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Evaluation the Efficacy of Fungicides and Bio-agents against *Fusarium oxysporum* under *in vitro* and *in vivo* Conditions

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A B S T R A C T

An experiment was carried out to evaluate the efficacy of fungicides (Calcium chloride, Mancozeb, Carbendazim), bio-agents (*Trichoderma harzianum, T. viride* and *Pseudomonas fluorescens*) against root rot of fennel (*Fusarium oxysporum*). All the treatments found effective against *Fusarium oxysporum*. Maximum inhibition per cent was recorded on Carbendazim (T_1-99.44%) which was statistically superior on Mancozeb (T_3-98.77%) and T_3 statistically superior on Calcium chloride (T_2-81.33%) which was statistically at par with *T. harzianum (*T_5-79.44%) followed by *Trichoderma viride* (T_4-76.88%) *Pseudomonas fluorescens* (T_6- 72.66%) while the minimum in control (T_0-51.00%) under *in vitro* conditions. The experiment was laid out in RBD with six treatments and three replications for *in vivo* studies. Among all the treatments the minimum disease incidence (%) of root rot was recorded in T_1 - carbendazim (13.50%), followed by T_3-mancozeb (15.00%), T_2-Calcium chloride (18.65%), T_5-*Trichoderma harzianum* (21.00%) T_4-*Trichoderma viride* (21.65%), T_6-*Pseudomonas fluorescence* (25.27%). In the present investigation carbendazim showed least disease incidence of 13.50% followed by mancozeb, calcium chloride, *Trichoderma harzianum* which showed 15.00, 18.65 and 21.00% disease incidence, respectively.

Introduction

Fennel (*Foeniculum vulgare* Mill.) is one of the most important seed spices belong to family *Apiaceae*. All parts of fennel plant viz., roots, leaves and volatile oil present in the seeds have medicinal values. Leaves are diuretic and roots are purgative. Seeds are aromatic, stimulant carminative and useful in diseases of chest, lungs, spleen, and kidney. It is also used to cure the diseases like cholera, bite, disorders of nervous system, cough and cold, constipation, dysentery and diarrhea. It is considered a good vermicide against hook worm. Hot infusion of fruits is used in indigenous medicine to increase lacteral secretion and to stimulate sweating. The consumption of fennel seeds also aid weight loss and longevity. Fennel oil is used in preparation of medicines for treatment of post menopausal syndrome and regulate menstrual period (Agarwal *et al*., 2001). India is the largest producer, consumer and exporter of seed spices in the world. The major growing belt spreads from arid to semi-arid regions covering large area in Gujarat, Rajasthan and Uttar Pradesh. In the Uttar Pradesh area under fennel is 842 ha with production of 740 tones

and the productivity 380 kg/ha (Anonymous, 2011).

Fennel is attacked by a number of diseases *viz*. root rot (*Fusarium oxysporum*), damping off of seedling (*Phytophthora syringae*), powdery mildew (*Erysiphepolygoni* D.C. and *Leveillula taurica* (Lev.) Arnaud), leaf spot (*Cercospora traversiana* Sacc.) and collar rot (*Rhizoctoniasolani* Kuhn). In India, the disease has been prevalent in all the fennel growing states. It cause on an average 10 to 25 % loss in yield and the damage has been observed to extend up to 60 % and 43 % at seedling and adult stages. Root rot in fennel can be observed 30-35 days after sowing. The disease appears at seedling (September-October) and reproductive stages (February-March) of the crop under field conditions. The infected plants show yellowing and withering of upright foliage. The infection extends up to lower portion of the stem at ground level and a dark brown discolouration of the affected portion of the stem is observed with whitish fungus growth on the affected portion of the stem at ground level (Gupta and Srivastava, 1976). A major limiting factors in profitable cultivation of this crop in Uttar Pradesh is attack of several diseases mainly caused by fungi and amongst them root rot caused by *Fusarium oxysporum* causes considerable damage in semi-arid areas where soil are sandy loam.

Materials and Methods

In vitro efficacy of fungicides

Efficacy of two systemic fungicides against mycelial growth of *F. oxysporium* was tested by poisoned food technique (Nene and Thapliyal, 1979). Four different concentrations *viz.*, 50, 100, 200 and 300 ppm of each fungicide was tested. Required quantity of each fungicide was added separately to sterilized medium, mixed thoroughly and poured in sterilized 9 cm diameter glass petri plates and allowed to solidify. Five replications were maintained for each treatment. A control was also maintained where medium was not supplemented with any fungicides. Each plate was inoculated with 5 mm disc with the help of sterilized cork borer from the edge of the fungal culture and incubated at 25±2ºC for 7 days. The colony diameter (two diagonals) of the test fungus was recorded and per cent growth inhibition was calculated by following formula (Vincent's, 1947):

$$\text{Per cent growth inhibition} = \frac{C - T}{C} \times 100$$

where,

C = diameter of the colony in check (average of both diagonals)
T = diameter of colony in treatment (average of both diagonals)

In vitro efficacy of bioagent

In vitro efficacy of three bio-control agents *i.e. Trichoderma harzianum, T. viride* and *Pseudomonas fluorescens* were tested by using dual culture plate method on PDA medium (Singh *et al.*, 2005). PDA was used as the basal medium. 20 ml of sterilized melted PDA was poured in each 9 cm diameter glass Petri plates and allowed to solidify. After 12 hours of pouring, these Petri plates were inoculated with 5 mm discs with the help of sterilized cork borer from the edge taken from 7 days old culture of *F. oxysporum* and antagonistic agents. Both were placed separately at equal distance on the periphery of the Petri plates. Inoculated Petri plates were incubated at 25±2ºC in BOD incubator for 7 days. After the incubation area of inhibition zone was measured (cm) from the difference between mycelial growth of the pathogen and antagonists. Inhibition zone was measured (cm) in comparison with the control

(Yadav and Majumdar, 2004). Five replications were maintained for each treatment. A field trial was conducted during *Rabi* season 2013-14 at Department of Plant Pathology, Sam Higgin bottom Institutes of Agriculture, Technology and Sciences, Allahabad (Deemed to be University) UP, India to test the efficacy of six different fungicides; bio-agents against fennel root rot disease. Fennel crop variety Co-1 was raised during *Rabi* season in a randomized block design with three replications. A spacing of 50 x 30 cm was adopted in plots of 2 x 1 m and six treatments were imposed viz., Calcium chloride (2%), Carbendazim @ (50% WP), Mancozeb @ (75% WP), *Trichoderma harzianum* (10g/l), *Trichodermaviride* (10g/l) and *Pseudomonas fluorescens* (10g/l) upon untreated control. The fungicides were sprayed soon after the first appearance of the disease. The treatments were repeated three times at 15 days interval and a total of three sprayings were taken up for each fungicides, bio-agents and neem leaf extract. Observations on the disease intensity were recorded 10 days after the last spray using the scale of 0-9 (Mayee and Datar, 1986) on randomly selected 5 plants of upper, middle and lower leaves. From total grades of 25 leaves, the percent disease index (PDI) was calculated.

Disease intensity (%) =

$$\frac{\text{Sum of all disease rating}}{\text{Total number of leaves} \times \text{maximum grade}} \times 100$$

Results and Discussion

Radial growth and per cent inhibition of *F. oxysporum*

The results (Tables 1 and 2 and Fig. 1) revealed that average mycelial growth was recorded in test treatments from 0.05 to 4.41 cm. However, significantly minimum average mycelia growth was recorded in Carbendazim ($T_{1,}$ .05 cm). This was statistically superior on Mancozeb ($T_{3,}$ 11cm) statistically superior also on Calcium chloride (T_2- 1.68 cm) followed by *Trichoderma harzianum* ($T_{5,}$ 1.85 cm), *T. viride* ($T_{4.}$ 2. 08cm), *P. fluorescens* ($T_{6,}$ 2.46 cm) as compared to control ($T_{0,}$ 4.41cm).

The data presented on inhibition % of mycelial growth as influenced by fungicides and bio-control agents are given (Table 2). A significant difference the inhibition % of mycelia growth was observed among the treatment. Maximum inhibition per cent was recorded on Carbendazim (T_1, 99.44%) which was statistically superior on Mancozeb ($T_{3,}$ 98.77%) and T_3 statistically superior on Calcium chloride ($T_{2.}$ 81.33%) which was statistically at par with *T. harzianum* (T_5, 79.44%) followed by *T. viride* (T_4, 76.88%) *P. fluorescens* (T_6, 72.66%) while the minimum in control (T_0, 51.00%). In *in vitro* evaluation of fungicides is a handy tool to test large number of fungicides in the present study, the Carbendazim among fungicide were found to be highly effective inhibiting.

The results of the present experiment evaluated that fungicides tested in this study exhibited antagonistic activity against *F. oxysporum*. The test antagonist *T. harzianum* (79.44%) was most effective antagonist and inhibited the radial growth of pathogen which was statistically at par with *T. viride* (76.88%) the probable reason for such finding may be the more than on mechanism like antibiosis, lysis and competition ware exhibited by *Trichoderma* spp., might have developed on the hyphae of other fungi. *T. harzianum and T. viride* an effective biological control agent against *Fusarium* spp (Sivan and Chet, 1986; Loban, 1990; Deshmukh and Raut, 1992; Verma and Dohroo, 2005).

Table.1 Inhibitory effect on radial growth of *Fusarium oxysporum*

Treatments	Radial growth of *Fusarium oxysporum (cm)*						
	24hrs	48hrs	72hrs	96hrs	120hrs	144hrs	168hrs
T_1-Carbendazim	00	00	00	00	00	.10	.25
T_2-Calcium chloride	.62	1.26	1.70	1.75	1.90	2.07	2.50
T_3- Mancozeb	00	00	00	00	00	.25	.55
T_4- *Tricoderma viride*	.20	1.20	1.60	2.25	2.90	3.10	3.31
T_5*T. harzianum*	.60	1.55	1.75	2.08	2.12	2.30	2.55
T_6- *Pseudomonas fluorecens*	.65	2.00	2.20	2.85	3.02	3.50	4.05
T_0- Control	.74	2.95	3.69	4.53	5.60	6.55	7.85
F-Test	S	S	S	S	S	S	S
SEm+-	8.41	3.72	3.05	2.082	1.345	1.72	1.529
CD at 5%	0.047	0.569	0.068	0.058	0.047	0.058	0.066

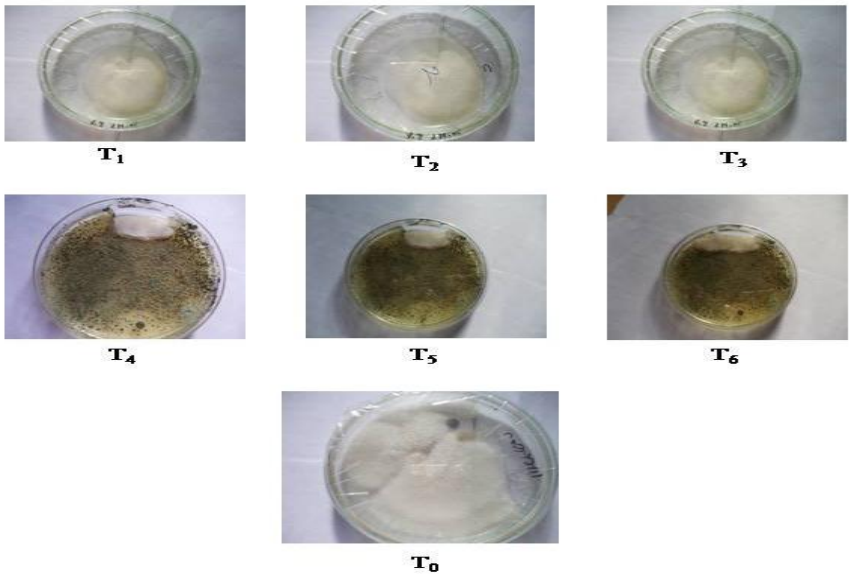
Table.2 Radial growth and per cent inhibition of *Fusarium oxysporum* as affected by different treatments

Treatments	Radial growth of pathogen (cm)	Per cent inhibition
T_1-Carbendazim	.05	99.44
T_2- Calcium chloride	1.68	81.33
T_3- Mancozeb	.11	98.77
T_4- *Trichoderma viride*	2.08	76.88
T_5- *T. harzianum*	1.85	79.44
T_6- *Pseudomonas fluorescens*	2.46	72.66
T_0- Control	4.41	51.00

Table.3 Per cent disease incidence of root rot on fennel as affected by different treatments (in Field and pot condition)

S. No.1	*Treatments*	*Dose (g/kg)*	**Per cent disease incidence**	**Per cent disease control**
1.	Carbendazim	2g	13.50	74.36
2.	Calcium chloride	3g	18.65	64.58
3.	Mancozeb	3g	15.00	71.51
4.	*Trichoderma viride*	4g	21.65	58.88
5.	*Trichoderma harzianum*	4g	21.00	60.11
6.	*Pseudomonas fluorescens*	10g	25.27	52.03
7.	Check (inoculated)		52.65	-
	overall mean		23.96	
	F-test		S	
	SEm+-		1.02	
	CD at 5%		1.23	

Fig.1 Inhibitory effect on radial growth of *Fusarium oxysporum*



Per cent disease incidences of root rot on fennel as affected by different treatments (in field and pot condition)

Efficacy of bio- agents, calcium chloride and fungicides at their respective does were further tested *in vivo* condition against *F. oxysporum*, root rot causing pathogen, with respect of per cent disease incidence of fennel.

The data presented in table 3 showed that all the treatments are significantly effective over control. Among all the treatments the minimum disease incidence (%) of root rot was recorded in T_1 - carbendazim (13.50%), followed by T_3- mancozeb (15.00%), T_2 - calcium chloride (18.65%), T_5 - *T. harzianum* (21.00%) T_4 - *T. viride* (21.65%), T_6- *P. fluorescence* (25.27%). In the present investigation carbandazim showed least

disease incidence of 13.50% followed by mancozeb, calcium chloride, *Trichoderma harzianum* which showed 15.00,18.65 and 21.00% disease incidence, respectively. Jahagirdar *et al*., (2002) reported that seed treatment with biocontrol agent, *Trichoderma viride* (4 g/kg) of seed is recommended as ecofriendly, approach for the management of chickpea wilt. Ghasolia and Jain (2003) reported that the efficacy of the carbendazim, thiram, captan, tebuconazole against *Fusarium* wilt in cumin and they found that carbendazim treated seed showed highest value for seed germination, root and shoot length and vigour index and lower pre- and post emergence mortality and also number of seedlings showing wilt symptoms.

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