

IN VITRO CONTROL OF *FUSARIUM SOLANI* AND *F. OXYSPORUM* THE CAUSATIVE AGENT OF BRINJAL WILT

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Abstract

Seven soil fungi viz. *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Penicillium* sp., *Trichoderma harzianum* and *T. viride* associated with the rhizosphere, non-rhizosphere and rhizoplane of brinjal plants were selected to observe their antagonistic potential against the test fungi *Fusarium oxysporum* and *F. solani*. Out of seven soil fungi *T. harzianum* was found most effective to control the growth of both the test fungi. Plant parts extract of *Allium sativum*, *Asparagus racemosus*, *Azadirachta indica*, *Cassia alata*, *Ocimum sanctum*, *Zingiber officinale* and *Datura metel* were evaluated for their *in vitro* efficacy at 5, 10 and 20% concentration against the test fungi. *Datura metel*, and *C. alata* and *A. indica* was found most efficient inhibitor of *F. solani* and *F. oxysporum*, respectively. Five fungicides viz., agridazim 50 wp, cozeb 80 wp, newban 50 wp, sunvit 50 wp and vitavax 200 B were evaluated for their *in vitro* efficacy at 500 ppm concentration against *F. solani* and *F. oxysporum*. Vitavax 200B was found most efficient inhibitor of both the test fungi.

Introduction

Brinjal (*Solanum melongena* L.) is the most popular vegetables in Bangladesh and grows in an area of about 29,132 ha producing about 1,87,705 in tons⁽¹⁾. Among the diseases that attack brinjal, wilt is one of them caused by *Fusarium oxysporum* f. sp. *melongenae* and *F. solani*⁽²⁾. Among the destructive diseases of brinjal, wilt has significant impact on the yield of the crop.

Now-a-days, many inorganic fungicides are used frequently to control plant diseases. Therefore, it is necessary to evaluate the efficacy of the fungicides against the targeted pathogens of brinjal. Various workers in different countries of the world evaluated the efficacy of various fungicides against different brinjal pathogens⁽³⁻⁵⁾.

In the recent years scientists have begun to understand the consequences of the widespread and repeated use of chemical fungicides that threaten human interest. Fungi are potentially better biocontrol agents suggesting one of the alternatives. Presently, different species of *Trichoderma* are used successfully to control different plant pathogens⁽⁶⁻¹⁰⁾. Plant parts and their constituents of some higher plants have already been reported to be of successful nature of fungitoxicants because of their lesser phytotoxicity, systemicity, easily biodegradability and favourable effects for the growth of the host⁽¹¹⁻¹⁴⁾.

In Bangladesh, little information is available regarding the mycoflora of rhizosphere of brinjal and their role in biological control. Considering the above mentioned facts present investigation was undertaken with a view to *in vitro* controlling of *Fusarium solani* and *F. oxysporum*, the natural enemies of brinjal.

Materials and Methods

A total of 21 soil fungi were isolated from the rhizosphere, non-rhizosphere and rhizoplane of healthy and wilted brinjal plants following tissue planting and serial dilution plate method from the selected cultivar during December 2011 to August 2012 directly from the field of BADC in Gazipur. Seven soil fungi *viz.*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Penicillium* sp., *Trichoderma harzianum* and *T. viride*. were selected as the antagonists on the basis of their growth rate. *Fusarium oxysporum* and *F. solani* was selected as the test fungi. The antagonistic behavior of the selected antagonists against the test fungi was determined by colony interaction, volatile and non-volatile metabolites.

The antagonistic action of the selected fungi against the test fungi were performed by dual culture technique as described by Bashar and Rai⁽⁶⁾. Assessment of colony interaction between the test fungi and soil fungi were done in terms of grades which were determined following the model of Skidmore and Dickinson⁽¹⁵⁾. The parameters used for the assessment of colony interaction were the width of inhibition zone, intermingled zone and per cent inhibition of radial growth⁽¹⁶⁾.

The effect of volatile and non-volatile substances of the soil fungi on the growth of the test fungi were performed following the methods described by Bashar and Rai⁽⁶⁾.

Leaves of *Azadirachta indica* A Juss., *Ocimum sanctum* L, *Datura metel* L., *Cassia alata* L., roots of *Asparagus racemosus* L., bulbs of *Allium sativum* L. and tubers of *Zingiber officinale* Rosc were selected for screening of antifungal activity against the test fungi. The toxicity of the crude plant extract was determined against the test fungi following poisoned food technique⁽¹⁷⁾. The per cent inhibition of mycelial growth over control was calculated using the formula described by Bashar⁽³⁾.

In vitro fungitoxicity of five fungicides *viz.*, agridazim 50 wp, cozeb 80 wp, newban 72 wp, sunvit (copper oxychloride) and vitavax 200 B (carboxin, 37.5%, thiram, 62.5%) were tested against the test fungi at 500 ppm concentration following poisoned food technique⁽¹⁷⁾.

Results and Discussion

The results of colony interactions between the test fungi and the soil fungi have been summarized in Table 1. Grade 3 was found to be the common type of colony interaction. From the results it is evident that out of seven soil fungi examined only *Aspergillus niger*, *T. harzianum* and *T. viride* exhibited strong antagonistic effects against the test fungi.

All the tested soil fungi inhibited the radial growth of the test fungi in varied degrees in dual culture experiments on agar plates. In case of *F. solani*, *T. harzianum* showed highest inhibition of radial growth (78.6%) followed by *A. niger* (56.25%) and *T. viride* (50%). Least inhibition of radial growth was noticed with *Aspergillus terreus* (23.53%). The maximum inhibition of *F. oxysporum* was exhibited by *T. harzianum* (70.59%) followed by *A. niger* (65.21%), *T. viride* (64.24%) and *A. terreus* (53.33%). Least inhibition of radial growth was noticed with *A. fumigatus* (Table 1). High antagonistic activity of the *Trichoderma* spp. observed against the test fungi might be due to fast growing nature, rapid sporulation and toxic metabolite producing capacity. Similar observation was recorded by other workers against many plant pathogens⁽⁶⁻¹⁰⁾. Inhibition of growth of the test fungi by *Aspergillus* spp. is also in confirmatory with the similar reports of Upadhyay and Rai in case of other pathogenic fungi⁽¹⁸⁾.

Table 1. Colony interaction between soil fungi and test fungi.

Soil fungi	Grade*	% inhibition of radial growth	
		<i>Fusarium solani</i>	<i>F. oxysporum</i>
<i>Aspergillus flavus</i>	3	43.33	57.14
<i>A. fumigatus</i>	3	34.78	34.78
<i>A. niger</i>	3	56.25	65.21
<i>A. terreus</i>	3	23.53	53.33
<i>Penicillium</i> sp.	3	37.50	52.00
<i>Trichoderma harzianum</i>	3	78.60	70.59
<i>T. viride</i>	3	50.00	64.24

* = Grades from 1 (mutually intermingling growth) to 5 (mutual inhibition at a distance), based on Skidmore and Dickinson (1976). 3 = Intermingling growth where the fungus under observation has ceased growth and is being overgrown by another colony.

The growth inhibition of fungi on agar plates at a distance due to other fungi is commonly described due to the production of toxic metabolites by the antagonists which diffuse into the medium and cause growth inhibition. Competition for the nutrients, change in pH, nutrient imbalance, mechanical obstruction and hyperparasitic behavior are involved in the colony interactions^(15,16,18,19).

Volatile substances emanating from the cultures of the soil fungi inhibited the radial growth of the test fungi to varied degrees. From the results it is evident that out of the seven soil fungi examined only *T. harzianum*, *Penicillium* sp. and *A. niger* exhibited better antagonistic effect against the test fungi (Table 2). In case of *Fusarium solani*, *T. harzianum* showed highest inhibition of radial growth (38.55%) followed by *Penicillium* sp. (29.48%) and *A. niger* (26.33%) whereas *T. harzianum* showed 31.21% inhibition of radial growth of *F. oxysporum* which was followed by *Penicillium* sp. (27.06%) and *A. niger* (20.15%). In case of each test fungus *A. fumigatus* showed least inhibition of the test fungi (Table 2).

The growth inhibition of the test fungi may be attributed to the presence of growth inhibitory substances in the metabolites⁽¹⁵⁾. The present observation suggests that there were qualitative and quantitative differences in the volatile substances produced by various soil fungi as they exhibited different degrees of growth inhibition of the test fungi. Some protective compounds recently isolated from endophytes are taxol, oocydin A, cryptocin, ambuic acid and jesterone^(20,21).

Table 2. Per cent inhibition of radial growth of the test fungi by volatile substances of soil fungi.

Name of soil fungi	% inhibition of radial growth of the test fungi	
	<i>Fusarium solani</i> *	<i>F. oxysporum</i> *
<i>Aspergillus flavus</i>	14.79	15.78
<i>A. fumigatus</i>	13.06	12.25
<i>A. niger</i>	26.33	20.15
<i>A. terreus</i>	15.75	19.00
<i>Penicillium</i> sp.	29.48	27.06
<i>Trichoderma harzianum</i>	38.55	31.21
<i>T. viride</i>	34.24	29.75

*= Test fungi.

All the antagonists showed varied degree of growth inhibition of the test fungi at different concentrations. Out of the seven antagonists, *T. harzianum* showed 87.17% inhibition of *F. solani* at 20% concentration which was followed by *T. viride* (79.49%) and *A. terreus* (76.92%). Similarly, the maximum inhibition of radial growth was observed with *T. harzianum* (86.01%) against *F. oxysporum* which was followed by *A. terreus* (83.33%) and *T. viride* (82.05%) at the same concentration. The inhibition of the test fungi increased with the increase of concentration of culture filtrates in the culture medium (Table 3).

The inhibition of the radial growth of the test fungi due to non-volatile metabolites may be attributed to the production of antibiotic substances in the culture filtrates^(22,23), nutrient impoverishment^(7,8) and alteration of pH of the culture medium due to staling growth substances. Table 3 also shows that the per cent inhibition of the test fungi owing to soil fungi also differed. The differences might be due to differences in nature, quality and quantity of the inhibitory substances produced by the soil fungi^(10,22-24).

Results of plant extracts on the radial growth of *Fusarium solani* and *F. oxysporum* are presented in Table 4. All the plant extracts showed varied degree of growth inhibition of the test fungi at different concentrations. Out of the seven plant extracts, *Datura metel* showed 66.67% inhibition of *F. solani* which was followed by *A. racemosus* (65.71%) and *A. indica* (57.14%). The highest inhibition of growth of *F. oxysporum* was observed with *Cassia alata* (74.68%) which was followed by *A. indica* (62.03%) and *A. racemosus* (56.96%).

The inhibition of the test fungi increased with the increase of the concentration of plant extracts in culture medium (Table 4).

Table 3. Effect of non-volatile substances of soil fungi on the radial growth of the test fungi at different concentrations.

Name of soil fungi	% inhibition of radial growth at different concentrations					
	<i>Fusarium solani</i> *			<i>F. oxysporum</i> *		
	5	10	20	5	10	20
<i>Aspergillus flavus</i>	43.59	53.85	74.36	25.00	50.00	66.66
<i>A. fumigatus</i>	33.33	56.41	71.36	25.00	69.44	80.56
<i>A. niger</i>	48.71	64.10	74.36	16.57	27.78	72.22
<i>A. terreus</i>	48.72	53.85	76.92	27.77	38.89	83.33
<i>Penicillium</i> sp.	38.46	58.97	69.23	58.33	72.22	80.56
<i>Trichoderma harzianum</i>	76.92	82.05	87.17	75.00	82.05	86.01
<i>T. viride</i>	51.28	56.41	79.49	72.22	77.78	82.05

*= Test fungi

Table 4. Effect of plant extracts on the radial growth of the test fungi at different concentrations.

Name of plant	% inhibition of radial growth at different concentrations					
	<i>Fusarium solani</i> *			<i>F. oxysporum</i> *		
	5	10	20	5	10	20
<i>Allium sativum</i>	19.87	27.56	53.85	17.63	25.0	34.21
<i>Asparagus racemosus</i>	40.00	42.86	65.71	44.30	46.83	56.96
<i>Azadirachta indica</i>	21.43	34.30	57.14	24.05	41.77	62.03
<i>Cassia alata</i>	36.10	44.40	50.00	46.83	49.36	74.68
<i>Datura metel</i>	16.67	22.20	66.67	10.13	17.72	34.18
<i>Ocimum sanctum</i>	11.10	24.36	48.72	6.89	13.17	16.96
<i>Zingiber officinale</i>	19.48	44.67	49.35	23.68	34.21	39.47

* = Test fungi.

The inhibitory effect of the plant extracts might be attributed to the presence of antifungal compounds. Antifungal activity of different plant extracts have been reported earlier by several investigators against a number of plant pathogens^(11,13,14,25).

The data with regard to fungicidal effect on the radial growth of *Fusarium solani* and *F. oxysporum* are presented in Table 5. All the fungicides inhibited the radial growth of the test fungi. The extent of growth inhibition, however, varied amongst the five fungicides. The results of the evaluation of fungicides on radial growth of *F. solani* and *F. oxysporum* showed that all the selected fungicides strongly inhibited the growth of the test fungi at 500 ppm concentrations.

The highest inhibition of growth of *F. solani* was observed with vitavax 200B (97.87%), which was followed by agridazim 50 wp (97.23%), cozeb 80 wp (96.81%), sunvit 50 wp (96.81%) and newban 72 wp (94.89%). In case of *F. oxysporum*, vitavax caused the maximum inhibition (98.73%). Agridazim 50 wp, cozeb 80 WP, newban 72 wp and sunvit 50 wp were responsible for 98.61, 95.95, 96.46 and 91.14% inhibition of *F. oxysporum*, respectively at 500 ppm concentration.

Table 5. Fungitoxicity of fungicides on the radial growth of the test fungi at 500 ppm concentration.

Name of fungicides	% inhibition of radial growth	
	<i>Fusarium solani</i> *	<i>F. oxysporum</i> *
Agridazim 50 wp	97.23	98.61
Cozeb 80 wp	96.81	95.95
Newban 72 wp	94.89	96.46
Sunvit 50 wp	96.81	91.14
Vitavax 200 B	97.87	98.73

*= Test fungi.

Laboratory evaluation of fungicides revealed that all the fungicides caused maximum inhibition of *Fusarium solani* and *F. oxysporum* at 500 ppm concentration (Table 5). Similar observations have also been reported for other fungicides by Vishwakarma and Basu Chaudhury⁽²⁶⁾ and Bashar⁽³⁾. *In vitro* efficacy of various fungicides against the test fungi indicates that vitavax 200B and agridazim 50 wp showed promising results as compared to others. Present study revealed that *Trichoderma* spp., *Datura metel*, *Cassia alata* and *Vitavax* can effectively control the growth of *Fusarium* spp. the natural enemies of brinjal plant.

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