.21
7	Control	11.55± 1.15	12.00 ± 2.12	12.15 ± 2.31	12.30 ± 1.53

Maximum vegetative growth at 5 ppm concentration was supported by gibberellic acid which was significantly higher than growth in kinetin ($t=0.0279$, $df=6$, $p<0.05$), indole butyric acid ($t=0.0544$, $df=6$, $p<0.05$), Mixture ($t=0.0369$, $df=6$, $p<0.05$) and control ($t=0.0612$, $df=6$, $p<0.05$) (Table-10).

Next best vegetative growth was supported by nephthyl acetic acid which has been recorded to be significantly higher than growth in IAA (t=0.0435, df=6, p<0.05), kinetin (t=0.0176, df=6, p<0.05), indole butyric acid (t=0.0475, df=6, p<0.05), mixture (t=0.0357, df=6, p<0.05) and Control (t=0.0546, df=6, p<0.05). In comparison the growth with gibberellic acid was found to be non significant. Least vegetative growth was recorded in indole butyric acid (Table-8) which was significantly higher than growth in mixture (t=0.0239, df=6, p<0.05). In comparison the growth in Control was found to be non significant.

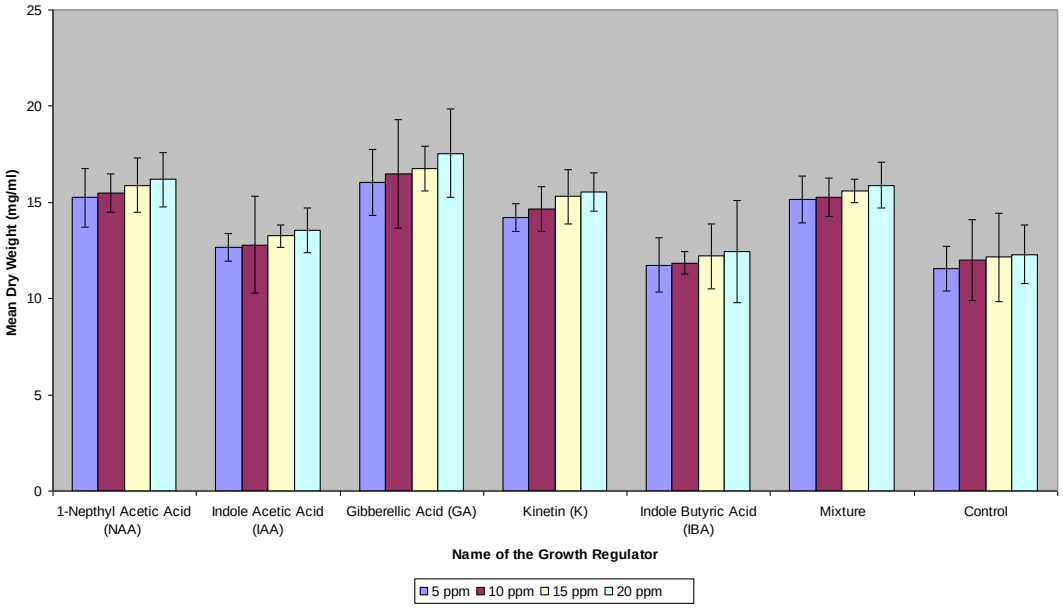

Fig. 3- Histogram showing effect of different concentration of growth regulators for mycelial growth of *L. squarrosulus*.

Table 10 t-value showing the effect of 5 ppm concentration of different growth regulators on mycelial growth of *L. squarrosulus*.

Sr. No.	Growth regulator		1 Nepthyl Acetic Acid	2 Indole Acetic Acid	3 Gibberellic Acid	4 Kinetin	5 Indole Butyric Acid	6 Mixture	7 Control
		Dry wt. (mg/ml)	15.25	12.65	16.05	14.20	11.75	15.15	11.55
1	Nepthyl Acetic Acid	15.25	--	0.0435*	0.0097	0.0176*	0.0475*	0.0357*	0.0546*
2	Indole Acetic Acid	12.65		--	0.0514*	0.0436*	0.0161*	0.0101*	0.0230*
3	Gibberellic Acid	16.05			--	0.0279*	0.0544*	0.0369*	0.0612*
4	Kinetin	14.20				--	0.0438*	0.0397*	0.0554*
5	Indole Butyric Acid	11.75					--	0.0239*	0.0031
6	Mixture	15.15						--	0.0316*
7	Control	11.55							--

* Significant at 0.05 level

Maximum vegetative growth at 10 ppm concentration was recorded in gibberellic acid which was significantly higher than growth in kinetin (t=0.0171, df=6, p<0.05), indole butyric acid (t=0.0455, df=6, p<0.05), mixture (t=0.0293, df=6, p<0.05) and control (t=0.0359, df=6, p<0.05) (Table-11).

Table 11 t-values showing the effect of 10 ppm concentration of different growth regulators on mycelial growth of *L. squarrosulus*.

Sr. No.	Growth regulator		1 Nepthyl Acetic Acid	2 Indole Acetic Acid	3 Gibberellic Acid	4 Kinetin	5 Indole Butyric Acid	6 Mixture	7 Control
		Dry wt.(mg/ml)	15.50	12.80	16.50	14.65	11.85	15.25	12.00
1	Nepthyl Acetic Acid	15.50	--	0.0281 *	0.0094	0.0157 *	0.0893 *	0.0130 *	0.0422*
2	Indole Acetic Acid	12.80		--	0.0276 **	0.0188 *	0.0103 *	0.0060	0.0068
3	Gibberellic Acid	16.50			--	0.0171 *	0.0455 *	0.0293 *	0.0359 *
4	Kinetin	14.65				--	0.0614 *	0.0010	0.0310 *
5	Indole Butyric Acid	11.85					--	0.0169 *	0.0019
6	Mixture	15.25						--	0.0269 *
7	Control	12.00							--

* Significant at 0.05 level ** Significant at 0.01 level

Next best vegetative growth was recorded in nepthyl acetic acid which was significantly higher than growth in indole acetic acid (t=0.0281, df=6, p<0.05), kinetin (t=0.0157, df=6, p<0.05), indole butyric acid (t=0.0893, df=6, p<0.05), mixture (t=0.0130, df=6, p<0.05) and control (t=0.0422, df=6, p<0.05). In comparison the growth with gibberellic acid was found to be non significant. Least vegetative growth was recorded in indole butyric acid (Table-11).

Maximum vegetative growth at 15 ppm concentration was recorded in gibberellic acid which was significantly higher than growth in kinetin (t=0.0225, df=6, p<0.05), indole butyric acid (t=0.0619, df=6, p<0.05), mixture (t=0.0279, df=6, p<0.05) and control (t=0.0504, df=6, p<0.05) (Table-12).

Next maximum vegetative growth was recorded in nepthyl acetic acid which was significantly higher than growth in indole acetic acid (t=0.0491, df=6, p<0.05), gibberellic acid (t=0.0132, df=6, p<0.05), indole butyric acid (t=0.0468, df=6, p<0.05), and control (t=0.0391, df=6, p<0.05). In comparison the growth in kinetin and mixture was found to be non significant. Amongst the different growth regulators used, least vegetative growth was recorded in indole butyric acid (Table-12).

Maximum vegetative growth at 20 ppm concentration was recorded in gibberellic acid which was significantly higher than growth in kinetin (t=0.0224, df=6, p<0.05), indole butyric acid (t=0.0410, df=6, p<0.05), mixture (t=0.0259, df=6, p<0.05) and control (t=0.0535, df=6, p<0.05) (Table-11). Next best vegetative growth was recorded in nepthyl acetic acid which was significantly higher than growth in indole acetic acid (t=0.0411, df=6, p<0.05), gibberellic acid (t=0.0141, df=6, p<0.05), kinetin (t=0.0106, df=6, p<0.05), indole butyric acid (t=0.0353, df=6, p<0.05), mixture (t=0.0160, df=6, p<0.05) and control (t=0.0530, df=6, p<0.05). Least vegetative growth was recorded in indole butyric acid (Table -13).

Discussion

During the present investigation, for vegetative growth of *L. squarrosulus* nicotinic acid @ 15.5 mg/ml of concentration in basal medium gave best mycelial growth on dry weight basis. Wuyep et al. (2003) also employed malt extract agar incorporated with 0.0002% NaH_2PO_4, 0.001% $FeSO_4.7H_2O$, 2.0% thiamine hydrochloride and 2% H_3BO_3 for maintenance of culture of this mushroom. Atri et al. (2010) observed for maximum vegetative growth of *L. connatus* thiamine @ 0.01 mg/100 ml of concentration gave best vegetative growth in basal medium on dry weight basis. Kaur and Lakhanpal (1995) reported maximum mycelial growth in case of *Lentinula edodes* in medium with 20 ppm of thiamine. Hiroe and Ikuda (1960) and Ishikawa (1967) have also reported thiamine stimulating best mycelial growth in case of *Lentinula edodes.* Out of the growth regulators employed, best mycelial growth on dry weight basis in *L. connatus* has been documented in 5 ppm

Table 12 t-values showing the effect of 15 ppm concentration of different growth regulators on mycelial growth of *L. squarrosulus*.

Sr. No.	Growth regulator		1 Nepthyl Acetic Acid	2 Indole Acetic Acid	3 Gibberellic Acid	4 Kinetin	5 Indole Butyric Acid	6 Mixture	7 Control
		Dry wt. (mg/ml)	15.9	13.25	16.75	15.30	12.20	15.60	12.15
1	Nepthyl Acetic Acid	15.9	--	0.0491 *	0.0132 *	0.0085	0.0468 *	0.0058	0.0391 *
2	Indole Acetic Acid	13.25		--	0.0768 *	0.0380 *	0.0162 *	0.0673 *	0.0130 *
3	Gibberellic Acid	16.75			--	0.0225 *	0.0619 *	0.0279 *	0.0504 *
4	Kinetin	15.30				--	0.0392 *	0.0132 *	0.0329 *
5	Indole Butyric Acid	12.20					--	0.0230 *	0.0004
6	Mixture	15.60						--	0.0010
7	Control	12.15							--

* Significant at 0.05 level

Table 13 t-values showing the effect of 20 ppm concentration of different growth regulators on mycelial growth of *L. squarrosulus*.

Sr. No.	Growth regulator		1 Nepthyl Acetic Acid	2 Indole Acetic Acid	3 Gibberellic Acid	4 Kinetin	5 Indole Butyric Acid	6 Mixture	7 Control
		Dry wt. (mg/ml)	16.20	13.55	17.55	15.55	12.45	15.90	12.30
1	Nepthyl Acetic Acid	16.20	--	0.0411 *	0.0141 *	0.0106 *	0.0353 *	0.0160 *	0.0530 *
2	Indole Acetic Acid	13.55		--	0.0438 *	0.0371 *	0.0107 *	0.0173 *	0.0184 *
3	Gibberellic Acid	17.55			--	0.0224 *	0.0410 *	0.0259 *	0.0535 *
4	Kinetin	15.55				--	0.0309 *	0.0180 *	0.0502 *
5	Indole Butyric Acid	12.45					--	0.0369 *	0.0013
6	Mixture	15.90						--	0.0010
7	Control	12.30							--

* Significant at 0.05 level

concentration of indole -3- butyric acid in the basal medium. Kaur and Lakhanpal (1995) reported maximum mycelial growth of *Lentinula edodes* in gibberellic acid.

Manjunathan and Kaviyarasan (2010) observed that thiamine gave best among the vitamins followed by biotin and togoferrol. Lander (1954) also found that thiamine stimulates mycelial growth of *Cercospora arachidicola* Hori in liquid culture. Madunagu (1988) also observed that thiamine is required for good growth in mushrooms. Luo (1993) reported that, different vitamins produce different effects on mycelial growth within a certain concentration range. Nolan (1970) observed that combined amino acids stimulate much growth than single amino acids. Adejoye et al. (2007) reported riboflavin and pyridoxine promoted the best growth of 85 and 100.45 mg/30 cm in *Schizophyllum commune* There are no such studies available for comparing the results in *L. squarrosulus*, however Treschow (1944) documented the requirement of biotin and thiamine for mycelial growth in case of *Agaricus bisporus*.

In case of *Lentinula edodes*, Han et al. (1981) obtained the best mycelial growth with 10 mg/l of gibberellic acid. Chang and Miles (1989) studied the effect of indole-3-acetic acid on the mycelial growth in *Lentinula edodes*, and reported that in all the concentrations of indole-3-acetic acid, growth was higher in comparison to control. Sladky and Tichy (1974) applied indole-3-acetic acid, gibberellic acid and kinetin to *Lentinus trigrinus* cultivated on small "Cellulose cylinders" and results indicated

that solution of 100 ppm IAA and 400 ppm gibberellic acid increased mean sporophore size by 25% and 16%, respectively. In present study at 20 ppm concentration maximum mycelial growth of 17.55 mg/ml was recorded in gibberellic acid and least mycelial growth of 12.45 mg/ml was there in Indole-3-butyric acid. So far there is no work of this type by any worker on *L. squarrosulus.*

Acknowledgements

Thanks are due to Head Department of Botany Punjabi University Patiala for providing laboratory facilities and to U.G.C. for grant under ASIST and DRS (SAP III) Programs.

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