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RESEARCH ARTICLE

Effect of epidemiological factors on the vegetative growth of certain wild edible mushrooms from the Eastern Ghats of Tamil Nadu

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ABSTRACT

Studies were conducted to ascertain the most suitable substrate and growing conditions for the best vegetative growth of some wild edible mushrooms (*Russula parazurea, Volvariella volvacea, Calvatia bicolor, Agaricus bitorquis, Termitomyces eurrhizus* and *Tricholoma caligatum*) found along the Eastern Ghat regions of Tamil Nadu. Potato dextrose yeast agar medium followed by Potato dextrose coconut extract agar medium were found to be superior in supporting the vegetative growth of all the selected mushrooms. PDY broth infused with sorghum flour and horsegram flour @ 2 per cent significantly enhanced the bio mass production of the mushrooms. *A. bitorquis, V. volvacea, R. parazurea* and *C. bicolor* preferred pH 6.5. *R. parazurea , T.eurrhizus* and *C. bicolor* preferred 26ºC and 28ºC, respectively.

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INTRODUCTION

Mushrooms are considered as good vegetable which consist of higher protein, fibre and carbohydrate and lesser content of fat, vitamins and minerals (Patrabansh and Madan, 1997). India with its diversity of soil and climatic conditions supports a rich variety of edible mushroom flora. In India, about 300 species under 70 genera have been reported as edible (Purkayastha and Chandra, 1985), but many of them are collected from natural habitats by the local people for their use and marketing due lack of cheap and successful technology for the production of many mushroom species with higher biological efficiency. Studies to evaluate suitable substrate and growth conditions are need of the hour to bring new edible mushrooms under artificial conditions to meet the growing demand for consumption and exploitation.

Surveys were conducted during the monsoon periods on the hilly tracts of Kolli hills, Shevaroy hills, and Jevadu hills along the Eastern Ghat regions of Tamil Nadu and about six different varieties of edible mushrooms (*Russula parazurea* J. Schff. (Section Heterophyllae), *Volvariella volvacea* (Bull. ex Fr.) Singer, *Calvatia* sp. aff. *Calvatia bicolor, Agaricus bitorquis, Termitomyces* sp. aff. *Termitomyces eurrhizus* and *Tricholoma caligatum*) were collected with the help of the local people and identified at Agharkar Research Institute, a National Fungal Culture Collection of India (NFCCI).

For each mushroom, there exists an optimum temperature, optimum pH and an optimum humidity, just as there exists upper and lower limits outside of which the fungus cannot form fruit or cannot even survive (Giovanni, 1981). In an attempt to cultivate these mushrooms under artificial conditions, experiments were undertaken to ascertain the most suitable solid and liquid media, organic additives to the medium, optimum temperature and pH which encourage the maximum vegetative growth and bio mass production of the above mushrooms.

MATERIAL AND METHODS

Effect of different culture media on the vegetative growth of the collected mushrooms :

Nine different media *viz.*, Corn meal agar, Czapek's dox agar, Potato dextrose coconut water agar, Malt agar, Oat meal agar, Potato dextrose agar, Potato dextrose yeast agar, Tapioca agar and Tapioca dextrose agar were tested for their efficacy in supporting the mycelial growth of the collected edible mushrooms. The media were prepared and taken in Petri plates to assess their effect on the mycelial growth. The experiment was replicated thrice. The plates were inoculated with nine mm mycelial discs obtained from the actively growing seven day old culture of each mushroom separately and incubated at room temp. (28±2°C). The radial growth was recorded periodically up to 14 days and expressed in mm.

Effect of different additives on the *in vitro* growth of the collected mushrooms :

The effect of various organic additives *viz.*, rice flour, wheat flour, tapioca flour, horsegram flour, sorghum flour, blackgram flour, greengram flour, soya flour @ 2 per cent level were infused into PDY broth in order to evaluate their effect on the bio mass production of the collected mushrooms**.** Medium devoid of supplements served as control and three replications were maintained for each treatment. The additive impregnated media were inoculated with seven mm mycelial discs obtained from the actively growing seven day old culture of the collected mushrooms and incubated at room temp. (28±2°C). To assess the fungal biomass, after the incubation period the mycelial mat was filtered through previously weighed Whatman No. 1 filter paper. The dry weight of the fungal mass was determined after drying in a hot air oven at 105ºC to a constant weight.

Effect of pH on the vegetative growth of the collected mushrooms :

Potato dextrose yeast agar (PDYA) was used for this experiment. The pH of both solid medium was adjusted to levels of 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0. After sterilizing they were inoculated with nine mm mycelial discs of actively growing seven day old culture of the collected mushrooms and incubated at room temp. (28±2°C). The treatments were replicated thrice.

Effect of temperature on the vegetative growth of certain wild edible mushrooms :

The tissues of different mushrooms inoculated in sterilised Petriplates separately were incubated at different temp. *viz.*, 20, 22, 24, 26, 28, 30 and 32. The treatments were replicated thrice. The radial growth was recorded periodically up to 14 days and expressed in mm.

Statistical analysis :

Statistical evaluation was done using analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The statistical significance was expressed at $P<0.05$.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under following heads :

Effect of culture media on the vegetative growth of wild edible mushrooms :

Among the nine different solid culture media, Potato dextrose yeast agar (PDYA) medium, Potato dextrose coconut water agar medium supported the maximum vegetative growth followed by Potato dextrose agar medium (PDA). Of all the

Table 1 : Effect of different culture media on the vegetative growth of certain wild edible mushrooms

Tr. No.	Medium	Radial growth (mm)					
		A. bitorquis 8th day	*V. volvacea* 8th day	*T. eurrhyzus* 14th day	*R. parazurea* 11th day	*C. bicolor* 14th day	*Tricholoma* sp. 14th day
1.	Corn meal agar	84.9_c	80.0_e	64.0_e	61.9_e	54.1_h	61.5_f
2.	Czapek's dox agar	80.4_e	82.1_d	61.2_f	68.6_d	68.3_d	58.7_g
3.	PDA + coconut water	90.0_a	90.0_a	72.1_a	88.6_b	73.6_a	67.6_b
4.	Malt agar	87.6_b	87.0_b	70.0_b	55.8_f	71.4_b	62.1_e
5.	Oat meal agar	82.2_d	84.3_c	66.7_c	66.1_d	67.2_e	64.2_c
6.	Potato dextrose agar	90.0_a	90.0_a	66.2_d	82.0_c	70.0_c	63.7_d
7.	Potato dextrose yeast agar	90.0_a	90.0_a	72.0_a	90.0_a	71.7_b	69.5_a
8.	Tapioca agar	72.4_f	75.2_g	59.3_g	59.1_f	57.4_g	56.8_h
9.	Tapioca dextrose agar	82.3_d	78.2_f	67.0_c	61.4_e	63.2_f	64.6_c
Chlamydospore formation	Potato dextrose yeast agar, PDA & PDCA	-	++++	-	-	-	-
Chlamydospore formation	Tapioca agar	-	+	-	-	-	-

Values not sharing a common superscript differ significantly at $P<0.05$ (DMRT)

\+ Low density; ++ Medium density; +++ High density; ++++ Very high density

mushrooms tested, the least growth was observed by Tapioca agar medium (Table 1).

The linear growth of *A. bitorquis* and *V. volvacea* in PDYA, PDCWA and PDA medium recorded minimum duration of 8 days to cover the Petriplate. *R. parazurea* took 11.1 days to cover the Petri plate in PDYA. *C. bicolor* and *T. eurrhyzus* recorded the maximum of 73.6 and 72.1 mm, respectively in PDCA medium which was at par with PDYA medium on the 14 th day. Tapioca agar medium showed a slow rate of growth and low mycelial density when compared to the other cultures. The lag phase in the growth of certain species on specific synthetic media is probably due to a component of the medium which is inhibitory to the growth. Further studies would, however, need to be conducted in order to test this assumption. The chlamydospore formation in PDYA medium was maximum followed by PDA, PDCA which were at par with each other in the formation of chlamydospore in *V. volvacea*. Tapioca agar medium recorded the minimum density of chlamydospores and the presence of HCN in the tapioca extract might be the reason for the inhibition of chlamydospore formation in the Tapioca agar medium.

It is discernable from the data (Table 2) that the media supplemented with various additives except blackgram flour showed enhanced mycelial growth of the fungus when compared to control. Sorghum flour was found to be superior in influencing the maximum bio mass in *A. bitorquis* (2.10g), *R. parazurea* (1.91g), *T. eurrhizus* (1.36g) and *T. caligatum* (1.76g). *V. volvacea* grew best in the medium infused with horsegram flour (2.15g). Wheat flour enhanced the mycelial growth of *C. bicolor* (1.43g) Sorghum floor followed horsegram flour and soya flour influenced the bio mass production of all the mushrooms in the decreasing order of merit.

The following findings also tend to support to the present results. Sangeetha *et al.* (2004) reported that media

Table 2 : Effect of different additives on *in vitro* growth of the collected mushrooms

Sr. No.	Additives (@ 2 % level)	Mycelial dry weight (g)					
		A. bitorquis 8th day	*V. volvacea* 8th day	*R. parazurea* 11th day	*T. eurrhyzus* 14th day	*C. bicolor* 14th day	*T. caligatum* 14th day
1.	Rice flour	1.85_{d}	2.03_{c}	1.61_{d}	1.36_{a}	1.24_{e}	1.48_{d}
2.	Wheat flour	1.62_{e}	1.92_{e}	1.52_{e}	1.27_{b}	1.43_{a}	1.47_{c}
3.	Tapioca flour	1.56_{f}	1.69_{f}	1.58_{d}	1.06_{f}	1.25_{e}	1.38_{d}
4.	Horse gram flour	2.03_{b}	2.15_{a}	1.81_{b}	1.26_{b}	1.25_{e}	1.63_{b}
5.	Sorghum flour	2.10_{a}	2.10_{b}	1.91_{a}	1.36_{a}	1.38_{b}	1.76_{a}
6.	Black gram flour	1.32_{h}	1.42_{h}	1.33_{g}	1.22_{c}	1.13_{g}	1.19_{f}
7.	Green gram flour	1.48_{g}	1.58_{g}	1.40_{f}	1.18_{d}	1.28_{d}	1.22_{e}
8.	Soya flour	1.91_{c}	1.97_{d}	1.68_{c}	1.01_{g}	1.10_{h}	1.55_{c}
9.	control	1.45_{h}	1.33_{i}	1.41_{f}	1.34_{e}	1.32_{c}	1.20_{f}

Values not sharing a common superscript differ significantly at P<0.05 (DMRT)

Table 3 : Effect of pH on the vegetative growth of certain wild edible mushrooms

Tr. No.	Potato dextrose yeast agar medium (pH)	Radial growth (mm)					
		A. bitorquis 8th day	*V. volvacea* 8th day	*R. parazurea* 11th day	*T. eurrhyzus* 14th day	*C. bicolor* 14th day	*Tricholoma* sp. 14th day
1.	5.0	52.1_{f}	76.1_{b}	62.1_{f}	48.6_{e}	54.8_{c}	52.1_{e}
2.	5.5	71.2_{c}	75.2_{b}	70.6_{e}	55.1_{d}	56.4_{c}	60.6_{d}
3.	6.0	86.1_{b}	90.0_{a}	80.3_{c}	58.3_{d}	75.3_{a}	60.3_{d}
4.	6.5	90.0_{a}	90.0_{a}	90.0_{a}	60.8_{c}	73.8_{a}	60.8_{d}
5.	7.0	90.0_{a}	90.0_{a}	85.1_{b}	70.1_{a}	72.6_{b}	75.1_{a}
6.	7.5	74.4_{c}	74.4_{c}	78.0_{d}	65.0_{b}	70.4_{b}	72.0_{b}
7.	8.0	65.2_{d}	65.2_{d}	55.5_{g}	65.5_{b}	56.4_{c}	65.5_{c}
8.	8.5	55.9_{e}	45.0_{e}	49.8_{h}	39.8_{f}	48.1_{d}	39.8_{f}
9.	9.0	33.8_{g}	$32._{f}$	32.9_{i}	22.9_{g}	34.9_{e}	22.9_{g}
Chlamydospore formation	6.5	-	++++	-	-	-	-
Chlamydospore formation	6.0	-	+++	-	-	-	-

Values not sharing a common superscript differ significantly at P<0.05 (DMRT)
\+ Low density; ++ Medium density; +++ High density; ++++ Very high density

supplemented with gram powder enhanced the growth and chlamydospore density of *V. volvacea*. In the present study also, addition of horsegram flour resulted in enhanced mycelial growth and chlamydospore density of *V. volvacea*. The addition of these gram flours could have increased the nutrient content of the substrate which might be attributed as the reason for the enhanced growth of the fungus as stated by El-Kattan *et al.* (1991).

Effect of pH on the vegetative growth of wild edible mushrooms:

The pH of the medium has a greater impact upon the growth of any organism. It had been well established that hydrogen ion conc. (pH) in the growing media influenced the growth and metabolism of any of the mushroom fungi (Zervakis *et al.*, 2004; Sharma *et al.*, 2005). A pH of 6.5 was found to be optimum for the maximum biomass production by *V. volvacea* (Akinyele and Adetuyi, 2005). The present findings corroborate with these earlier reports.

Similarly, among the different pH tested *viz.*, (5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5 and 9) pH 6.5, 7.0 and 6.0 was found optimum in the decreasing order of merit for all the tested mushrooms. A pH of 6.5 recorded the fastest growth of *A. bitorquis, V. volvacea* coveritng the Petriplate on the 8th day and *R.parazurea* on the 11th day. However, pH 7 was at par with 6.5 for *A. bitorquis* and *V. volvacea*. *Tricholoma* sp. and *T. eurrhyzus* preferred pH of 7 recording (75.1 and 70.1 mm, respectively) on the 14th day of observation. At pH below 5.5 and above 7.5, a drastic reduction in the mycelial growth of the fungus was observed. *T. eurrhyzus* showed the least growth at all pH among all the mushrooms in *in vitro* conditions. Formation of chlamydospores was at its maximum in the culture with pH 6.5 and 6.0.

According to Hopkins (1995), at a very strong acidic or alkaline pH, cell wall may corrode and selective permeability function of the cell membrane may be impaired, which might be the reason for the drastic reduction in the mycelial growth. Also, enzymes have an optimal pH range within which they are most effective and therefore substrate pH affects fungal growth and productivity (Przybylowicz and Donoghue, 1990) and the synthesis of various fungal metabolites (Moore-Landecker, 1982).

Effect of temperature on the vegetative growth of certain wild edible mushrooms :

Temperature plays an important factor in the growth and development of edible mushrooms. Tropical mushrooms required high temp. conditions (25- 45^{0}C) for their cultivation (Agarwala, 1973). The cultivation of *V. volvacea* should be done during the periods between May and September at a temp. range of 30- 42^{0}C (Suman and Sharma, 1999). It was reported that *Flammulina velutipes* can be grown when the temp. is between 10 to 25^{0}C, *Agaricus bisporus* at 14-25^{0}C. *A. bitorquis* at 24 to 34^{0}C and *Pleurotus ostreatus* at 8–25^{0}C (Kapoor, 1989; Akinyele and Adetuyi, 2005).

The tissues of the selected mushrooms inoculated in sterilised Petriplates separately were incubated at different temp., showed significant variations in the level of the mycelial growth. From the readings of the Table 4, it is evident that among the different levels of temp. maximum growth (90 cm) was observed with *V. volvacea* on (7.21days) at 32 ºC, *A. bitorquis* on (8.88 days) at 24ºC, *R. parazurea* covered the the petriplate on the 11.19 day at 26ºC. *C. bicolor* and *T. eurrhyzus* recorded (78 and 70 mm), respectively at 28º C on the 14th day of observation.

Temperature affects vegetative and reproductive growth primarily because enzymes, which are essential for the degradation of the substrate, have an optimal temperature range within which they are most active. Temperature therefore determines the fungal metabolic activity (Przybylowicz and Donoghue, 1990) and the synthesis of various fungal metabolites (Moore-Landecker, 1982).

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Table 4 : Effect of temperature on the vegetative growth of certain wild edible mushrooms

Temperature (ºC)	Radial growth (mm)				
	Volvariella volvacea 7th day	*Agaricus bitorquis* 8th day	*Russula parazurea* 11th day	*Termitomyces eurrhizus* 14th day	*Calvatia bicolour* 14th day
20	33.8_g	81.8_b	61.4_d	41.4_e	41.4_e
22	42.0_f	82.0_b	84.8_b	53.2_d	53.2_d
24	59.8_e	90.0_a	90.0_a	61.4_c	61.4_c
26	65.4_d	90.0_a	90.0_a	64.8_b	64.8_b
28	72.9_c	77.0_c	77.0_c	70.0_a	78.0_a
30	85.4_b	53.8_d	63.8_d	61.0_c	61.0_c
32	90.0_a	37.0_e	33.0_e	31.0_f	31.0_f

Values not sharing a common superscript differ significantly at P<0.05 (DMRT)

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