IN VITRO CONTROL OF *COLLETOTRICHUM DEMATIUM* CAUSING ANTHRACNOSE OF SOYBEAN BY FUNGICIDES, PLANT EXTRACTS AND *TRICHODERMA HARZIANUM*

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ABSTRACT

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A study was conducted in the Microbiology Laboratory of Plant Pathology Department, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, during 2005 to 2006 to control *Colletotrichum dematium* causing anthracnose of soybean with fungicides, plant extracts and *Trichoderma harzianum*. Five fungicides namely Tilt-250 EC, Vitavax-200, Rovral 50 WP, Dithane M-45 and Cupravit were evaluated at 100, 200 and 400 ppm for their efficacy against the radial colony growth and mycelial dry weight of *C. dematium*. The complete inhibition was obtained with Tilt-250 EC at all the selected concentrations. The highest concentration of Vitavax-200 inhibits 77.41% and 83.45% radial growth and mycelial dry weight, respectively and significantly superior to Rovral 50 WP at the highest concentration. Dithane M-45 and Cupravit were found to be significantly inferior against the test pathogen. Plant extracts of garlic, ginger, onion and neem at three different concentrations (5%, 10% and 20%) were evaluated against the radial growth and mycelial dry weight of *C. dematium.* Among the four plant extracts, garlic extract at 20% concentration was appeared to be best in inhibiting the radial growth and mycelial dry weight of the test pathogen followed by onion, ginger and neem extracts and each extracts were significantly different from each other. A total of 20 *T. harzianum* isolates collected from rhizosphere and rhizoplane of different crops were screened against *C. dematium* following dual plate culture technique. The screened isolates of *Trichoderma* showed significantly variable antagonism ranging from 50.93 to 89.44% reduction of the radial growth of *C. dematium.* Among the promising antagonists, the isolate T3 of *T. harzinum* showed the highest 89.44 % inhibition of radial growth of *C. dematium.*

Key words: Anthracnose, *Colletotrichum dematium*, Fungicides, Plant Extract and *Trichoderma harzianum*

INTRODUCTION

Soybean (*Glycine max* L. Merr.) has received a great deal of attention all over the world as an important source of protein to alleviate the protein deficiency. It is comparatively cheaper than the animal sources of protein such as meat, fish, milk, egg etc. It contains 40-45% protein, 18-20% edible oil, 24-46% carbohydrate and a good amount of vitamins (Kaul and Das, 1986). As legume crop it is capable of utilizing atmospheric nitrogen through biological nitrogen fixation. Soybean fixes about 270 kg N/ha compared to 58 to 157 Kg N/ha by other pulses (Hoque, 1978). As a result the crop is less dependent on chemical nitrogenous fertilizers. Most of the people of Bangladesh now consume soybean oil in their daily dishes due to its high quality. A large number of soya products are successfully developed and some of them such as soyaflour, soyamilk, soyabicuits, soyabread, soyachanachur etc are commercially produced in Bangladesh. At present the total acreage under this crop is about 5000 hectares and total production is about 3750 tons all over the country (BBS, 1998).

All parts of the soybean plant are susceptible to diseases. More than 100 pathogens are known to affect soybean, of which 35 are of economically important (Sinclair and Backman, 1989). Soybean diseases reduce yield, on an average of 10 to 30% in most production area (Sinclair 1994). No exact data on yield losses due to diseases of soybean is available in Bangladesh. The total yield loss due to disease during 1994 in 10 countries with greatest soybean production was 28.5 × 106 metric t, valued at U.S. $6.29 × 109 million (Wrather *et al.* 2001). In Bangladesh soybean suffers from many diseases among them anthracnose caused by *Colletotrichum dematium* is the predominant one (Fakir, 1979 and Bhuiyan and Fakir 1993). Methods for complete control of soybean seed borne diseases are yet to be developed. Management strategies for these diseases include use of presumed disease free seeds, resistant cultivars and fungicidal sprays. Seed treatment is one of the best methods to manage seed-borne diseases. The continuous and indiscriminate use of chemicals to manage the crop disease results in accumulation of harmful chemical residues in the soil, water and grains. Development of fungicide-resistant biotypes of the pathogen is a major constraint to control the major seed-borne pathogens of soybean. In recent years, considerable success has been achieved by introducing antagonists to control seed-borne fungal pathogens. A considerable work has been done in controlling seedling diseases of many crops caused by *Rhizoctonia solani* and *Sclerotium rolfsii* both *in vitro* and pot culture experiments by using *Trichoderma* (Akhter 1999, Pradeep *et al*. 2000, Raihan *et al.* 2003, Haider 2005). Only a limited trial has been done in inhibiting the growth and development of *C. dematium*, *F. oxysporum* and *M. phaseolina* by using *Trichoderma* (Iqbal *et. al*. 1994). Complete elimination of *C. dematium* from soybean and other crops is very difficult by any single approach of control. Some plant extracts also found to be most effective in reducing the growth and

development of many pathogens (Hossain *et al*. 1993; Rahman 1992; Suratuzzaman *et. al.* 1994, Raihan *et al*. 2003, Begum and Bhuiyan 2006).

To develop a sustainable integrated control strategy against the major pathogens of soybean it is essential to ensure the *in vitro* efectivity of *Trichoderma*, some fungicides and some plant extracts. Considering the present situation, the present study has therefore been undertaken with the objective to evaluate the efficacy of fungicides, plant extracts and *Trichoderma* against the major seed-borne pathogen *C. dematium* of soybean.

MATERIALS AND METHODS

An experiment was conducted to control the major seed-borne fungi *Colletotrichum dematium* causing anthracnose of soybean by fungicides, plant extracts and *Trichoderma* in the laboratory. The experiment was conducted in the Microbiology Laboratory of Plant Pathology Department, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, during 2005 to 2006.

Laboratory evaluation of fungicides on the growth of C. dematium

Five fungicides namely Cupravit, Dithane M-45, Rovral 50WP, Tilt 250EC and Vitavax-200 were tested in *in vitro* to evaluate their effect on colony growth of *C. dematium* following poison food technique (Dhingra and Sinclair 1985). All fungicides were used at 100 ppm, 200 ppm and 400 ppm. Fungicidal suspensions of different concentrations were prepared by dissolving requisite quantities of each fungicide in warm PDA. The fungicides were thoroughly mixed with the medium by shaking with hands before autoclaving. About 15 ml of sterilized medium was poured in each 9 cm sterilized petridish. After solidification, the plates were inoculated by placing 5 mm discs of 3 days old PDA cultures of *C. dematium*. Three replicated plates were used for each concentration of every fungicide. Three replicated PDA plates received no fungicides served as control. The inoculated plates were incubated at 28 C and data on the radial colony diameter was recorded after 4-5 days of incubation when the the growth of the control plates completely covered the plate. Diameter of the colonies on PDA with and without fungicide was measured from the bottom side of the petridishes.

Inhibition of radial growth was computed based on colony diameter on control plate using the following formula as stated by Sundar *et al*. (1995).

$$\% \text{ Inhibition} = \frac{X-Y}{X} \times 100$$

Where,

X= Growth of control plate

Y= Growth of fungicide treated plate

To determine the effect of the fungicides on mycelial dry weight of *C. dematium*, potato dextrose (PD) broth was used. The medium was prepared by mixing infusion of 200 g peeled potato; 20 g dextrose in 1000 ml distilled water. After cooking the medium was poured into 250 ml conical flask at the rate of 100 ml per flask. Requisite quantity of each fungicide was added to the broth to have concentration of 100, 200, 400 ppm. Three replicated flasks were used for each dose of the five fungicides. The contents of the flasks were autoclaved at 121 C under 1kg/cm^2 for 20 minutes. The flasks were placed inside a clean bench for cooling ambient temperature. The flasks were inoculated with mycelial discs of 5 day's old culture of *C. dematium*. The discs were cut with a flame sterilized cork borer (5 mm). Inoculation was done by putting one mycelial disc per flask with a flame-sterilized needle. Additional three flasks containing the PD broth receiving no fungicides were used as control. The inoculated flasks were incubated at room temperature for 15 days. At the end of incubation, the cultures in all flasks were filtered separately through pre-weighted filter paper. Dry weight of mycelium was obtained by subtracting weight of filter paper from weight of filter paper and mycelium. Inhibition of mycelial dry weight was determined by comparing the growth in control flasks following the formula mentioned earlier.

Evaluation of plant extracts on the growth of C. dematium

Another *in-vitro* test was conducted to determine the effect of plant extracts on radial colony growth of *C. dematium* following poison food technique as described by Begum and Bhuiyan (2006). Water extracts of garlic (*Allium sativum*), ginger (*Zingiber officinale*), onion (*Allium cepa*) and neem (*Azadirachta indica)* leaves were tested. Stalk solutions of the materials were prepared by blending 100g of each plant material in 100 ml of sterilized water in a blender. PDA medium was amended with individual extract at 0, 5, 10 and 20% (v/v). Requisite quantity of individual plant extract was added to the 100 ml conical flask with PDA medium to have concentrations of 0, 5, 10 and 20% (v/v). After thorough mixing with plant extracts the medium was autoclaved and approximately 15 ml of melted PDA mixed with extracts was poured into each 90 mm petridish. After

solidification, the plates were inoculated by placing 5 mm discs of 3 days old PDA cultures of *C. dematium.* Similar procedure was followed as described earlier in the evaluation of fungicides against the growth of *C. dematium*. Inhibition of radial growth was computed based on colony diameter on control plate using the same formula (Sundar *et al.* 1995) as described earlier.

To determine the effect of the plant extracts on mycelial dry weight of *C. dematium*, potato dextrose (PD) broth was used. Similar procedure was followed as described earlier in the evaluation of fungicides against the hyphal dry weight of *C. dematium*. Similar procedure has been followed for taking the mycelial dry weight as stated earlier.

Screening of T. harzianum isolates against C. dematium

A total of 20 isolates of *Trichoderma* were isolated from rhizosphere and rhizoplane soils of bean, rice, chilli, tomato, sunflower, soybean, cauliflower, brinjal, okra and wheat field of BARI and BSMRAU and farmer's fields of Gazipur by soil dilution plate technique and root washing methods (Tuite 1969). All the collected *Trichoderma* isolates were identified as *Trichoderma harzianum*. The isolates of *Trichoderma* were purified in acidified agar (P^H 4.5) using hyphal tip technique. After purification they were maintained as stock culture in PDA slants at 10 C for future use.

An *in vitro* screening experiment was conducted to find out the antagonistic effect of all the isolated 20 *T. harzianum* isolates against *C. dematium* on PDA by dual culture technique (Dhingra and Sinclair 1985). Discs of mycelium (5mm diameter) of each of the selected fungal isolates were cut from the edge of an actively growing fungal colony with a cork borer. Test plates were prepared by pouring 20 ml of PDA per plate. After solidification, one mycelial disc of individual isolate of *T. harzianum* and one disk of test fungal pathogen was placed simultaneously on the edge of the each PDA petri plate at opposite direction. Three replicated plates were used for each isolate of *Trichoderma* and test pathogen. The plates were arranged on the laboratory desks following completely randomized design. The plates received only mycelial discs of the test pathogens served as control. The plates were incubated in the laboratory having ambient temperature of 25±3 C until mycelium of the test pathogens *C. dematium* cover the whole control plate. Thereafter inhibition percentages of *C. dematium* was calculated based on the growth of the pathogen on PDA plates following the formula as suggested by Sundar *et al.* (1995).

Experimental design and Data Analysis

The experiments were conducted following Completely Randomized Design (CRD) with three replications. Data were analyzed by using MSTAT- C program. The significant difference, if any, among the means were compared by Duncan's Multiple Range Test (DMRT). Whenever necessary the data were transformed before statistical analysis following appropriate method.

RESULTS AND DISCUSSION

Effect of fungicide on the growth of C. dematium

Five fungicides tested in the present study gave appreciable inhibition in colony growth and mycelial growth in the Table 1. Among five fungicides all concentrations Tilt-250 EC completely inhibit the mycelial growth of *C. dematium.* Vitavax-200 and Rovral 50WP gave 68.20-77.41 % and 55.26-68.20%, respectively inhibition of the radial colony growth of the test pathogen. Dithane M-45 and Cupravit were appeared to be significantly inferior in comparison to other fungicides in inhibiting the colony growth. Complete inhibition of mycelial dry weight *C. dematium* was achieved with all the concentration of Tilt-250 EC. Mycelial dry weight was reduced by 83.45% and 73.52% with the highest concentrations of Vitavax-200 and Rovral 50% WP, respectively where Vitavax 200 was significantly superior to Rovral 50% WP but significantly inferior to Tilt-250EC. Dithane M-45 at 400 ppm inhibited only 28.61% mycelial dry weight and statistically inferior to Rovral 50% WP but superior to Cupravit which was appeared to be significantly inferior in comparison to all other fungicides. The trends in efficacy of all fungicides at all concentrations were almost similar as observed in case of inhibition of radial colony growth of the fungus. Among five tested fungicides, Tilt-250 EC appeared to be the best one in inhibiting the hyphal growth of *C. dematium* which was followed by Vitavax-200, Rovral 50WP, Dithane M-45 and Cupravit. The present results are in partial agreement with other investigators who observed that Tilt 250 EC, Vitavax-200 and Rovral 50 WP were most effective against *Colletotrichum* spp. In different crops (Alabi *et al.* 1986, Hossain 1989, Rahman *et al.* 1993, Islam *et al.* 2002). Dithane M-45 was noted as poor performing fungicides against *C. dematium.* Its poor performance against some *Colletotrichum* spp. was also reported by Islam *et al.* (2002) and Sharif (2005).

Effect of plant extracts on the growth of C. dematium

Among the tested plant extracts garlic at the highest concentration was found to be best in the reduction both the radial colony diameter and mycelial dry weight followed by the highest 20% of onion. The rate of reduction was corroborated with its concentrations in case of all the tested plant extracts. Ginger at the highest concentration gave 39.83% and 42.55% reduction in colony diameter and dry mycelium weight, respectively and significantly inferior to onion but superior to neem extract at all the concentrations. Neem extract gave the highest 32.47% reduction in colony diameter and 34.23% reduction in mycelium dry weight at the highest 20% concentrations (Table 2) and significantly inferior to all other extracts. Result of the experiment showed that the most effective material is garlic, which was followed by onion, ginger and neem. The result supports the observation of other investigator (Singh *et al.* 1997, Mala *et al*. 1998 and Harbant *et al.* 1999) who found garlic extract was very effective in controlling the anthracnose pathogen *Colletotrichum* spp. in different crops.

Effect of antagonist against C. dematium

The results of the screening of 20 isolates of *T. harzianum* against *C. dematium* on PDA plates are presented in Table 3 and Figure 1. Among the selected isolates T3 was appeared to be most effective against the test pathogens showing 89.44% inhibition of colony growth and significantly higher compared to the other isolates. Identically higher inhibition of radial growth against *C. dematium* was recorded with the isolates T2 and T5. The isolate T8 inhibited 64.44% radial growth of the pathogen but significantly inferior to the isolate T2 and T5. The lowest inhibition in radial growth against *C. dematium* was recorded with the isolate T14 but statistically similar to T19 and T20. Findings of the present investigation are in agreement with several investigators who observed the significant reduction of the growth of anthracnose pathogen *C. dematium* in different crops in presence of *T. harzianum* (Moon *et. al.* 1988, Sundar *et.al.* 1995, D'Souza *et al.* 2001, Rajathilagam and Kannabiran 2001).

The efficacy of the individual component of an integrated measure against the target pathogen should be Cleary understood to develop an integrated control strategy. The results of the current laboratory evaluation of fungicides, plant extracts and *Trichoderma* isolates against *C. dematium* of soybean will fulfill the prerequisite criteria for the selection of appropriate dose of fungicide and plant extract and specific antagonist fungal isolate to develop an eco-friendly integrated disease management of anthracnose of soybean in the field.

Table 1. Effect of fungicide on the growth of *Colletotrichum dematium*

Fungicides	Concentration (ppm)	% Inhibition	
		Radial growht	Mycelial dry weight
Tilt-250 EC	100	100 a* (10.02)**	100 a (10.02)
	200	100 a (10.02)	100 a (10.02)
	400	100 a (10.02)	100 a (10.02)
Vitavax-200	100	68.20 c (8.29)	75.52 c (8.70)
	200	76.32 b (8.76)	82.27 b (9.10)
	400	77.41 b (8.83)	83.45 b (9.16)
Rovral 50 WP	100	55.26 d (7.47)	59.57 d (7.75)
	200	65.13 c (8.10)	70.21 c (8.41)
	400	68.20 c (8.29)	73.52 c (8.604)
Dithane M-45	100	20.17 fg (4.55)	21.75 fg (4.72)
	200	22.59 f (4.80)	24.35 f (4.98)
	400	26.54 e (5.20)	28.61 e (5.39)
Cupravit	100	18.20 g (4.32)	19.62 g (4.48)
	200	20.17 fg (4.55)	21.75 fg (4.72)
	400	22.15 f (4.75)	23.87 f (4.93)
Control		7.6 (cm)	0.37gm

*Values within in a column with a common letters do not differ significantly (P=0.05), ** Figures within parenthesis are arcsin transformation ($Y = \sin \sqrt[-1]{X}$) value.

Table 2. Effect of plant extracts on the growth of *Colletotrichum dematium*

Plant extracts	Concentration (%W/V)	% Inhibition	
		Radial growth	Mycelial dry weight
Garlic	5	41.78 d	44.66 c
	10	48.70 c	51.51 b
	20	72.73 a	73.25 a
Onion	5	37.44 e*	40.63 cd
	10	48.92 c	53.07 b
	20	53.47 b	58.01 b
Ginger	5	38.96 de	41.94 c
	10	36.79 e	39.25 cd
	20	39.83 de	42.55 c
Neem	5	21.65 h	23.49 f
	10	25.97 g	28.18 ef
	20	32.47 f	34.23 de
Control		7.7 cm	0.338 gm

*Values within in a column with a common letters do not differ significantly (P=0.05)

Table 3. Inhibition of radial growth of *Colletotrichum dematium* by selected *Trichoderma harzianum* isolates in dual plate technique

Trichoderma isolates	% inhibition of radial growth
T1	62.22 de*
T2	67.22 b
T3	89.44 a
T4	58.15 f
T5	66.48 b
T6	55.00 g
T7	61.26 de
T8	64.44 c
T9	53.89 g
T10	53.33 gh
T11	54.82 g
T12	62.96 cd
T13	58.70 f
T14	50.93 I
T15	60.18 ef
T16	54.26 g
T17	61.11 de
T18	63.15 cd
T19	51.67 hi
T20	52.96 ghi
Control	9.0 (cm)

*Values within in a column with a common letters do not differ significantly (P=0.05

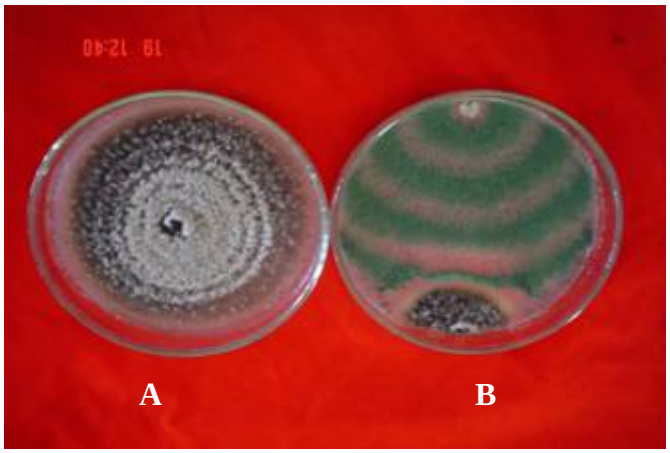

Figure I. Dual culture of *C. dematium* and *T. harzianum* isolate T3 on PDA plate [A: colony growth of *C. dematium* in absence of isolate T3; B: inhibition of colony growth of *C. dematium* in presence of isolate T3]

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