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In-vitro antifungal activity of certain phytoextracts against *Trichoderma viride*- a pathogen of *Auricularia delicata* (Fr.) Henn.

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In vitro antifungal study was conducted to determine the effect of 7 (seven) phytoextracts *viz. Tamarindus indica, Vitex trifolia, Azadirachta indica, Tagetes erecta, Allium sativum, Solanum nigrum* and *Adhatoda vasica* on the mycelial growth of *Trichoderma viride* - a pathogen for *Auricularia delicata.* Among 7 (seven) phytoextracts, the highest degree of *T. viridi* growth inhibition was shown by *Solanum nigrum* (54.15%) followed by *Vitex trifolia* (33.77%) and *Adhatoda vasica* (28.68%) whereas in *Auricularia delicata* the highest growth inhibition was recorded by phytoextracts of *Tamarindus indica* (64.49%) followed by *Solanum nigrum* (55.24%) and *Azadirachta indica* (10.88%) respectively.

Key words : Phytoextracts, antifungal activity, *Trichoderma viride*, pathogen of *Auricularia delicata*

INTRODUCTION

Auricularia (Jew's ear mushroom) is a popular edible fungus of the tropics and sub-tropics especially in the Asian countries. Among the different mushroom species it ranks fourth in terms of global production (4,85,000 tones per year) (Chang, 1999). However, the association of a number of undesirable fungi during different stages of crop growth has posed a serious threat to its higher production and productivity. Goltapeh *et al.* (1989) have reported *Cladobotryum verticillatum* (Link ex. S.F.Gray) Huges as a pathogen for infecting fruitbodies of *Auricularia polytricha.* Bhandal and Mehta (1987) have also reported green mould pathogens during the cultivation of *Auricularia polytricha.*

Varying crop losses during the cultivation of *Auricularia* due to *Trichoderma viride* have been reported by previous workers (Beach, 1939; Gandy and Edwards, 1953). Shiddique *et al.* (2004) and Mandvi and Singh (2005) have also reported the efficacy of leaf extracts of angiospermic plants against the moulds and other pathogenic fungi of mushrooms. These phytoextracts have fungitoxic substances that are eco-friendly as compared to synthetic chemical fungicides, which often imposes undesirable side effects. In the present study, an effort has been made to evaluate the efficacy of 7 (seven) phyto-extracts against *Trichoderma viride* - a pathogen for *Auricularia delicata* in *in vitro* condition.

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MATERIALS AND METHODS

Isolation and identification of pathogen

The pathogen (*Trichoderma viride*) was isolated from both the substrate and the fruiting bodies of *Auricularia delicata* with the help of sterilized needle and finally transferred to Petriplates containing PDA medium under aseptic condition and incubated at 25±1°C.

After 7 days, the colonies so developed were examined directly under a microscope for identification. The pathogen was identified as *Trichoderma*

viride (Pers. ex. S.F. Gray) by studying its morphological and microscopic characters and by comparing with standard literatures. Pure culture of the pathogen was maintained on PDA slants for further analysis.

Preparation of Phytoextracts

Fresh leaves of *Tamarindus indica, Vitex trifolia, Azadirachta indica, Tagetes erecta, Allium sativum, Adhatoda vasica* and seed of *Solanum nigrum* were collected and washed thoroughly in distilled water and processed in a grinder mixer with sterile distilled water (1:1, w/v). The homogenate was filtered through double layer muslin cloth and finally through Whatmann No.I filter paper. Prior to use, the aqueous extracts were sterilized at 121°C for 15 minutes.

In-vitro screening of phyto extracts

Poison food technique of Falck (1907) (Seth *et al.,* 1986-87) was used for *in vitro* study. Phytoextracts at 5 (five) concentrations (1%,5%,10%,15% and 20%) were added to the sterilized PDA medium in conical flasks, mixed thoroughly and 20 ml of this mixture was transferred to each Petriplate (9 cm diameter). Petriplates devoid of any phytoextracts served as control. In each case, 3 (three) replicates were taken. The Petriplates were inoculated aseptically with colony bits (7 mm) removed from 4 days old pure culture of *Trichoderma viride* and 7 days old culture of *Auricularia delicata* respectively, and incubated at 26±1°C in B.O.D. incubator till the mycelial growth in the control reaches a maximum growth. The diameter of the colonies were measured after every twenty four hours and average values, compared with check, were taken as a measure of fungitoxicity. Growth inhibition (%) of test fungus was determined by using the formula given by Vincent, (1947) (Pani and Patra, 1997).

Growth inhibition (%) = Growth in control-Growth in treatment/Growth in control × 100

RESULTS AND DISCUSSION

The effect of seven (7) phytoextracts (*Tamarindus indica, Vitex trifolia, Azadirachta indica, Tagetes erecta, Allium sativum, Solanum nigrum* and *Adhatoda vasica*) on the linear growth (colony diameter) of *Trichderma viride*, pathogen of edible mushroom *Auricularia delicata* is provided in Table 1. The degree of inhibition on the mycelial growth of *T. viride* and *A. delicata* by seven phytoextracts as compared to the untreated plate are presented in Table 2. Inhibitory effects of the seven extracts on each test fungus were different. No complete growth suppression of *T. viride* and *A. delicata* was recorded in all the concentrations of the seven extracts. The highest degree of growth inhibition of *T. viride* was shown by the extract of *Solanum nigrum* (54.15%) followed by *Vitex trifolia* (33.77%) and *Adhatoda vasica* (28.68%) extracts (Table 2). Regarding *A. delicata*, the highest growth inhibition was recorded against *Tamarindus indica* extract (64.49%) followed by *Solanum nigrum*(55.24%) and *Azadirachta indica* (10.88%) extracts respectively. However, no growth inhibition of *A. delicata* was recorded at all the five concentrations from the extracts of *Vitex trifolia, Tagetes erecta, Allium sativum* and *Adhatoda vasica*. Moreover the phytoextracts of these four plants enhanced the mycelial growth of *A. delicata* over control instead of growth inhibition.

The presence of naturally occurring substance in the plants with antifungal properties have been recognized and tested against a wide range of fungi infecting many crops and commercially important plants (Singh and Dwivedi, 1990). The fiudings of the present study agree with the observations of shiddinque *at al.* (2004) and Mandvi and Singh (2005).

It was confirmed that all the plant extracts tested were found to be non-phytotoxic but fungicidal in nature. Thus, the plant extracts screened, were markedly different in their fungitoxicity and differential responses shown by the test fungi which were due to different metabolisms of the fungus. Thus, the study opens up a new approach to botanical fungicides that can be used as biofungicide to replace the synthetic chemicals.

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Tabla 1 : Effect of 7 (seven) phytoextracts against the mycelial growth of *Trichoderma viride* and *Auricularia delicata*.

Phytoextracts	Part used	*Trichoderma viride*						*Auricularia delicata*					
		Radial growth (mm) after 48 h.						Radial growth (mm) after 168 h.					
		0% (Control)	1%	5%	10%	15%	20%	0% (Control)	1%	5%	10%	15%	20%
Tamarindus indica	leaf	87.00	81.00	72.66	49.33	32.00	24.50	69.00	88.00	86.66	80.00	72.16	63.00
Vitex trifolia	leaf	86.83	79.66	85.33	87.66	81.00	78.33	75.33	81.00	79.83	73.66	67.00	57.50
Azadirachta indica	leaf	86.00	81.00	83.33	80.00	80.66	60.00	67.33	83.83	78.83	75.83	69.66	62.66
Tagetes erecta	leaf	84.50	87.66	82.66	88.00	79.83	75.50	74.66	82.83	84.50	81.33	74.33	65.66
Allium sativum	leaf	90.00	76.33	79.00	80.16	81.83	86.83	61.50	90.00	90.00	90.00	90.00	86.00
Solanum nigrum	seed	86.50	56.66	49.16	37.66	36.33	34.16	76.33	45.83	44.83	44.50	41.16	39.66
Adhatoda vasica	leaf	87.16	64.50	76.16	82.66	85.00	78.33	63.66	79.66	77.50	76.33	69.00	62.16
±S.E.		0.15	0.32	1.52	1.05	2.60	0.36	0.28	1.45	0.72	1.25	1.85	0.49
C.D. at 5%		0.29	0.70	3.62	2.47	6.19	0.82	0.58	3.45	1.69	2.95	4.38	1.16

Tabla 2 : Inhibition rate of the mycelium growth (%) of *Trichoderma viride* and *Auricularia delicata* by 7 (seven) phytoextracts.

Phytoextracts	Part used	*Trichoderma viride*					*Auricularia delicata*				
		Per cent inhibition of mycelium growth over control (mm) after 48 h.					Per cent inhibition of mycelium growth over control (mm) after 168 h.				
		1%	5%	10%	15%	20%	1%	5%	10%	15%	20%
Tamarindus indica	leaf	0.00	0.00 (5.30)	28.50 (0.00)	53.62 (0.00)	64.49 (0.00)	0.00 (17.39)	0.39	8.04	17.05	27.58
Vitex trifolia	leaf	6.71	0.00 (13.27)	0.00 (16.36)	0.00 (7.52)	0.00 (3.98)	0.00 (5.74)	8.06	15.16	22.83	33.77
Azadirachta indica	leaf	2.52	0.00 (23.76)	0.00 (18.81)	0.00 (19.79)	10.88 (0.00)	0.00 (20.30)	8.33	11.82	19.00	27.13
Tagetes erecta	leaf	1.97	0.00 (10.71)	0.00 (17.86)	0.00 (6.92)	0.00 (1.12)	0.00 (17.41)	0.00	3.75	12.03	22.29
Alliun sativum	leaf	0.00	0.00 (28.45)	0.00 (30.34)	0.00 (33.05)	0.00 (41.18)	0.00 (24.11)	0.00	0.00	0.00	4.44
Solanum nigrum	seed	47.01	35.59 (0.00)	50.66 (0.00)	52.40 (0.00)	55.24 (0.00)	25.76 (0.00)	48.17	48.55	52.41	54.15
Adhatoda vasica	leaf	8.60	0.00 (19.63)	0.00 (29.84)	0.00 (33.52)	0.00 (23.04)	0.00 (1.31)	11.08	12.42	20.83	28.68

*The values in parentheses denote the percentage of increase growth over control.

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