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Effect of Vitamin on Mycelial Growth of *Tricholoma crassa*

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Abstract

Tricholoma (Fr.) Quel is well recognized genus of the family Tricholomataceae containing about one hundred species *Tricholoma* sp. is an ectomycorrhizal fungus which has immense potential in medicinal world as it has been found to have antibiotic substances and is beneficial to stomach and intestinal ailments besides being an excellent nutritious edible fungus having protein (14.60 to 18.43%), carbohydrate (70.99 to 78.52%) and ash (6.88 to 10.58%) on percentage dry weight. Among seven vitamins tested at 50 and 100 ppm concentration, nicotinic acid at 50 ppm gave maximum mycelial growth of *Tricholoma crassa*. In case of folic acid, thiamine, nicotinic acid and pyridoxine, the mycelial growth was inhibited at higher doses (100 ppm) as compared to lower doses (50 ppm).
Key words : *Tricholoma crassa*, Mycelial growth, Vitamins.

Tricholoma (Fr.) Quel is well recognized genus of the family Tricholomataceae containing about one hundred species (1). *Tricholoma* sp. is an ectomycorrhizal fungus which has immense potential in medicinal world as it has been found to have antibiotic substances (2) and is beneficial to stomach and intestinal ailments (3) besides being an excellent nutritious edible fungus having protein (14.60 to 18.43%), carbohydrate (70.99 to 78.52%) and ash (6.88 to 10.58%) on percentage dry weight. India Samajpati (4) cultivated this mushroom on wheat straw and on paddy straw and found it to be an excellent edible mushroom. In Rajasthan, this mushroom was successfully cultivated by Zacharia (5) on wheat straw. Matsutake is the common name for a group of mushrooms of *Tricholoma* spp. that the Japanese have considered an autumn delicacy for many hundreds of years. They grow in a symbiotic relationship on the roots of various soft wood trees such as *Pinus* spp. and angiosperms such as oaks. Matsutake fruiting bodies usually form in rings and arise from more or less circular zones of intense fungal activity called shiro which first appear when the trees are about 20 years old (4—5 cm high). In India, mushroom cultivation has great potential due to favorable weather conditions, abundant cheaper agro-waste and cheaper availability of labor. Today it is much more encouraging with an overall increase in production by five times during the last decade. However, this quantity is obviously too small if the vast market potential of this great country is to be fully exploited. In Rajasthan, mushroom cultivation is on small scale and at present commercially only two species *Agaricus bisporus* and *Pleurotus* spp. are grown. The major mushroom growing districts of Rajasthan are Udaipur, Jaipur, Ajmer, Bikaner, Bhilwara, Kota, Bundi, Jodhpur, Jalore, Sikar, Sriganganagar, Banswara, Tonk and Pali. Total production of mushroom during 1997-1998 was 200 tonnes in the state. The estimated total production for the year 2002-2003 is of 300 metric tonnes. Thus, per capita production is meager. Moreover, the climate of the state is more conducive for growing of tropical mushroom (March to October) as compared to temperate mushroom (October to March). Therefore, more emphasis is given to develop the cultivation technology of summer mushroom *Calocybe indica* and *Tricholoma* spp. as it can fetch more market price from international market.

Methods

All the experiments related to research work were conducted at Mushroom Research laboratory, Department of Plant Pathology, R. C. A., Udaipur.

Cleaning of Glasswares

Throughout the study corning and borosil made

Table 1. Effect of vitamin on the mycelial growth of *Tricholoma crassa*. *Average of four replication.

	Vitamins	Concentration (ppm)	Mycelial growth* (mm)	Per cent increase/ decrease
1.	Folic acid	50	83.75	10.75
		100	80.75	6.78
2.	Thiamine	50	87.75	16.04
		100	78.50	3.80
3.	Riboflavin	50	85.62	13.22
		100	88.75	17.36
4.	Nicotinic acid	50	89.50	18.35
		100	79.62	5.28
5.	Pyridoxine	50	79.87	5.62
		100	75.37	– 0.33
6.	Ascorbic acid	50	76.37	0.99
		100	82.25	8.76
7.	Biotin	50	71.25	– 5.77
		100	77.37	2.31
8.	Control	–	75.62	00.00
	SE ±		1.83	
	CD at 5%		5.23	
	CD at 1%		6.99	

glasswares were used. The glasswares were cleaned with 6% chromic acid ($K_2 Cr_2O_7$ 60 g, conc H_2SO_4 60 ml, distilled water 1000ml) followed by several washings in running tap water and finally cleaned with distilled water and air dried before use.

Sterilization

Glasswares. Petriplates were sterilized by keeping in hot air oven at 180 C for 120 minutes. After cooling at room temperature these glassware were used for different experimentation purpose.

Substrate. For sterilization of substrate, the wheat straw was presoaked in chemical solution of formaldehyde (2%) dichlovos (0.5%) and bavistin (50 ppm) for 14—18 hours. After this excess water was strained/decanted. The chemicals were used to check the weed fungi and insect pest present in wheat straw.

Cropping Room. Cropping room was pre-sterilized by spraying formaldehyde solution (0.2%) with foot sprayer. After spraying room was kept air tight for 48 hours and after 48 hours fresh air was introduced by air circulation duct, then rooms were used for cropping purpose.

Casing Material. Casing materials were sterilized by using 2% formaldehyde solution and keeping it for 48 hours in a polythene sheets /closed containers and then opened to remove the fumes.

Media. Media used was sterilized at 15 p.s.i. at 121 C for 20 minutes in an autoclave. The cork borer, inoculation needle, forceps etc were initially dipped in rectified spirit and finally sterilized on spirit lamp and allowed to cool down before use.

Supplements. Different supplements like wheat bran, rice bran, neem cake, maize meal, cotton seed cake, cotton linter, termitorium soil were sterilized with 2.0% formaldehyde solution by standard method.

Culture. Culture of *Tricholoma crassa* was procured from Mr S. Tonty Aporn working at division of Plant Pathology and Microbiology, Department of Agriculture in the city of Bangkok, Thailand.

Multiplication of Culture. Culture was multiplied and maintained in test tubes having malt extract agar (1.0%) medium by inoculating tubes with mycelial bit from pure culture tubes (Plate la). The newly inoculated slants were incubated at 28 ± 1 C.
Composition of malt extract agar medium (1%) was as follows : Malt extract 10 g, agar-agar 20 g distilled water 1000 ml, pH 7.0.

Preservation of Besidiospores

The preservation of basidiospores was done by the spore print technique. A mature fresh, healthy fruit body was harvested and the stipe was cut. Whatman No. 1 filter paper was sterilized by dipping the paper wholly in rectified spirit and was allowed to air dry. The pileus of the mushroom was kept on sterilized filter paper in such a way that gills face the filter paper and it was covered with a beaker then kept overnight. The basidiospores after dispersal from the gills, stuck to the filter paper and this paper was again air dried and wrapped in an aseptic polythene bags and kept safely for further multiplication. Some crystals of silica gel-G was also kept to avoid moisture.

Preparation of Spawn

Spawn was prepared in polypropylene bags or bottles. Healthy sorghum seeds were cleaned and soaked in water overnight and then boiled in water upto semi cooked condition. After boiling, excess of water decanted off. Then grains were allowed to sur-

face dry by spreading on bloating sheet for an hour. These seeds were then mixed with 4 per cent calcium carbonate ($CaCO_3$) on grain weight basis to adjust the pH of grain at 7.0—7.8 and to check the coagulation of the grains. About 500 g of grains were filled in each bag (upto 3/4th of total capacity), then plugged tightly with non-absorbent cotton and sterilized at 15 p.s.i. and 121 C for one and half hour. After cooling to room temperature each bag was kept in laminar air flow cabinet and given UV-light exposure for 15—20 minutes for surface sterilization, each bag was inoculated with mycelial bits of 10 days old mushroom culture and then incubated at 28±1 C adequate growth was obtained.

Addition of Spawn

Grain spawn prepared as per method given in 3.8 was added at 5% and was mixed with substrate in three layers in polythene bags. The substrate filled in sterilized polythene bags (32 × 22 cm) of 1 kg capacity. The mouth of bags were tied with rubber bands and the bags were kept in cropping room in which a constant humidity (70%) and temperature (28 ± 1 C) were maintained.

Effect of Vitamin on Mycelial Growth and Yield of Tricholoma crassa

Six important vitamins viz., folic acid, thiamine, riboflavin, nicotinic acid, pyridoxine biotin and ascorbic acid each at 50 and 100 ppm concentration were tested for their value in supporting maximum growth of the *Tricholoma crassa.* These above vitamins were added in malt extract medium before pouring in petriplates and inoculated with culture of *Tricholoma crassa* as described earlier.

Results and Discussion

In this experiment, seven, vitamins viz., folic acid, thiamine, riboflavin, nicotinic acid, pyridoxine, ascorbic acid and biotin each at (50 and 100 ppm) were tried to see their effect on the mycelial growth of *T. crassa*. Maximum mycelial growth was recorded with nicotinic acid (50 ppm) 89.50 mm followed by riboflavin (100 ppm) 88.75 mm, thiamine (50 ppm) 87.75 mm, riboflavin (50 ppm) 85.62 mm, folic acid (50 ppm) 83.75 mm, ascorbic acid (100 ppm) 82.25 mm, folic acid (100 ppm) 80.75 mm, pyridoxine (50 ppm) 79.87 mm, nicotinic acid (100 ppm) 79.62 mm, thiamine (100 ppm) 78.50 mm, biotin (100 ppm) 77.37 mm, ascorbic acid (50 ppm) 76.37 mm, pyridoxine (100 ppm) 75.37 mm, and minimum in biotin (50 ppm) 71.25 mm (Table 1). In folic acid, thiamine, nicotinic acid and pyridoxine, the mycelial growth was inhibited at higher doses (100 ppm) as compared to lower doses (50 ppm).

Significant increase in mycelial growth was obtained in folic acid (50 ppm), thiamine (50 ppm), riboflavin (50 and 100 ppm), nicotinic acid (50 ppm) and ascorbic acid (100 ppm) at 5 and 1% level of significance. Kaur and Lakhanpal (6) recorded maximum mycelial growth of *Lentinus edodes* with thiamine hydrochloride (20 ppm) followed by nicotinic acid (50 ppm) and riboflavin (100 ppm). Jandaik and Kapoor (7) studied partial deficiency of thiamine and biotin in case of *Pleurotus sajor-caju, Podaxis pistillaris* and *Phellorinia inquinans*. They also recorded the optimum concentration 50 and 100 µg/liter. of thiamine and biotin respectively for maximum mycelial yield. Das et al. (8) found the folic acid (5 ppm) and ascorbic acid (2.5 ppm) gave best results in terms of mycelial growth and mushroom yield of *Pleurotus florida*.

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