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Efficacy of Fungi Toxicants and Bio Control Agents against *Fusarium oxysporum* f. sp. *ciceri*

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A B S T R A C T

Bio control agents (*Trichoderma viride*, *Pseudomonas fluorescens*) and various fungitoxicants were tested for their efficacy in controlling the Fusarium wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri in vitro* and *in vivo*. *In vivo* conditions soil inoculated with *Fusarium oxysporum* f. sp. *ciceri* was conducted to compare the efficacy of different treatments viz. seed treatment with bio-control agents and fungitoxicants in the management of chickpea wilt. All the treatment significantly reduced the wilt incidence. Bavistin, Thiram and *Trichoderma viride* were the most effective and reduced the wilt incidence as compared to inoculated control respectively whereas neem leaf and neem bark was the least effective over inoculated control. Seed treatment with *Pseudomonas fluorescens* and *Trichoderma viride* effectively enhancing the growth of chickpea viz. Shoot length, root length, shoot weight and root weight as compared to control. *In vitro* condition all the treatments used *in vivo* conditions were evaluated at different concentrations for their efficacy is significantly inhibited the radial growth of *Fusarium oxysporum* f. sp. *ciceri.* All the treatments were effective and significantly reduced the radial growth of *Fusarium oxysporum* f. sp. *ciceri.*

Introduction

India grows a variety of pulse crops under a wide range of agro climatic conditions and it is the most important pulse crop recorded production of 5.77 million tonnes/year. (Masood Ali and Shiv Kumar 2005). Generally chickpea (*Cicer arietinum* L.) is grown under rainfed situation, but it responds to variable irrigation (Chundawal *et al.*, 1976). Pulses are an important source of protein in vegetarian diets being leguminous crops, processing root nodules, they fix atmospheric nitrogen. They are thus not dependent on industrially fixed nitrogen, a process requiring energy but upto 30kg N/ha to the soil and

improve its fertility (Ahlawat *et al.*, 1997-98). In general also play a unique role in restoring the soil fertility by denitrogen fixation through symbiosis of root nodules bacteria of rhizobium species and perhaps due to this reason they have the ability to give good basis per year more than 75 percent (about $40X10^6$ tonnes per year) is contributed by the pulse-rhizobium association (Kush and Mishra 1981).

Chickpea is affected by the so many diseases, among all wilt is most important disease in India losses about 10-40% chickpea crop. In chickpea more than one conidium are formed of these toxins like zearalenone and sporofusariun are produced from *Fusarium*. Fusarium oxysporum is one of the most common fungi occurring in all types of soil throughout the world (Burgess, 1981). Fusarium are known to suppress Fusarium wilt of chickpea such as *Fusarium oxysporum* f. sp. *ciceri* (Alaboutte and Singh, 2002) The pathogen is a soil inhabitant, between crops it survives in infected plant debris in the soil as mycelium and in all its spore forms but most commonly, especially in the cooler distances by means of water and contaminated farm equipments and over long distances primarily in infected transplants or in the soil carried with them, usually once an area becomes infected with Fusarium, it remains so indefinitely. In the present investigation various fungal viz. *Trichoderma viride* and bacterial biocontrol agents viz. *Pseudomonas fluorescens* chemicals like carbendazim, thiram and plant extracts viz. neem leaf extract and neem bark extracts were used to control the Fusarial wilt under *in vitro* and *in situ* conditions.

Materials and Methods

The investigation *in vitro* and *in vivo* was carried out Advanced Centre for Rainfed Agriculture, Dhiansar.

In vitro investigation was carried out in radial growth (in solid medium). Isolation, identification and purification of test fungus *Fusarium oxysporum* f. sp. *ciceri* and bio control agents viz. *Trichoderma viride* and *Pseudomonas fluorescens* used in the experiment was carried out in laminar air flow under aseptic conditions. In case of Fusarium PDA medium was used and infected chickpea plant showing characteristic symptoms was used, washed the infected portion of plant were washed and three times sterilized with 0.1% $HgCl_2$ foe 1-2 seconds. Before culturing infected plants were viewed under microscope for ascertaining examination of conidia, by applying $HgCl_2$ tissues get surface sterilized so as to minimize the contamination. Already sterilized, melted PDA medium was transferred into petriplates and then small pieces of infected roots of the chickpea were kept on semi-solidified media inside petriplates. The whole process was done under laminar flow and petriplates were kept under BOD incubator after 3 days white cotton growth of mycelium was observed in petriplates and slight portion was taken under microscope for confirming the pathogen. The mycelium of fungus is cottony, hyaline, branched, septate multinucleate. The fungus can be cultured on simple media, growing profusely and produces three types of asexual spores, i.e. macroconidia, microconidia and chlamydospores, with in test tissue as well as in cultures. Similarly commercial bio control agents viz. *Trichoderma viride* trade name Nisarga bacterial bio control agents viz. *Pseudomonas fluorescens* trade name Sparsh marketed by Agro chemicals 1st main road, Mahalaxmi, layout Bangalore 560086, India.

Viability and population assessment test of the product

Commercial formulation of *Trichoderma viride* and *Pseudomonas fluorescens* were tested for their viability and population

assessment test before using in the experiment. 1g of product was weighed and made upto 10 ml. suspension was taken and transferred to 9ml. of sterilized water in a test tube (1:100). Serial dilution was made similarly transferring 1ml of the suspension to the subsequent tubes to get 1:1000000 dilution 1ml of the 1000000 suspension was transferred to sterile petriplates. 15 ml of melted and cooled PDA was poured in petriplates for assessment of *Trichoderma viride* and *Pseudomonas fluorescens*. The plates were rotated gently and allowed to solidify. The plates were incubated at room temperature, after 48 hours average number of colonies per plates was calculated. Five colonies in case of *Trichoderma* were found and the no. of c. f. u. present in 1g of the product was calculated by formulae:

$$\text{c. f. u. in /g product} = \frac{\text{No. of colonies}}{\text{Amount placed X dilution}}$$

c. f. u. of *Trichoderma viride* in 1g product = $(5/1 \text{ X } 10^{-6}) = 5\text{X } 10^{6}$ c. f. u/g

c. f. u. of *Pseudomonas fluorescens* in 1g product = $(6/1 \text{ X } 10^{-6}) = 6 \text{ X } 10^{6}$ c. f. u/g

Fungal biocontrol agents viz. *Trichoderma spp*. have been widely explored and recommended against many soil borne soil fungal diseases (Elad, Y., Chet, I and Katan, J. 1980).The use of *Trichoderma a*s a fungal biocontrol agent in the control of plant pathogens along with other disease management (Papavizas 1980), use of bacteria for management of plant diseases and yield improvement has increased steadily since the mid 1960b (Baker, 1987). *In vitro* the fungal, bacterial, chemicals and plant extracts were tested against Fusarial wilt in solid as well as in liquid. The fungitoxicants used in the experiment were evaluated by poisoned food technique methods (Nene and Thapiyal 2000) in it incorporation of nutrient medium with a toxic chemical and then allowing test fungus to grow on such a poisoned food.

In the *in vivo* experiment the seeds were dressed as per the treatment. The test fungus Fusarium was multiplied on sorghum medium, 100g of sorghum was crushed and sorghum was taken in conical flasks and was sterilized in an autoclave at temp. 121^{0}C and 15lbs pressure for 20 minutes. Inoculate each conical flask with carried sorghum with 2 discs measuring 5mm of test fungus at 25^{0}C. The pathogen *Fusarium oxysporum* f. sp. *ciceri* multiplied on sorghum medium was applied @100g/plot. Seeds were dressed with commercial formulation of *T. viride* and *P. fluorescens* 4g/kg seed, similarly carbendazim and thiram 3g/kg of seed before sowing seeds treated were kept in shade for few hours before sowing to dry up. Similarly the neem leaf extract and neem bark extract @ 4g/kg of seed before sowing treated were kept in shade for few hours before sowing to dry up.

Statistical analysis: In the investigation Randomized Block Design was adopted. The analysis of variance technique was applied for drawing conclusion from the data. The calculated values of F were compared with the tabulated value at 5% level of probability for the appropriate degrees pf freedom the skeleton of analysis of variance table. The data obtained were statistically analyzed using "Analysis of variance" technique and "Critical difference" as by P. G. Panse and P. V. Sukhamte. Indian Council of Agriculture Research New Delhi (1967).

Results and Discussion

Under *in vitro* conditions with dual culture techniques on solid media the minimum growth of *Fusarium oxysporum* f. sp. *ciceri* was recorded from day 1 to day 8 after every 24 hours.

Table.1 Antagonistic efficacy of fungi toxicants and bio control agents against *Fusarium oxysporum* f. p. *ciceri* (Dual culture technique)

Treatments	Radial growth of *Fusarium oxysporum* f. sp. *ciceri* (mm) in days							
	D1	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**
T_1(inoculated control) *F. o.*	11.99	18.36	28.21	37.44	47.24	56.22	67.77	80.68
T_2 (*F. o. + T. v.)*	10.61	9.63	8.61	7.61	6.61	5.03	3.64	2.35
T_3 (*F. o + P. f.)*	5.40	5.80	6.20	6.65	7.25	7.62	8.52	8.61
T_4 (*F. o* + NL extract6%)	3.94	5.10	6.03	6.54	7.73	8.41	9.41	10.20
T_5 (*F. o* + NB extract6%)	3.10	5.03	6.13	6.93	8.17	9.27	10.12	10.55
T_6 (*F. o* + carbendazim.100ppm)	3.30	5.31	6.30	7.61	8.54	9.35.	9.82	9.07
T_7 (*F. o* + Thiram100ppm)	2.97	5.19	6.18	7.28	8.30	9.12	10.07	11.35
CD	0.244	0.241	0.178	0.548	0.186	0.579	0.266	0.404
SE (m)	0.080	0.079	0.058	0.179	0.061	0.189	0.887	0.132

Each value is mean of three replicates

F. o = *Fusarium oxysporum*, T. v. = *Trichoderma viride*, P. f. = *Pseudomonas fluorescens*, NL= Neem leaf, NB= Neem bark. D = Days

Table.2 Effect of biocontrol agents and fungitoxicants on the wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* (pooled data of both the years)

Treatments	30 DAS		60 DAS	
	Wilt incidence	**% reduction in wilt incidence over inoculated control**	**Wilt incidence**	**% reduction in wilt incidence over inoculated control**
T_1(inoculated control) *F. o.*	20.3	00	30.60	00
T_2 (*F. o. + T. v.)*	3.30	83.70	5.60	81.70
T_3 (*F. o + P. f.)*	8.00	57.63	11.60	62.09
T_4 (*F. o* + NL extract6%)	12.00	40.88	14.60	52.29
T_5 (*F. o* + NB extract6%)	13.30	34.48	15.30	50.00
T_6(*F. o* + carbendazim 100ppm)	2.60	87.19	3.60	88.66
T_7 (*F. o* + Thiram100ppm)	3.00	85.22	5.00	83.60
T_8 (uninoculated)	11.30	44.30	16.30	46.73
CD	0.863		1.188	
SE (m±)	1.852		2.548	

Each value is mean of three replicates

F. o = *Fusarium oxysporum*, T. v. = *Trichoderma viride*, P. f. = *Pseudomonas fluorescens*, NL= Neem leaf, NB= Neem bark. D = Days

Table.3 Effect of bio control agents and fungitoxicants on the growth parameters after 30 DAS and 60 DAS

Treatments	Shoot length (cm)		Root length (cm)		Shoot weight (g)		Root weight (g)	
	30DAS	**60DAS**	**30DAS**	**60DAS**	**30DAS**	**60DAS**	**30DAS**	**60DAS**
T_1(inoculated control) *F. o.*	5.00	13.05	4.60	5.27	3.20	6.60	1.90	4.80
T_2 (*F. o.* + *T. v.*)	17.30	36.80	6.80	12.33	18.60	33.50	15.30	21.40
T_3 (*F. o* + *P. f.*)	19.78	41.20	7.35	15.06	21.40	39.60	20.60	23.03
T_4 (*F. o* + NL extract6%)	14.80	25.30	6.20	8.63	10.00	20.81	9.00	13.43
T_5 (*F. o* + NB extract6%)	14.79	24.70	6.07	8.33	9.00	21.50	8.50	13.60
T_6(*F. o* + carbendazim 100ppm)	12.00	22.00	5.85	8.10	8.90	20.80	8.40	13.50
T_7 (*F. o* + Thiram100ppm)	12.30	22.50	5.90	8.16	9.30	21.20	8.70	13.30
T_8 (uninoculated)	9.50	16.00	5.15	6.93	5.90	19.03	5.30	9.70
CD	1.15	1.00	0.233	0.448	1.03	0.72	0.810	1.063
SE (m±)	2.46	2.145	0.500	0.960	2.21	1.54	1.740	2.280

Each value is mean of three replicates.

In case of bio control agents the 5mm disc was placed at the periphery of the petriplate and test fungus was placed on the another periphery of the petriplates. At day 1 the radial growth was minimum with neem bark extract (3.10mm) followed by carbendazim (3.30mm), after 24 days at 2nd day the minimum radial growth was with neem bark extract (5.03mm) followed by neem leaf extract (5.10mm), at third day the minimum radial growth was with neem leaf extract (6.03mm) followed by neem bark extract. At the 4th day the minimum radial growth was with neem leaf extract (6.54mm) followed by neem bark extract (6.93mm), again after 24 hours on 5th day the minimum radial growth was with *Trichoderma viride* (6.61mm) followed by *Pseudomonas fluorescens* (7.25mm) (Table 1).

At 6th day the minimum radial growth was with the *Trichoderma viride* (5.03mm) followed by *Pseudomonas fluorescens* (7.62mm). With the increase in days the radial growth was minimum with the biocontrol agents. At the 7th day the radial growth of pathogen was minimum was with *Trichoderma viride* (3.64mm) followed by *Pseudomonas fluorescens* (8.52mm). At the 8th day the minimum radial growth of pathogen *Fusarium oxysporum* f. sp. *ciceri* was with *Trichoderma viride* (2.35mm) followed by *Pseudomonas fluorescens* (8.61mm).

In vivo experiment

Effect of bio-control agents and fungitoxicants on the wilt incidence: It was revealed that the seed treatment with *Trichoderrma viride*, *Pseudomonas fluorescens*, Bavistin, thiram, neem leaf extract and neem bark extract in all the treatments were significantly effective in controlling the wilt incidence as compared to inoculated control. At 30 DAS and 60 DAS the minimum wilt incidence was recorded with bavistin and followed by thiram and next followed by *Trichoderma viride*. Seed dressing with thiram eradicate seed borne inoculum and seed treated with Bavistin decreased *Fusarium oxysporum* f. sp. *ciceri*. Seed treatment with Bavistin increased number of nodules/plant and maximum grain yield in *cicer arietinum* were obtained. Seed treatments are required to completely eradicate the disease and the antagonist moved to the rhizosphere and survived well in it controlled chickpea wilt. Similarly *Trichoderma viride* was used which showed profound effect over *Fusarium oxysporum* f. sp. *ciceri* causing wilt of *Cicer arietinum*. Plants effects were effective in decreasing the prevalence of seed borne fungi. Similar findings have been reported by earlier scientists viz. Zaman *et al.*, (1997), Ushamalini *et al.*, (1997), Hemani and Bhatnagar *et al.*, (1996) (Table 2).

Effect of seed treatment of antagonist on the shoot length, root length, shoot weight and root weight of chickpea of chickpea under field condition: The seed treatment with *Trichoderma viride, Pseudomonas fluorescence,* bavistin, thiram, neem leaf extract and neem bark extract in all the treatments were significantly effective in enhancing the growth of chickpea i.e. shoot length, root length, shoot weight and root weight production as compared to control. The treatment T_3 (*Fusarium oxysporum f. sp. ciceri* + *Pseudomonas fluorescens*) was more effective in comparison to other treatments at 30DAS and 60DAS (Table 3). Moreover fluorescent Pseudomonas produced plant growth promoting substances such as auxin and gibberlins and enhanced growth of plant and its yield. Enhancement of growth of chickpea viz. shoot length, root length, shoot weight and root weight confirm to the earlier workers (Defago *et al.*, 1990; Rangeshwaran and Prasad, 2000).

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