

RESEARCH ARTICLE

Estimation of deteriorative effect of *Fusarium oxysporum* and *Aspergillus niger* on fenugreek seed germination, seedling vigour and *in vitro* efficacy of fungicides

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ABSTRACT

In the present investigation, seed and soil inoculations were used to prove the pathogenicity of *Fusarium oxysporum* and *Aspergillus niger*, isolated from fenugreek seeds. Out of both, *Fusarium oxysporum* proved to be highly virulent as it caused higher per cent pre and post-emergence mortality (14.50% and 8.75%) and seedlings showed 49.55 per cent symptoms, whereas *Aspergillus niger* was observed to be less pathogenic because it caused lower per cent pre and post-emergence mortality (6% and 4.55) and seedlings showed (31.25%) symptoms. In *in vitro* test, complete inhibition was observed on Bavistin at all concentrations used. Whereas, 90.40 per cent growth inhibition occurred on Thiram at lowest concentration used *i.e.* 50 ppm. Captan and Raxil were not so effective at lower concentrations.

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INTRODUCTION

Fenugreek (*Trigonella foenum-graecum*), an annual legume native to the Mediterranean region, locally known as *Methi*, is cultivated not only as a leafy vegetable but also for medicinal purposes (Som and Maity, 1993). It is cultivated in counties India, Argentina, Egypt, Southern France, Morocco and Lebanon. Green methi is a good source of iron (Fe) as well as other minerals for human beings (Chhibba *et al.*, 2000). Seeds contain proteins 26 per cent, water-soluble polysaccharide (galactmannan) 20 per cent, hemi-cellulose and cellulose 24.5 per cent, water 9 per cent, fat (fenugreek oil) 7 per cent, lignin 2.5 per cent and saponin 8-10 per cent. The crop suffers severely from few seed-borne diseases including wilt caused by *Fusarium oxysporum* Schlecht which also affects seed germination, vigour index, plant growth and the grain yield (Shivpuri and Bansal, 1987 and Hashmi, 1988).

MATERIALS AND METHODS

Two tests were performed :

Soil inoculation technique :

Fusarium oxysporum and *Aspergillus niger* observed on fenugreek seeds were grown, separately on autoclaved rice medium (20g rice + 10 ml distilled water) contained in 250 ml conical flasks. These flasks were inoculated with spore/mycelial suspension prepared from 7 days old fungal culture. Flasks were shaken every day to avoid clumping. Autoclaved soil (Soil : FYM = 3:1, autoclaved at 1.045 kg/cm² for 1 hour for 3 consecutive days) was filled up in 30 cm earthen pots (pre-sterilized with 0.1 per cent HgCl₂ solution for 3 minutes followed by 3 washing with sterilized distilled water) and were inoculated with *Fusarium* sp. separately. For inoculation, the upper 4 cm layer of the soil was thoroughly mixed with rice medium supporting fungal growth. The pots were covered with polythene bags and left for 24 hours in a cage house

(22°-25°C). On next day, apparently healthy surface sterilized seeds were sown in pots at the rate of 10 seeds/pot with 5 replications. Apparently healthy surface sterilized seeds sown in uninoculated sterilized soil served as control. The pots were watered regularly. Observations on pre- and post-emergence mortality and per cent seedlings showing disease symptoms were recorded on 10th and 15th days of sowing, respectively.

Seed inoculation technique :

One hundred apparently healthy surface sterilized seeds were taken. The seeds were then rolled, separately on 7 days-old sporulating culture of each *Fusarium oxysporum* and *Aspergillus niger* thriving on PDA contained Petri dishes. Inoculated seeds were sown in 30 cm earthen pots (Pre-sterilized and having autoclaved soil as described earlier 3.6.1) at the rate of 10 seeds per pot with 5 replications. The uninoculated surface sterilized apparently healthy seeds served as control. These pots were kept in cage house (22°C – 25°C). The pots were watered regularly. Observations on pre- and post-emergence mortality and per cent seedling showing disease symptoms were recorded on 10th and 15th day of sowing, respectively.

In vitro efficacy of fungicides :

In this experiment the fungicides like, Thiram, Captan, Raxil and Bavistin were tested *in vitro* using poisoned food technique (Schimitz, 1930). Desired quantities of individual fungicide solution/suspension prepared in sterilized distilled water was added aseptically to 100 ml of sterilized PDA medium in 150 ml flask separately so as to get concentrations of 50, 100, 150, 200, 250 and 500 ppm. The flasks were shaken several times to ensure proper and uniform distribution of the fungicide. About 20 ml of medium mixed with fungicide was poured separately in sterilized Petridishes and allowed to solidify. Each Petridish containing medium mixed with fungicide was inoculated with 2 mm size mycelial bit cut from periphery of 7 days old culture of *F. oxysporum* grown on PDA in Petridishes. Inoculated dishes were incubated at

temperature 25±1°C in B.O.D. incubator. Medium without fungicide served as control. The experiment was conducted in Completely Randomized Design with three replications. The linear growth of fungus was recorded after 14 days of incubation and per cent inhibition was calculated according to Vincent's formula (1927) :

$$\% \text{ inhibition} = \frac{C-T}{T} \times 100$$

C = Diameter of the colony in control

T = Diameter of the colony in treatment

RESULTS AND DISCUSSION

The results of the present study as well as relevant discussions have been presented under following sub heads:

Soil inoculation test :

In soil inoculation, *Fusarium oxysporum* caused higher pre-and post emergence mortality (14.50% and 8.75%, respectively) and 49.55 per cent seedlings showed wilting, browning of roots, tip burning of young leaves, yellowing, black lesions or streaks on roots or stem base and stunting, while *Aspergillus niger* was less pathogenic which caused 6.25 per cent pre and 4.55 post emergence mortality and 31.25 per cent seedlings showed root rot, tip burning of leaves, seed rot and seedling decay (Table 1).

Seed inoculation test :

Inoculation of healthy seeds with *Fusarium oxysporum* caused both pre and post-emergence mortality in comparison to control and symptoms were wilting, yellowing, tip burning of young leaves and stunting which were observed on stems and leaves. In seed inoculation, *Fusarium oxysporum* caused higher pre and post-emergence mortality (29.50% and 11.24%, respectively) and 44.75 per cent seedlings showed wilting, yellowing, tip burning of young leaves, and stunting symptoms, while *Aspergillus niger* was less pathogenic which caused 8.50 per cent pre and 6.74 per cent post-emergence

Seed borne fungi	Germination (%)	Per cent seedling mortality		Per cent seedling showing symptoms*	Elongation (cm)*		Vigour index	Type of symptoms
		Pre-emergence	Post-emergence		Root	Shoot		
<i>Fusarium oxysporum</i>	65.00	14.50	8.75	49.55	2.15	2.75	318.50	Yellowing, wilting, stunting tip burning of young leaves, browning of roots dark brown lesions on roots or stem base.
<i>Aspergillus niger</i>	70.00	6.25	4.55	31.25	3.25	2.50	402.50	Root rot, seedling decay, seed rot and tip burning of young leaves.
Control	85.00	0.00	0.00	0.00	5.60	2.90	722.50	-

* Based on the emerged seedlings. Number of seed tested 100

mortality and 27.75 per cent seedlings showed seed rot, seedling decay and tip burning of leaves (Table 2). Pathogenic nature of *Fusarium oxysporum* through seed has also been reported by Hashmi (1988), Shivpuri and Bansal (1987).

In vitro efficacy of four fungicides against *F. oxysporum* at six different concentrations *i.e.* 50, 100, 150, 200, 250 and 500 ppm was assessed. The observations recorded on per cent inhibition of growth are presented in Table 3. A perusal of the data revealed that all the fungicides were superior in inhibiting the growth of the fungus over check. Bavistin gave complete inhibition of mycelial growth of the fungus at all the concentrations. Thiram significantly inhibited the growth of the fungus at 50 ppm concentration and gave complete inhibition of mycelial growth at 150, 200, 250 and 500 ppm concentrations. Captan and Raxil moderately inhibited the mycelial growth of *F. oxysporum* at 50 ppm and 100 ppm. There was a drastic increase in growth inhibition of the fungus with increased concentrations of fungicides. It can be concluded

that out of four fungicides, Bavistin was the best with complete inhibition of the mycelial growth followed by Thiram, Captan and Raxil in order of their decreasing efficacy. In *in vitro* test, all the fungicides namely, Bavistin (Carbendazim), Thiram, Captan and Raxil inhibited the fungal growth of *Fusarium oxysporum* in Petridishes at all the concentrations tested. Complete inhibition was observed on Bavistin at all concentrations used. Whereas, 90.40 per cent growth inhibition occurred on Thiram at lowest concentration used *i.e.* 50 ppm. Captan and Raxil were not so effective at lower concentrations. Further, growth of the fungus was inhibited with increasing the concentration of all the fungicides. Sharma and Jain (1984) observed that under *in vitro* conditions seed borne *Fusarium* spp. were more sensitive to Bavistin (Carbendazim) and Thiram. Ahmad and Abu (1989) also reported that the systemic fungicides Bavistin and Benlate, at different concentrations, were highly effective against *Fusarium oxysporum* f.sp. *sesami*.

Table 2: Pathogenicity of seed borne *Fusarium oxysporum* and *Aspergillus niger* of fenugreek by seed inoculation technique

Seed borne fungi	Germination (%)	Per cent seedling mortality		Per cent seedling showing symptoms*	Elongation (cm)*		Vigour index	Type of symptoms
		Pre-emergence	Post-emergence		Root	Shoot		
<i>Fusarium oxysporum</i>	67.00	29.50	11.24	44.75	2.00	2.50	301.50	Yellowing, wilting, tip burning of young leaves and stunting.
<i>Aspergillus niger</i>	72.00	8.50	6.74	27.75	3.00	2.75	414.00	Seed rot, tip burning of young leaves and seedling decay
Control	85.00	0.00	0.00	0.00	5.60	2.90	722.50	-

* Based on the emerged seedlings. Number of seed tested 100

Table 3: *In vitro* efficacy of fungicides against *Fusarium oxysporum* at different concentrations

Sr. No.	Fungicide	Per cent growth inhibition at various concentrations (ppm)*						Average (Fungicides)	Average (Concentrations)
		50	100	150	200	250	500		
1.	Thiram	90.40	95.00	100.00	100.00	100.00	100.00	84.83	57.29
		(71.95)	(77.08)	(90.00)	(90.00)	(90.00)	(90.00)		
2.	Captan	16.36	25.70	60.90	85.00	95.90	100.00	56.85	48.35
		(23.81)	(30.46)	(51.30)	(67.21)	(78.32)	(90.00)		
3.	Raxil	17.46	48.75	67.40	89.00	97.10	100.00	60.81	67.70
		(24.65)	(44.25)	(55.18)	(70.63)	(80.19)	(90.00)		
4.	Bavistin	100.00	100.00	100.00	100.00	100.00	100.00	90.00	
		(90.00)	(90.00)	(90.00)	(90.00)	(90.00)	(90.00)		
5.	Control (only distilled water)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)		
		S.Em±			C.D. at 5%				
	Fungicides	0.61			1.68				
	Concentrations	0.55			1.53				
	F×C	1.36			3.76				

Figures in parentheses are angular transformed values

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