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Studies on the effect of different trace elements and vitamins on mycelial growth of Shiitake mushroom (*Lentinula edodes*) (Berk.) Pegler

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Abstract
Shiitake mushroom (*Lentinula edodes*) commonly known as Golden oak mushroom, is the second most popular edible mushroom in the world which contributes about 26% in the production which is due to both its high nutritional content and potential therapeutic uses. The present investigation was done to evaluate the effect of three trace elements *viz.* Manganese, Iron and Zinc @ 2 ppm, 3 ppm and 4 ppm concentrations and three vitamins *viz.* Thiamine, Nicotinic acid and Ascorbic acid @ 30 ppm and 50 ppm. The results indicated that in case of different trace elements, maximum (90.00 mm) mycelial growth of Shiitake (LE-22104) was discovered on Manganese @ 3 ppm and minimum (68.67 mm) mycelial growth found on control. Similarly, maximum dry mycelial weight was found in Manganese @ 3 ppm (4.58 mg/100 ml) and minimum dry weight was found in control (1.10 mg/100 ml). In case of different vitamins, maximum mycelial growth was observed in thiamine @ 50 ppm (90.00 mm) and minimum mycelial growth was observed in control which was devoid of vitamins (68.67 mm). Similarly, maximum dry mycelial weight was found in thiamine@ 50 ppm (4.26 mg/100 ml) and least dry weight was found in control (1.12 mg/100 ml).

Keywords: Shiitake mushroom, mycelial growth, spawn growth, dry mycelial growth, trace elements, manganese, pigeon pea flour

Introduction
Mushroom is a ubiquitous group of fungi with many uses. Mushroom is not a taxonomic group but do include well over 14,000 species which have macroscopic fruit-bodies, which are large enough to be seen by the naked eye. Mushrooms are fleshy mysterious life forms which have attracted the attention of naturalists before the invention of microscope. References of mushrooms can be seen in almost every civilization records, whether they are Romans, Greeks or Chinese. Some used them as food while others considered them as medicine or for spiritual purposes (Rai and Arumuganathan, 2003) [13].

Shiitake (*Lentinula edodes*) are the mushrooms that the Chinese affectionately named "Xiang-gu" or "Shiang-gu", "the fragrant mushrooms", while '*edodes*' refers to the eatable in Latin (Halpren, 2007) [3]. *L. edodes* contains proteins (2.22-2.60% fresh and 25.9% dry weight), lipids (primarily linoleic acid), water-soluble carbohydrates (0.45-0.72 g/100 g dry weight), total carbohydrates (67.0%) (Terashita *et al.*, 1990) [16], insoluble (41.6%) and soluble (3.4%) fibre (Yoshida *et al.*, 1987) [19], minerals (especially calcium), and vitamins B2 and C (Liu and Bau, 1980; Ying, 1987) [11]; [18]. The fruiting bodies contain a high amount of ergosterol, between 873 and 4381 IU/100 g dry weight, a pro-vitamin that converts to vitamin D in the presence of sunlight (Ying, 1987) [18].

Materials and Methods
Experimental site
The present research was carried out in the Mushroom Research & Training Centre of the Department of Plant Pathology at the Sardar Vallabhbhai Patel University of Agriculture and Technology in Modipuram, Meerut 250110 (Uttar Pradesh).

Establishment of pure culture
The present investigation was conducted to find out the effect of different trace elements on the mycelial growth, mycelial growth rate/day, dry mycelial growth and dry mycelial growth

rate/day of strain LE-22104 of Shiitake mushroom. Potato dextrose agar (PDA) medium was used as basal medium. Iron (Fe), Manganese (Mn) and Zinc (Zn) were the three trace elements utilised at 2 ppm, 3 ppm and 4 ppm concentrations. For the experiment, different salts consisting of these trace elements namely $FeSO_4.7H_2O$, $MnSO_4.7H_2O$ and $ZnSO_4.7H_2O$ were used. The stock solution of all these aforementioned trace elements were prepared and stock solution of 2 ppm concentration of trace elements were prepared by adding 0.4 mg of trace element in 200 ml of double distilled water; 3 ppm concentration of trace elements were prepared by adding 0.6 mg of trace element in 200 ml of double distilled water. Similarly, 4 ppm concentration of trace elements were prepared by adding 0.8 mg of trace element in 200 ml of double distilled water. The relevant trace element stock solution of each concentration was added into the basal medium. The basal medium devoid of any trace element was used as control. The medium was then sterilised and poured into 90 mm diameter Petri plates with 20 ml of media per plate. These plates were then inoculated centrally with a 9 mm diameter disc of a one-week-old Shiitake culture and incubated at 25±1 °C. For dry mycelial weight of aforementioned trace elements, Potato Dextrose Broth (PDB) was selected as the basal medium. The stock solution of different trace elements of different concentration was added into potato dextrose broth in the conical flask of 250 ml @ 100 ml in each flask. These flasks were then sterilised for 20 minutes at 121 °C and 15 psi pressure. These flasks were then inoculated with 9 mm discs of Shiitake mushroom. After that, the flasks were incubated at 25±1 °C for 15 days. The culture was then filtered with Whatman filter paper no.1, and the mycelium obtained was dried at 60 °C in a hot air oven for 48 hours before being measured on an electronic scale, for dry matter growth. For the experiment on effect of different vitamins on mycelial growth, mycelial growth rate/day, dry mycelial growth and dry mycelial growth rate/day of strain LE-22104 of Shiitake mushroom have been taken. The experiment was carried out using three vitamins of different doses *viz.* thiamine (Vitamin B_1) @ 30 ppm, nicotinic acid (Vitamin B_3) @ 30 ppm, ascorbic acid (Vitamin C) @ 30 ppm, thiamine (Vitamin B_1) @ 50 ppm, nicotinic acid (Vitamin B_3) @ 50 ppm and ascorbic acid (Vitamin C) @ 50 ppmand potato dextrose agar (PDA), was taken as control for mycelium growth. Stock solution for all the vitamins were prepared. In 200 ml of double distilled water, 6 mg of vitamin was added to create a stock solution with a 30 ppm concentration of vitamins. Similarly, 200 ml of double distilled water was diluted with 10 mg of vitamin to create a 50 ppm concentration of vitamins. Stock solution of vitamins were added in the PDA media separately and sterilized it in the autoclave at 121 °C and 15 psi for 20 minutes. 20 ml of sterilized PDA medium was dispensed in 90 mm sterilized Petriplate and three replicates of each concentration of each vitamin was prepared. Petriplates were inoculated with diameter disc of 9 mm of 7 days old actively growing culture of Shiitake mushroom. The inoculated plates were incubated at 25±1 °C in B.O.D. The observations of mycelial growth (mm) were recorded every 3 days until the colony covered the entire Petriplate, which was 90 mm in diameter. For the estimation of dry matter growth, the stock solution of three vitamins of different concentrations was added into Potato Dextrose Broth (PDB) then put into 250 ml conical flasks at 100 ml each flask and autoclaved it at 121 °C, 15 psi for 20 minutes. The sterilized conical flasks were inoculated with 9 mm diameter disc of 7 days old culture of *L. edodes*. The flasks were cultured for 15 days at 25 ± 1 °C, with three replications of each treatment. The culture was then filtered using Whatman filter paper no. 1, and the mycelium mat was dried in a hot air oven at 60 °C for 48 hours before being weighed on an electronic scale.

Statistical Analysis

The Complete Randomized Design (CRD) was applied and the data thus obtained were analyzed statistically. Analysis of variance (ANOVA) technique and critical difference (CD) was calculated at five percent level of significance for comparison with other treatment (Kumar *et al*., 2022a; Kumar *et al*., 2022b) [9, 8].

Results and Discussion

In present investigation of different trace elements on 15th day of inoculation as shown in Table- 01 and Fig. 01, maximum mycelial growth of strain LE-22104was observed in PDA + Manganese @ 3 ppm with mycelial growth 90.00 mm and growth rates 6.00 mm/day, followed by PDA + Manganese @ 2 ppm with mycelial growth 87.50 mm and growth rates 5.83 mm/day. The minimum mycelial growth was observed in control (PDA) with mycelial growth 68.67 mm and growth rates 4.57 mm/day which was significantly lower than all the other treatments and followed by PDA + Zinc @ 4 ppm with mycelial growth 76.00 mm and growth rates 5.06 mm/day. Similarly, in case of dry mycelium weight of Shiitake mushroom (LE-22104) on 15th day of observation, maximum weight of dry mycelium was obtained in PDB + Manganese @ 3 ppm (4.58 mg/100 ml with dry mycelial growth rate 0.30 mg/day) followed by PDB + Manganese @ 2 ppm (3.14 mg/100 ml with dry mycelial growth rate 0.20 mg/day). The minimum weight of dry mycelium was obtained in control (PDB) (1.10 mg/100 ml with dry mycelial growth rate 0.07 mg/day) which was followed by PDB + Zinc @ 4 ppm (1.40 mg/100 ml with dry mycelial growth rate 0.09 mg/day). The results are found in accordance with Kaur (1994) [6] reported that the maximum mycelial growth on *L. edodes* was supported by 2 ppm Manganese (39.53 mg), followed by 1 ppm Iron (37.99 mg) and minimum growth was supported by 1 ppm Zinc (22.93 mg). Lata (2018) [10] also concluded that the maximum mycelial growth on *Lentinus sajor-caju* (Fr.) Fr. (9.53 mg/ml) was recorded in the medium containing Manganese as trace element at 10 ppm concentration which was followed by Mixture (8.84 mg/ml) at 1 ppm concentration. Wuyep *et al*. (2003) [17] also documented the importance of metal ions Mn^{2+} and Ca^{2+}in stimulating the mycelial growth in case of *L. squarrosulus* (Mont.) Singer as compared to Mg^{2+} and K^{+} ions which did not stimulate the vegetative growth of mycelia in this mushroom.

The result revealed that in case of different vitamins on 15th day as shown in Table. 02 and Fig. 02, maximum mycelial growth of the strain (LE-22104) was observed in PDA + thiamine @ 50 ppm with mycelial growth 90.00 mm and growth rates 6.00 mm/day which was significantly higher than all other treatments and followed by PDA + ascorbic acid @ 50 ppm with mycelial growth 87.00 mm and growth rates 5.80 mm/day. The minimum mycelial growth was observed in control (PDA) with mycelial growth 68.67 mm and growth rates 4.57 mm/day which was followed by PDA + nicotinic acid @ 30 ppm with mycelial growth 77.00 mm and growth rates 5.13 mm/day. Similarly, in case of dry mycelium weight on 15th day of observation, maximum weight of dry mycelium was obtained in PDB + thiamine @ 50 ppm (4.26 mg/100 ml), followed by PDB + ascorbic acid @ 50 ppm (3.88 mg/100

ml). The minimum weight of dry mycelium was obtained in control (PDB) (1.12 mg/100 ml) which was followed by PDB + nicotinic acid @ 30 ppm (1.90 mg/100 ml). The findings of the present experiment having similarities with the results of Kaur and Lakhanpal (1995) [7] who reported that the maximum mycelial growth was recorded in 20 ppm thiamine followed by 50 ppm nicotinic acid. Hiroe and Ikuda (1960) [4] as well as Ishikawa (1967) [5] also reported that the thiamine played the major role in stimulating the mycelial growth.Kaur (1994) [7] also observed that at 100 ppm concentration thiamine hydrocloride supported maximum mycelial growth which was non-significantly different from all other combinations. Atri *et al*. (2010) [2] 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. Manjunathan and Kaviyarasan (2010) [12] reported that the maximum effective vitamin was thiamine which was followed by biotin and tocoferrol. Atri and Guleria (2013) [1] also reported that the maximum mycelial growth of 6.30 mg/ml was recorded at 10 ppm concentration of Thiamine.

Table 1: Effect of different trace elements (inorganic source) on mycelial growth of Shiitake mushroom (*L. edodes*)

S. No.	Treatment	Mycelial Growth (mm)					Growth rate (mm/day)	Dry mycelial weight (mg/100 ml)	Dry matter growth rate mg/day
		3rd day	6th day	9th day	12th day	15th day			
1	PDA+ Manganese @ 2 ppm	13.16	30.00	46.67	69.97	87.50	5.83	3.14	0.20
2	PDA+ Iron @ 2 ppm	12.08	27.41	42.83	66.00	83.67	5.57	2.54	0.17
3	PDA+ Zinc @ 2 ppm	11.91	26.75	41.16	65.16	82.00	5.46	2.40	0.16
4	PDA+ Manganese @ 3 ppm	14.00	31.50	49.16	70.25	90.00	6.00	4.58	0.30
5	PDA + Iron @ 3 ppm	12.58	27.75	44.41	67.41	84.30	5.62	2.78	0.18
6	PDA +Zinc @ 3 ppm	10.83	24.91	38.16	60.50	78.16	5.21	1.50	0.10
7	PDA + Manganese @ 4 ppm	12.91	29.25	45.50	68.30	86.50	5.76	2.96	0.19
8	PDA+ Iron @ 4 ppm	11.50	25.75	40.50	62.67	81.67	5.44	1.92	0.12
9	PDA+ Zinc @ 4 ppm	10.83	24.41	35.00	59.00	76.00	5.06	1.40	0.09
10	PDA (Control)	10.16	23.60	33.08	42.16	68.67	4.57	1.10	0.07
11	CD at 5%	0.810	0.806	2.494	1.422	1.182	-	0.330	_
12	SE (m)	0.273	0.271	0.840	0.479	0.398	-	0.111	_

Average of three replications

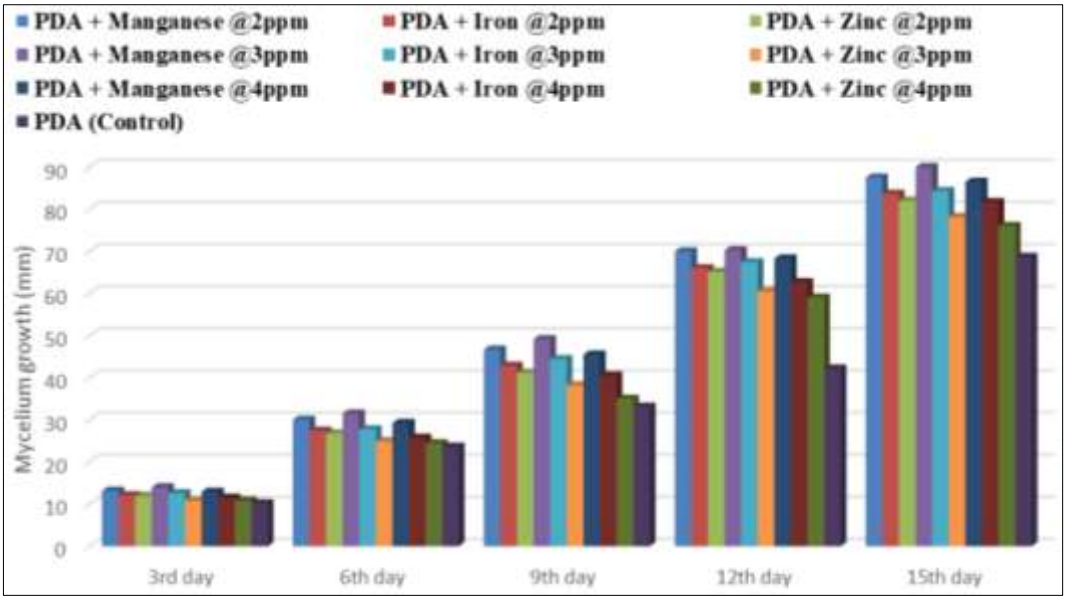

Fig 1: Effect of different trace elements (inorganic source) on mycelial growth of Shiitake mushroom (*L.edodes*)

Table 2: Effect of different vitamins on mycelial growth of Shiitake mushroom (*Lentinula edodes*)

S. No.	Treatment	Mycelial Growth (mm)					Growth rate (mm/day)	Dry mycelial weight (mg/100 ml)	Dry matter growth rate mg/day
		3rd day	6th day	9th day	12th day	15th day			
1	PDA+ Thiamine @ 30 ppm	11.83	28.83	43.33	65.83	81.50	5.43	2.80	0.18
2	PDA+ Nicotinic acid @ 30 ppm	9.83	24.67	39.50	61.00	77.00	5.13	1.90	0.12
3	PDA+ Ascorbic acid @ 30 ppm	11.00	25.83	41.33	63.83	79.50	5.30	2.71	0.18
4	PDA+ Thiamine @ 50 ppm	15.50	35.16	48.83	72.50	90.00	6.00	4.26	0.28
5	PDA + Nicotinic acid @ 50 ppm	13.00	31.83	45.16	68.00	84.67	5.64	3.43	0.22
6	PDA + Ascorbic acid @ 50 ppm	14.30	32.33	47.50	70.16	87.00	5.80	3.88	0.26
7	PDA (control)	10.16	23.60	33.08	42.16	68.67	4.57	1.12	0.07
8	CD at 5%	0.841	1.431	1.453	1.579	1.091	-	0.263	-
9	SE (m)	0.275	0.467	0.475	0.516	0.356	-	0.086	-

Average of three replications

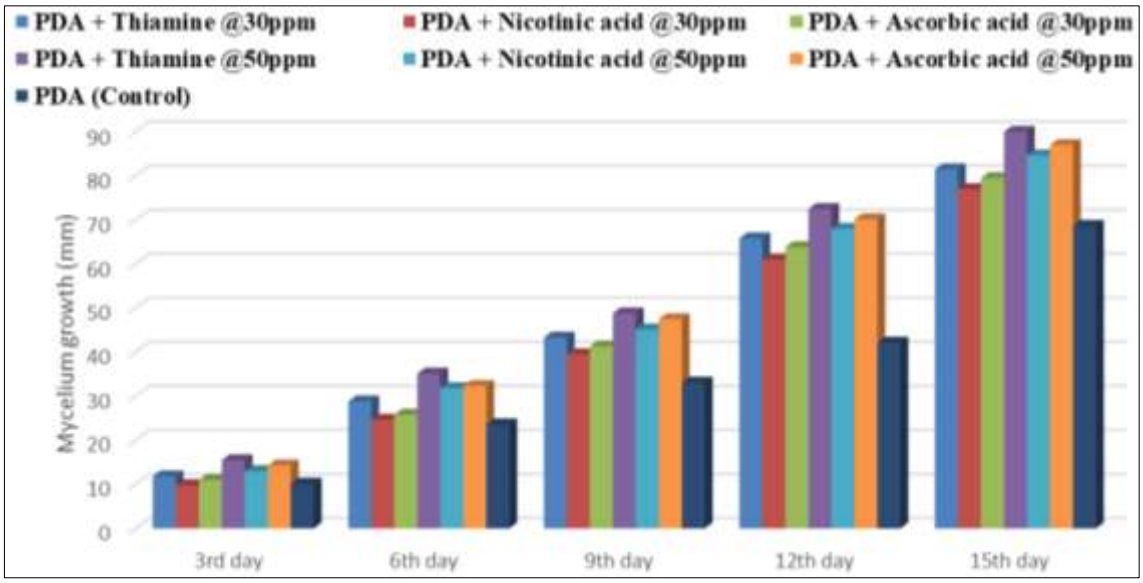

Fig 2: Effect of different vitamins on mycelial growth of Shiitake mushroom (*Lentinula edodes*)

Conclusion

Regarding the various trace elements evaluated for mycelia growth, Manganese @ 3 ppm was found to have the highest mycelial growth, mycelial growth rate/day, dry mycelia growth, and dry mycelia growth rate/day. For the various vitamins, Thiamine @ 50 ppm was found to have the highest mycelial growth, mycelial growth rate/day, dry mycelia growth, and dry mycelia growth rate/day. On the basis of present research, we conclude that Manganese @ 3 ppm and Thiamine @ 50 ppm is the best supplement to enhance the mycelial growth of *L. edodes* (LE-22104).

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