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Cultural Characteristics, Virulence and *In-vitro* Chemical Control of *Fusarium oxysporum* f. sp. *phaseoli* of Bush bean (*Phaseolus vulgaris* L.)

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Abstract

An experiment was conducted at Microbiology Laboratory of Plant Pathology Department, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) during 2007 to determine the virulence and variation in symptom development by *Fusarium oxysporum* f. sp. *phaseoli* isolates at different growth stages such as emergence and early vegetative stage, branching and rapid vegetative growth stage and early flowering stage of Bush bean, and in-vitro control of the pathogen with the selected fungicides. Eight isolates of this pathogen were collected from different pathology laboratory of BARI, BAU and BSMRAU. IS3 isolate collected from Bushbean seeds were found most virulent in pathogenicity test such as pre-emergence mortality, root rot, root lesion, leaf yellowing and wilting when this isolate was inoculated at different growth stages of bush bean. Four fungicides such as Vitavax, Rovral, Cupravit and Aimcozim were evaluated invitro to test the efficacy against isolate IS3. Aimcozim at different concentration (50-400 ppm) was found most effective in in-vitro evaluation.

Keywords: *Fusarium oxysporum* f. sp. *phaseoli, in-vitro* chemical control, Bush bean

1. Introduction

Bush bean (*Phaseolus vulgaris* L.) is the second legume vegetable in the world and its adaptation in this country is increasing due to its high nutritive value and export potentiality. Various saprophytic and parasitic fungi infect this crop which deteriorates the seeds both in quality and quantity (Papavizas and Lewis, 1975; Kulsherstha *et al.*, 1976). George (1985) reported the main seed-borne pathogens of *Phaseolus* spp. which are *Fusarium oxysporum* f. sp. *phaseoli* (Yellows and wilt) and *F. solani* f. sp. *phaseoli* (Root rot). Fusarial wilt caused by *Fusarium oxysporum* f. sp. *phaseoli* is capable of penetrating intact root tissue, older parts of root, hypocotyl tissue through wounds or natural openings (Dongo & Müller, 1969; Duque & Müller, 1969) has been reported to occur in common bean in all bean producing regions by many workers (Rodolfo-Velasqez and Schwartz, 2000; Chandel, 2000; Cavalcanti *et al.*, 2002; Alves-Santos *et al.*, 2002; Schwartz and McMillan, 1989; Buruchara and Camacho, 2000). In Bangladesh, among the soil borne pathogens, root rot or wilt caused by *Fusarium oxysporum* f. sp. *phaseoli* is the most important one (Mozumder, 2004).

Buchvarova *et al*. (1989) found that Vitavax - 200 HP gave the best control *in vivo* but under *in-vitro* condition, the best were Mugibon,

Vitavax 200-NP and Homai 80 WP. On the contrary, Champawat (1990) found Bavistin [carbendazim] and RH 893 to be the most effective among 10 fungicides tested *in-vivo* and *in-vitro* against this pathogen which also enhanced seed germination and seedling vigour. Khatun (2006) reported the effectiveness of Vitavax -200 at different concentrations against *F. oxysporum* in a laboratory trial, while Rahman (2006) found all the selected concentrations of Aimcozim to completely inhibite the radial growth of *F. equiseti*.

The present study was therefore undertaken to study the pathogenicity and different symptoms caused by *Fusarium oxysporum* f.sp. *phaseoli* at different growth stages of Bush bean and to evaluate the efficacy of some selected fungicides against the growth of *F. oxysporum* f. sp. *phaseoli.*

2. Materials and Methods

A series of *in-vitro* experiments were conducted to determine the virulence and variation in symptoms produced by the pathogen at different growth stages of Bush bean, and *in-vitro* control of the pathogen with the selected fungicides. The experiments were conducted in the Microbiology Laboratory of Plant Pathology Department, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) during 2007.

2.1. Collection, multiplication and study of cultural characteristics of Fusarium oxysporum f. sp. *phaseoli isolates*

Eight isolates of *Fusarium oxysporum* f. sp. *phaseoli* were collected from Plant Pathology Laboratory of Bangladesh Agricultural Research Institute (BARI), Bangladesh Agricultural University (BAU), and the Microbiology Laboratory of Plant Pathology Department of BSMRAU. The isolates were multiplied on potato dextrose agar (PDA) at pH 7.0. Inoculum of the isolates of *Fusarium oxysporum* f. sp. *phaseoli* were prepared on autoclaved moist wheat grain with three days old PDA cultures of the pathogen. The colonized wheat grain was air dried for one week and stored at 10°C for further use. Two cultural characteristics such as colony colour and colony texture of the collected isolates were closely observed and recorded.

2.2. Virulence tests of the isolates

Virulence of the eight isolates of *Fusarium oxysporum* f. sp. *phaseoli* was evaluated by soil inoculation technique on Bush bean in a pot culture. Earthen pots were filled with sterilized soil (Sandy loam) at 1.5 kg/pot. Inoculum of isolates was thoroughly mixed with the soil at the rate of 20 g/kg soil 7 days before of seed sowing. Treatments consisted of eight isolates and an un-inoculated control where 20 g wheat grain without fungal inocula was used. Three replications were maintained for each treatment. Twelve seeds of a Bush bean variety "BARI Jharsheem-1" were sown in each earthen pot. Pots containing plants were arranged in a completely randomized design. The number of seedlings which showed five distinguished symptom such as pre-emergence mortality, root lesion, root rot, leaf yellowing and wilt were recorded from the artificial inoculation of most virulent isolate.

2.3. Effect of different growth stage of Bush bean on disease development

A separate pot culture experiment was conducted to observe the reaction of pathogen to the seedling and variation in disease development at the different growth stages of Bush bean. One selected virulent isolate of *F. oxysporum* f. sp. *phaseoli* was inoculated at different growth stage including seeds, emergence and early vegetative stage (7 days after emergence), branching and rapid vegetative growth stage (15 days after emergence) and early flowering stage (40 days after emergence) of seedlings. The experiment was conducted following similar method as described earlier in the pot culture for the pathogenicity tests of the selected isolates.

Pots containing plants were arranged in a completely randomized design. Data on pre-emergence and post emergence mortality of seedlings were recorded. Diseased seedlings were counted every alternate day and continued until 30 days after planting. To determine the cause of death of seedlings, the diseased seedlings were uprooted gently. Dead seeds were also collected from the pots. The causal pathogens associated with the dead seeds and the seedlings were isolated and then compared with the most virulent isolate. All the disease symptoms were expressed in terms of percentage which is actually the disease incidence. In case of variation in symptoms, the data were collected by observing the infected portion of the plants very closely.

2.4. Selection of fungicides

Four fungicides namely Aimcozime (Carbendazim), Cupravit (Copper oxychloride), Rovral 50WP (Iprodione) and Vitavax -200 (Carboxin + Thiram), were tested *in-vitro* to evaluate their efficacy on colony growth of *Fusarium oxysporum* f. sp. *phaseoli* following poisoned food technique on PDA Petri plates (Dhingra and Sinclair, 1985). All fungicides were used at 50, 100, 200 and 400 ppm concentration.

2.5. Effect of fungicides on radial growth of Fusarium oxysporum f. sp. *phaseoli*

The effect of fungicides on radial growth of selected pathogenic isolate of *Fusarium oxysporum* f. sp. *phaseoli* was determined on PDA medium. The medium was prepared, sterilized in autoclave at 121 °C for 15 minutes and requisite quantity of individual fungicide was added to the medium to have concentrations of 50, 100, 200 and 400 ppm. Approximately 20 ml of melted PDA mixed with fungicides was poured into each 90 mm petridish.

After solidification, the plates were inoculated by 5 mm discs of 3 days old PDA cultures of *F. oxysporum* f. sp. *Phaseoli* (IS 3). The discs were cut with flame sterilized cork borer (5 mm diameter). The inocula were placed at the center of the test plates using a flame sterilized needle at one disc per plate inside a clean bench and the plates were used for each dose of every fungicide and the plates were incubated at 28±1 °C till the fungus covered the PDA in control plates. Diameter of the colonies on PDA with and without fungicide was measured from the bottom side of the Petri dishes after 5 days of inoculation. Inhibition of radial growth was computed based on colony diameter on control plate using the following formula described by (Sunder *et al*., 1995):

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

Where,
X = Radial growth of control plates.
Y = Radial growth on fungicide treated plates.

2.6. Analysis of data

The number of distinguished symptom showing in seedlings was presented as percent of total number of seedlings infected. The treatments were arranged in a Completely Randomized Design (CRD). Data were analyzed for ANOVA using MSTAT-C program. Duncan's Multiple Range Test (DMRT) was used to compare the treatment means.

3. Results and Discussion

3.1. Cultural characteristics

The colony of the isolates was pinkish to whitish. The colony color of the isolates IS-1 was cottony white, while the isolate IS-6 was slightly yellowish. The textures of the colony of all the collected isolates were fluffy. The cultural characteristics of the tested *F. oxysporum* f. sp. *phaseoli* isolates are presented in Table 1. Two mycelia textures: fluffy and fibrous and three mycelia colors: white, purple and pink were observed in case of *Fusarium solani* f. sp. *phaseoli* by Mwang'ombe *et al*., 2008.

Table 1. Colony color and texture of the isolates of *Fusarium oxysporum* f. sp. *phaseoli* collected from different sources and cultured on PDA medium

Isolates	Place of collection	Source Crop	Colony color	Texture of colony
IS 1	BSMRAU	Black gram	Cottony white	Fluffy
IS 2	BSMRAU	Black gram	White	Fluffy
IS 3	BSMRAU	Bushbean	Pinkish	Fluffy
IS 4	BSMRAU	Bushbean	Pinkish	Fluffy
IS 5	BSMRAU	Bushbean	Pinkish	Fluffy
IS 6	BARI	Unknown	Slightly yellowish	Fluffy
IS 7	BAU	Unknown	Whitish	Fluffy
IS 8	BAU	Unknown	pinkish	Fluffy

Table 2. Virulence of eight isolates of *Fusarium oxysporum* f. sp. *phaseoli* against Bush bean variety BARI –Jharsheem 1

Isolates	% Pre-emergence mortality	% Post-emergence mortality	% Total mortality
IS1	8.33	8.33	16.67
IS2	8.33	0.00	8.33
IS3	80.56	16.67	97.22
IS4	66.67	16.67	83.33
IS5	66.67	13.89	80.55
IS6	11.11	8.33	19.44
IS7	11.11	0.00	11.11
IS8	5.56	0.00	5.56

Table 3. Disease reaction of *Fusarium oxysporum* f. sp. *phaseoli* (IS 3) at different growth stage of Bush bean

Stage of inoculation	Pre emergence mortality (%)	Root lesion (%)	Root rot (%)	Leaf yellowing (%)	Wilt (%)	Total infected plant (%)
Seed	100.00	0.00	0.00	0.00	0.00	No seedling emerged
Emergence and early vegetative growth stage (7 days old seedling)	0.00	50.00	19.44	16.67	5.56	91.67
Branching and rapid vegetative growth stage (15 days after emergence)	0.00	33.33	19.44	8.33	19.44	80.44
Early flowering stage (40 days after emergence)	0.00	50.00	5.56	5.56	8.33	69.44

3.2. Pathogenicity

The result of the pathogenicity test is presented in Table 2. The pathogenicity of the eight isolates of *Fusarium oxysporum* f. sp. *phaseoli* appeared to be highly variable in causing seedling mortality ranging from 5.56 to 97.22%. Three isolates collected from infected Bush bean plants namely isolate IS 3, IS 4 and IS 5 were found to be highly virulent and the total seedling mortality was recorded as 97.22, 83.33 and 80.55%, respectively. Rest of the isolates was observed as very low to moderately virulent in causing seedling mortality of Bush bean. The lowest virulence in causing seedling mortality was recorded with the isolate IS 8. Based on the present findings isolate IS3 was selected for further study.

3.3. Effect of different growth stages of Bush bean on disease development

Development of different disease symptoms caused by *F. oxysporum* f. sp. *phaseoli* was observed in pot culture experiment by inoculating the pathogen at four different growth stages of Bush bean. The results of the experiment are presented in the Table 3. The highest of 100% pre-emergence seedling mortality was observed when the pathogen was inoculated with the seeds at the time of sowing. The disease development was decreased with the increase of time of inoculation of Bush bean growth. Disease development caused by *F. oxysporum* f. sp. *phaseoli* clearly appeared as four distinct symptoms namely root lesion, root rot, leaf yellowing and wilt. These symptoms are in agreement with Buruchara and Camacho (2000) and George (1985). Among the disease symptoms appeared, root rot and wilt resulted in ultimate death of the seedlings. Inoculation at seven days old seedlings caused 91.67% plant infection but wilt symptom was only 5.56% while inoculation of 15 days old seedlings caused the highest wilting symptom of 19.44% followed by 8.33% in the inoculation of 40 days old seedlings. It can be concluded that early stage of the growth of Bush bean may be vulnerable to disease development by *F. oxysporum* f. sp. *phaseoli* and with the increase of age the host can resist the pathogen to develop disease. Again wilting and *Fusarium* yellowing in common bean by pathogenic isolate of *F. oxysporum* f. sp. *phaseoli* was also reported by Alves-Santos *et al*., (1999). Pastor-Corrales and Abawi (1987) reported leaf defoliation and discoloration of bush bean in case of artificial inoculation of *F. oxysporum* f. sp. *phaseoli.*

3.4. Effect of fungicides on Fusarium oxysporum **f. sp.** *phaseoli*

The results of the laboratory evaluation of the selected fungicides are presented in Table 4 and Fig. 1. The results exhibited that all the concentrations of Aimcozim and Vitavax-200 appeared to be the most effective against the selected pathogen. The lowest radial growth (5.53 mm) was observed in Aimcozim at 200 and 400 ppm and inhibited more than 89% radial growth even at the lowest concentration (50 ppm). The percent inhibition of radial growth was slightly increased with the increase of concentration of Aimcozim but it was statistically insignificant (92.58% at 100 ppm and 93.31% both in 200 and 400 ppm). The percent inhibition of radial growth was increased with the increase in the concentration of Vitavax 200. The lowest concentration of Vitavax 200 inhibited 70.57% radial growth which was statistically superior to all the concentrations of Cupravit and Rovral 50 WP. The lowest concentration of Cupravit and Rovral 50 WP was least effective in inhibiting the radial growth of *F. oxysporum* f. sp. *phaseoli*. Aimcozim and Vitavax-200 are the most effective against *F. oxysporum* f. sp. *phaseoli* even at the lower concentration, which are in full agreement with the findings of many investigators (Buchvarova *et al*., 1989; Kapoor and Kumar, 1991; Pant and Mukhopadhayay, 2001; Bhaskar *et al*., 2003; Khatun 2006 and Rahman, 2006). In a laboratory evaluation Karampour *et al*., (1996) observed 85.5% inhibition of the radial growth of *F. oxysporum* by Rovral which are not in agreement with the present study. This difference might be due to the different isolates and different locations of the pathogen.

Table 4. Sensitivity of *Fusarium oxysporum* f. sp. *phaseoli* (IS3) to different fungicides in *in- vitro* testing

Fungicides	Concentration (ppm)	Radial growth (mm)	Inhibition of radial growth %
Rovral 50 WP	50	55.17 c	33.25 k
	100	36.00 e	56.44 i
	200	32.83 f	60.27 g
	400	30.67 g	62.90 f
Vitavax-200	50	24.33 h	70.57e
	100	13.20 i	84.03 d
	200	11.77 j	85.79 c
	400	11.53 j	86.05 c
Cupravit 50WP	50	56.96 b	31.11l
	100	38.00 d	54.02 j
	200	34.00 f	58.86 h
	400	31.45 g	61.69 f
Aimcozim50 WP	50	8.370 k	89.88 b
	100	6.130 l	92.58 a
	200	5.530 l	93.31 a
	400	5.530 l	93.31 a
Control		82.65 a	0.000 m
CV%		2.65	1.07
LSD (0.05)		1.253	1.170

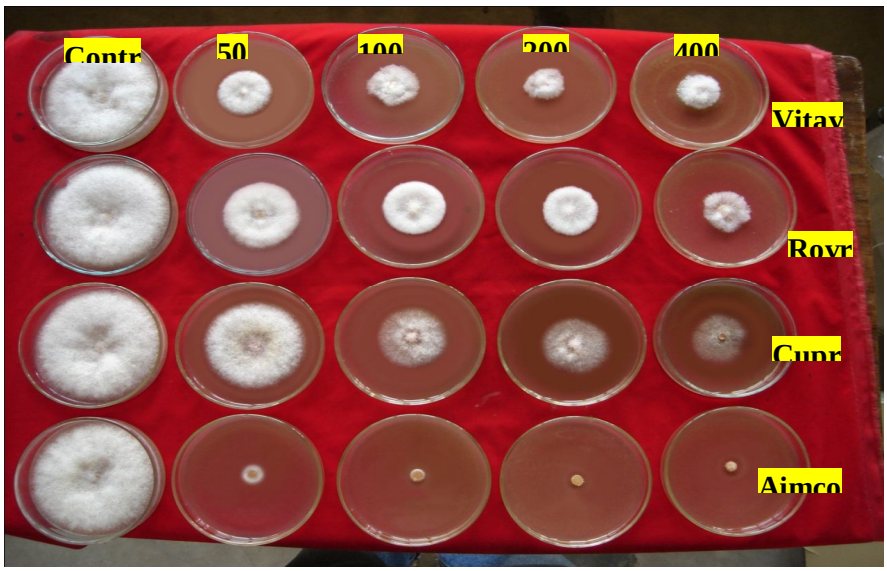

Fig. 1. Sensitivity of *Fusarium oxysporum* f. sp. *phaseli* (IS3) to different fungicides (5 days after inoculation)

4. Conclusions

The virulence of the isolates of *Fusarium oxysporum* f. sp. *phaseoli* are highly variable in causing seedling mortality of Bush bean. The disease development was decreased with the increase of the host (Bushbean) age. Aimcozim 50 wp at lowest concentration inhibit the fungal growth successfully in *in vitro* condition. Vitavax 200 is also one of the effective fungicides in inhibiting the radial growth of *Fusarium oxysporum* f. sp. *phaseoli*.

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