

## ANTAGONISTIC POTENTIAL OF RHIZOSPHERE FUNGI AGAINST LEAF SPOT AND FRUIT ROT PATHOGENS OF BRINJAL

MT AKTAR, KS HOSSAIN<sup>1</sup> AND MA BASHAR\*

*Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh*

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### Abstract

Antagonistic potentials of seven rhizosphere soil fungi viz., *Aspergillus flavus* Link., *A. fumigatus* Fresen., *A. niger* Tiegh., *A. terreus* Thom., *Penicillium* sp., *Trichoderma harzianum* Refat. and *T. viride* Pers. were tested in opposition to six pathogenic fungi viz., *Colletotrichum* sp., *Curvularia lunata*, *Fusarium moniliforme*, *F. oxysporum*, *F. semitectum* and *Phomopsis* sp. isolated from different leaf spots and fruit rots of brinjal. Out of seven soil fungi, *Trichoderma harzianum* was found most effective to control the growth of all the test fungi in the study of colony interactions and effects of volatile and non-volatile metabolites. This fungus may be exploited commercially to biocontrol the diseases.

### Introduction

Brinjal or eggplant (*Solanum melongena* L.) is an important solanaceous vegetable in Bangladesh. It is a good source of minerals and vitamins. It has several medicinal properties and more or less available round the year (Khan 1979). The crop is known to suffer from more than 70 different diseases in the world. Ten diseases have been recorded from Bangladesh. Among these leaf spots and fruit rots have significant impact on the crop yield (Ahmed and Hossain 1985).

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 (Vinalea *et al.* 2008). Colony interaction between non-pathogenic and pathogenic fungi for determination of antagonism of fungi isolated from different habitats including rhizosphere are studied (Skidmore and Dickinson 1976, Bashar and Rai 1994, Brozova 2002, and Prince *et al.* 2011). Different rhizodeposition of plant invites beneficial microbiota around the rhizosphere. Role of rhizosphere fungi as an effective antagonist against the pathogen of the same plant is also reported (Berg *et al.* 2005). Many microorganisms produce biologically active volatile substances which have been noticed inhibitory or stimulatory against the growth of fungi. The production of biologically active non-volatile toxic substances by fungi has been discussed by several workers (Dennis and Webster 1971a, Skidmore and Dickinson 1976). However in Bangladesh limited studies have been done in this regard (Ahmed and Hossain 1985). Hence, the present investigation was designed to evaluate antagonistic potentiality of some rhizosphere fungi *in vitro* against the pathogens of leaf spots and fruit rots of brinjal.

### Materials and Methods

Spotted leaves and rotten fruits of different brinjal cultivars were collected from March, 2010 to February, 2011 directly from farmers fields at Shibpur, Natore, seed production fields of BADC, Gazipur and research fields of the Botanical Garden, Dhaka University. Diseased samples were collected in separate sterile polyethylene bags, labeled properly and then brought to the

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\*Author for correspondence: <botanybashar@yahoo.com>. <sup>1</sup>Department of Botany, Jagannath University, Dhaka, Bangladesh.

laboratory for isolating associated fungi following standard blotter and agar plate methods. Among the isolated fungi *Colletotrichum* sp., *Curvularia lunata* (Wakker) Boedijn, *Fusarium moniliforme* Sheld., *F. oxysporum* Schltdl., *F. semitectum* Berk. & Rav. and *Phomopsis* sp. were selected as test pathogens owing to their higher percentage of occurrence and previous reports as pathogenic organisms (Anonymous 1997, Richardson 1979). Fungi were isolated from rhizospheric soil of the host following serial dilution method (Brown 2004). Among the isolated soil fungi, *Aspergillus flavus* Link, *A. fumigatus* Fresen., *A. niger* Tiegh., *A. terreus* Thom., *Penicillium* sp., *Trichoderma harzianum* Refai and *T. viride* Pers. were selected to test their antagonistic potential against the pathogens following dual culture technique described by Bashar and Rai (1994). In dual culture, assessment of colony interactions grading were done based on intermingling and inhibition zone. Per cent inhibition of the growth of the test fungi due to the presence of antagonists were also calculated (Skidmore and Dickinson 1976).

Effects of volatile and non-volatile metabolites of the selected soil fungi against the test pathogens were also studied following the methods described by Dennis and Webster (1971b) and Bashar and Rai (1994), respectively.

### Results and Discussion

Antagonistic potential of the selected seven soil fungi against the six test pathogens are presented in the Table 1. In this study, antagonistic relationships among the soil fungi and test pathogens ranged from grade 2 - 4. However, grade 2 was found to be the most commonly encountered type of colony interaction as 20 interactions out of 42 were incorporated in this grade, followed by grade 3 (13 out of 42). The grade 4 was recorded infrequently (09 out of 42). Among the 7 soil fungi only *Trichoderma harzianum* showed grade 4 interaction against all the 6 test pathogens followed by *Aspergillus niger*. In accordance with the present study Prince *et al.* (2011) also observed interactions at grade 4 between soil fungus *T. harzianum* and sugarcane red rot pathogen *Colletotrichum falcatum*.

Table 1 shows that volatile substances emanating from the soil fungi inhibited radial growth of the test pathogens to varied degrees ranging 5.0 - 22.60%. It is evident from the results that out of seven soil fungi examined, the highest inhibition was found by the volatile substance of *T. harzianum* against *Phomopsis* sp. followed by *F. semitectum* (21.50%). The 3rd highest (20.86%) inhibition was observed by the volatile substance of *T. viride* against *C. lunata* followed by substance of *T. harzianum* against *F. oxysporum* (20%). Barakat *et al.* (2013) reported that volatile metabolites produced by an isolate of *T. harzianum* inhibited mycelial growth of *Botrytis fabae* by 39.77% after six days of incubation. Difference in per cent inhibition with the present study might be due to the difference in organism involved in the interaction.

The antagonistic effects owing to non-volatile metabolites of the soil fungi against the test pathogens range from 25.23 to 65.50% (Table 1). It is evident from the data presented in the table that these inhibitions of the soil fungi were much higher than those of volatile ones. The highest inhibition was observed by the culture filtrate of *T. harzianum* against *C. lunata*, followed by *F. moniliforme* (65.25%), *F. oxysporum* (63.50%) and *Colletotrichum* sp. (62.50%). 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 and impoverishment of nutrient (Kexiang *et al.* 2002, Howell 2003, Wool and Larito 2007). It has also been reported that the antibiotic production varies depending on the comparing organisms. Differential sensitivity of the pathogen to the staling growth products of the fungi was also observed. This may be due to the genetic potentialities of the pathogen to tolerate a particular antibiotic substance and the chemical

properties of the staling substances. The environmental parameters, nutrients, media and the assay method also influences the antifungal activity of a fungus (Fravel 1988, Prince *et al.* 2011).

**Table 1. Antagonistic potential of rhizosphere fungi against leaf spots and fruit rots pathogens of brinjal.**

Name of soil fungi	Test pathogens					
	Co	Cu	Fm	Fo	Fs	Ph
<b>Grades in colony interactions</b>						
<i>Aspergillus flavus</i>	2	2	3	3	2	2
<i>A. fumigatus</i>	2	2	2	2	2	2
<i>A. niger</i>	3	4	4	3	2	3
<i>A. terreus</i>	2	2	2	2	2	2
<i>Penicillium</i> sp.	3	3	3	3	2	3
<i>Trichoderma harzianum</i>	4	4	4	4	4	4
<i>T. viride</i>	2	3	4	2	3	3
<b>Per cent inhibition by volatile substances:</b>						
<i>Aspergillus flavus</i>	11.78	10.50	8.25	18.00	8.00	10.50
<i>A. fumigatus</i>	11.00	7.90	7.50	12.00	7.75	9.50
<i>A. niger</i>	14.68	14.85	15.50	14.50	9.50	18.70
<i>A. terreus</i>	8.50	5.25	6.00	7.57	5.00	5.50
<i>Penicillium</i> sp.	12.45	14.78	10.25	16.00	9.45	19.25
<i>Trichoderma harzianum</i>	15.50	16.86	17.50	20.00	21.50	22.60
<i>T. viride</i>	11.00	20.86	15.00	10.25	13.50	15.65
<b>Per cent inhibition by non-volatile metabolites:</b>						
<i>Aspergillus flavus</i>	40.00	40.32	48.20	45.75	40.00	33.45
<i>A. fumigatus</i>	37.20	28.51	40.00	39.00	28.50	29.10
<i>A. niger</i>	52.56	52.50	60.50	52.00	45.00	45.03
<i>A. terreus</i>	30.50	30.50	30.50	30.50	30.50	30.50
<i>Penicillium</i> sp.	42.00	60.25	53.40	50.50	43.50	40.52
<i>Trichoderma harzianum</i>	62.50	65.50	65.25	63.50	55.00	50.84
<i>T. viride</i>	44.72	60.07	60.25	58.25	50.00	35.22
<b>Per cent inhibition in colony interaction:</b>						
<i>Aspergillus flavus</i>	54.19	51.78	66.66	65.62	47.36	46.03
<i>A. fumigatus</i>	50.25	45.52	50.00	51.50	46.15	41.47
<i>A. niger</i>	68.66	75.87	80.35	55.74	50.70	65.85
<i>A. terreus</i>	40.00	40.00	40.00	42.66	39.52	35.50
<i>Penicillium</i> sp.	56.25	76.53	67.62	65.25	50.00	63.23
<i>Trichoderma harzianum</i>	76.95	80.00	81.48	76.63	72.90	72.89
<i>T. viride</i>	57.24	75.50	80.26	55.35	56.52	53.58

Co = *Colletotrichum* sp., Cu = *Curvularia lunata*, Fo = *Fusarium oxysporum*, Fs = *F. semitectum*, Fm = *F. moniliforme* and Ph = *Phomopsis* sp.. Grades from 1 (mutually intermingling growth) to 5 (mutual inhibition at a distance), as proposed by Skidmore and Dickinson (1976) are as follows: Grade 1 = Mutual intermingling without any macroscopic signs of interaction. Grade 2 = Mutual intermingling growth where the growth of the fungus is ceased and being over growth by the opposed fungus. Grade 3 = Intermingling growth where the fungus under observation is growing into the opposed fungus either above (or) below. Grade 4 = Sight inhibition of both the interacting fungi with narrow demarcation line (1 - 2 mm). Grade 5 = Mutual inhibition of growth at a distance of > 2 mm.

Results in colony interaction tests show that radial growth inhibition of the test pathogen with the soil fungi was in the range of 35.50 - 81.48% and the highest growth inhibition of *Fusarium moniliforme* was obtained by *Trichoderma harzianum* followed by *Aspergillus niger* and *T. viride* (Table 1). The 4th - 6th positions were *T. harzianum* against *Curvularia lunata* (80%), *Colletotrichum* sp. (76.95%) and *F. oxysporum* (76.63%), respectively. In contrast with the present study Prince *et al.* (2011) reported 53.8% inhibition of *Botrytis fabae* by *T. harzianum* in colony interaction. The less inhibition might be due to the difference in the pathogen.

Results of this experiment reveal that out of seven soil fungi examined only *Trichoderma harzianum* exhibited strong antagonistic effect against all the test pathogens. This effect might be due to its fast growing nature, rapid sporulation and toxin producing capacity (Skidmore and Dickinson 1976). The occurrence of inhibition zone between some of the soil fungi and the test fungi could be considered as a result of the production of secondary metabolites, antibiotics, pH alteration and exhaustion of nutrients and space (Chakraborty *et al.* 2004).

*Trichoderma harzianum* is known to be capable of producing antibiotics which might have suppressed the growth of the test pathogens. These results are in agreement with the findings of Adriana and Sergio (2001), Krupke *et al.* (2003), Kexiang *et al.* (2002) and Shafiquzzaman *et al.* (2009).

The present observation suggests that *Trichoderma harzianum* may be exploited commercially as a biocontrol agent of leaf spots and fruit rots of brinjal.

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