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Integrated Management of Chickpea Wilt Incited by *Fusarium oxysporum* f. sp. *ciceris*

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ABSTRACT

Chickpea wilt incited by *Fusarium oxysporum* f. sp. *ciceris* is one of the severe diseases causes heavy losses (20-100%) depending upon stage of infection and wilting. Minimizing this disease can only be accomplished by careful crop management. Biological control is currently being painstaking for an increasing number of crops and managed ecosystems as the crucial technique of pest control. In this context an investigation was conducted to diminish wilt of chickpea by use of integrated disease management. It was observed that *in vitro* condition (dual culture technique) *Trichoderma viride* was highest inhibiting the growth of *Fusarium oxysporum* f. sp. *ciceris* at the ratio 1:4 followed by 1:2 and 1:1 in poisoned food technique maximum inhibition under field condition was obtained by 0.3% followed by 0.2 and 0.1%, doses of carbendazim. After that carbendazim it was neem cake at concentrations 7% followed by 5 and 3% in that order which give maximum inhibition of test pathogen under field condition. Lowest percentage of incidence of wilt (19.0%) was found with *T. viride* (T_2) followed by carbendazim (21.0%), neem cake (42.6%), carbendazim+neem cake (45.2%), carbendazim+*T. viride* (47.2%), neem cake+*T. viride* (48.2%). Pot culture studies revealed that the soil application of *T. viride* (4 g kg^{-1}) was found most effective treatment in reducing the incidence of chickpea wilt. Thus, chickpea wilt could be managed by the integration of various practices like, seed treatment with chemicals, seed and soil application of bioagents and amendment of soils with neem cakes.

Key words: *Cicer arietinum* L., Fusarium wilt, integrated disease management, carbendazim, neem cake, *Trichoderma viride*

INTRODUCTION

Pulse crops are an important source of food proteins, vitamins, lipids and certain minerals and generally grown under risk prone marginal lands. They are important crops for providing a high value food and nutritional security of a large fraction of vegetarian people of the world and are generally known as poor man's meat (Singh and Singh, 1992). Pulses being legume crops play a vital role in improving soil fertility and conserve natural resources which are essential for sustainable agriculture. Chickpea (*Cicer arietinum* L.) a self pollinating diploid (2n = 2x = 16) crop is the world's third most important legume. India is the principle chickpea producing country followed by Pakistan and Turkey (FAO, 2008).

The wilt of Chickpea incited by *Fusarium oxysporum* f. sp. *ciceris* is one of the serious diseases (Gupta *et al*., 1986). This pathogen is soil borne (Singh *et al*., 2009) and seed borne (Haware *et al*.,

1978) cause profound losses (20 to 100%) depending upon phase of illness and wilting (Haware and Nene, 1980). The spores of fungus enter in the plants passing through the roots. When the spores reach in vascular system they produce certain enzymes that disgrace the cell walls and obstruct the plant's transport system. Discoloration occurs inside tissues from the roots to the aerial parts, yellowing and wilting of the foliage occur and finally there is necrosis (Brayford, 1998; Leslie and Summerell, 2006). Chemical management of its infection by systematic fungicides is extravagant but also causes ecological problem. Hence, scientists are steadily looking out for non perilous and eco-friendly measures for plant disease management. Previously, a number of workers study various management practices by means of biological and chemical agents (Khan *et al*., 2001; Sibtain *et al*., 2001; Inam-ul-Haq *et al*., 2003; Kaur *et al*., 2007). In this context Bendre and Barhate (1998) concluded that crop yield loss could be minimized by adjustment of cultural practices, optimum use of chemicals, use of beneficial biological agents resistant varieties. Thus, the present investigation was planned to Isolate, identify pathogen from infected plants of chickpea and evaluate *in vitro* and *in vivo* the efficacy of carbendazim vis-a-vis neem cake and *Trichoderma viride* against *Fusarium oxysporum* f. sp. *ciceris*.

MATERIALS AND METHODS

Experimental detail: The present experiment was conducted at Department of Plant Protection, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad, India during Rabi 2009-10. The site of experiment is located at 25.87°N latitude and 81.54°E longitude and 98 m above sea level. This region has sub tropical climate with extremes of summer and winter. During winter season, especially in the month of December and January, the temperature drops down to as low as 2°C and during summer, it reaches up to 48°C. In laboratory all glass wares used were thoroughly cleaned with detergent, washed dried and sterilized at 150°C for 4 h and Potato Dextrose Agar (PDA) was used for isolation of fungus as method described by Aneja (2004). For isolation of fungus infected plants of wilt disease were cut into small bits of 2 mm surface sterilized by 0.1% mercuric chloride, rinsed 3 times by sterilized distilled water and transferred to petri plate containing PDA medium and incubated at 25°C. After 3 days, hyphal tip from periphery of colony growth was separated and transferred to another petri plates having medium to get pure culture. Identification of the pathogen was confirmed by studying morphological features of colony, spore characteristics and referring the relevant literature. Pathogenicity test and multiplication of *F. oxysporum* was done as per method of Nikam *et al*. (2007). Isolation and identification of fungal antagonists from rhizosphere soil was done as described by Rifai (1969). Incorporation of organic amendments into medium was done as mentioned by Jaganathan and Narsimhan (1988). Dual culture technique of Huang and Hoes (1976) was used for inoculation of antagonists of pathogen, where as poisoned food technique as described by Nene and Thapliyal (1979) was followed to determine percent mycelia inhibition. Field trial consisted of eight treatments one control and 3 replications with total number of 27 plots (each plot size 3×2 m). Treatments were carbendazim, *T. viride*, neem cake, carbendazim+neem cake, carbendazim+*T. viride*, neem cake+*T. viride*, carbendazim+neem cake+*T. viride*, uninoculated and inoculated control treatment *in vitro* experiment consisted of neem cake 3, 5 and 7% carbendazim 0.1, 0.2 and 0.3%. Each treatment was replicated 5 times. Dual culture technique consisted of treatments control, *F. oxysporum*+*T. viride* in 1:1 *F. oxysporum*+*T. viride* in 1:2 ratio, *F. oxysporum*+*T. viride* in 1:4 ratio. Each was replicated five times. Carbendazim was used at the rate of 2 g kg^{-1} seed through seed treatment (Nikam *et al*., 2007). Observations on disease intensity, plant growth parameters and yield were taken at 30, 60 and 90 Days After Sowing (DAS).

Statistical analysis: The data obtained on disease intensity, plant growth and yield parameters were analyzed statistically to test the significance of each character at 5% level of significance using procedure outlined by Fisher and Yates (1968).

RESULTS AND DISCUSSION

Biological control may be defined as the reduction in inoculums density producing activity of pathogen in its active or dormant state, by one or more organisms naturally or through manipulation of environment and host of the antagonists (Baker and Cook, 1974). Fungitoxic effect of three seed dressing fungicides alone and in combination was tested *in vitro* by applying poisoned food technique. The results obtained on the fungitoxicity of fungicides against *F. oxysporum* f. sp. *ciceris in vitro* are presented in Table 1. Our research finding indicates that maximum inhibition of pathogen was obtained by 7% neem cake (60.49%), followed by 5% neem cake (31.94%) and 3% neem cake (8.89%). The differences were significant compared to control. Similar results were reported by Nakkeeran *et al.* (2002) and Sharma and Gupta (2003).

Under *in vitro* condition maximum inhibition of *F. oxysporum* was done by 0.3% carbendazim (70.09%) followed by 0.2% carbendazim (50.69%), 0.1% carbendazim (25.92%), 7% of neem cake (20.38%), 5% neem cake (15%) and 3% neem cake (14%) and the difference in these were significant. T_6 was significantly better than other treatment where as differences between 3% neem cake and 5% neem cake and between 7% neem cake and 0.1% carbendazim were not significant (Table 2). These observations are in line with the results of Gupta *et al.* (1997). Regarding population density of *T. viride* (CFU×10^5 g^{-1} soil) in rhizosphere of chickpea field on 90 DAS Table 3 revealed that its highest colonization (41.81%) was in *T. viride* (4 g kg^{-1} of seed treatment)followed by carbendazim (39.27), neem cake (31.28) carbendazim+neem cake (18.99), neem cake+*T. viride* (13.51), carbendazim+*T. viride* (12.88) uninoculated (11.1) and

Table 1: Percent inhibition of *Trichoderma viride* on radial growth (mm) of *Fusarium oxysporum* f. sp. *ciceris* at different intervals

		Treatments (*F. oxysporum* f. sp. *ciceris*:*T. viride*)		
Hours	Control	1:1	1:2	1:4
24	80.500	14.180	30.633	37.333
48	40.500	15.833	20.970	16.387
72	20.000	7.220	33.497	17.257
96	0.000	6.890	31.947	60.490
SE±	8.983	2.885	4.779	6.423

Table 2: Percent inhibition of neem cake and carbendazim on radial growth (mm) of *Fusarium oxysporum* f. sp. *ciceris* at different intervals

		Treatments					
		Neem cake (%)			Carbendazim (%)		
Hours	T_0 Control	3	5	7	0.1	0.2	0.3
24	85.50	3.250	6.900	7.650	9.200	10.00	12.000
48	70.00	4.500	7.690	9.650	12.220	17.00	18.000
72	48.00	5.960	11.540	12.220	15.020	33.07	45.200
96	20.00	8.330	12.000	15.000	16.000	33.69	59.220
120	0.00	14.000	15.000	20.380	25.920	50.69	70.090
SE±	15.69	1.897	1.487	2.224	2.825	7.12	11.326

Table 3: Population density of *Trichoderma viride* in rhizosphere of chickpea field at different intervals

	Population density (CFU×10^5 g^{-1} soil)		
	DAS		
Treatments	0	45	90
T_0: Control (*Fusarium* inoculated)	1.660	3.290	6.513
T_1: Carbendazim	9.033	19.843	39.237
T_2: *Trichoderma viride*	12.360	27.797	47.810
T_3: Neem cake	7.843	14.860	31.287
T_4: Carbendazim+neem cake	7.327	11.220	18.993
T_5: Carbendazim+*Trichoderma viride*	6.067	9.437	12.883
T_6: Neem cake+*Trichoderma viride*	3.883	5.190	13.510
T_7: Carbendazim+neem cake+*Trichoderma viride*	3.287	4.957	6.920
T_8: Control (*Fusarium* uninoculated)	2.960	4.267	11.400
SE±	0.646	1.570	3.019

Table 4: Effect of carbendazim, neem cake and *Trichoderma viride* on dry shoot weight of chickpea (*Cicer arietinum* L.) at different intervals

	Dry shoot weight (g)		
	DAS		
Treatments	30	60	90
T_0: Control (*Fusarium* inoculated)	0.967	1.913	3.950
T_1: Carbendazim	2.313	3.927	7.947
T_2: *Trichoderma viride*	2.473	4.260	8.357
T_3: Neem cake	2.300	2.307	7.020
T_4: Carbendazim+neem cake	1.840	3.527	6.687
T_5: Carbendazim+*Trichoderma viride*	1.680	3.287	6.493
T_6: Neem cake+*Trichoderma viride*	1.667	2.620	5.360
T_7: Carbendazim+neem cake+*Trichoderma viride*	1.380	3.353	5.073
T_8: Control (*Fusarium* uninoculated)	1.667	2.520	4.620
SE±	0.117	0.207	0.429

inoculated control (6.51). All the treatments were significantly different from each other and superior to control. Sugha *et al.* (1995) evaluated 12 fungicides against Fusarium wilt of chickpea *in vitro* and *in vivo* under glass house and field conditions and reported Carbendazim (50 WP and 25 DS) and thiram alone and in combination as highly effective in inhibiting *in vitro* mycelial growth of the pathogen and in reducing wilt incidence both under glass house and field conditions.

With regard to effect of carbendazim (T_1) neem cake (T_3) and *T. viride* (T_2) on dry shoot weight (g), Table 4 shows that 90 DAS the highest dry weight of shoot was recorded in *T. viride* (8.35 g) followed by carbendazim (7.94 g) and neem cake (7.02 g), carbendazim+neem cake (6.68), carbendazim+*T. viride* (6.49), neem cake+*T. viride* (5.36), carbendazim+neem cake+*T. viride* (5.07), uninoculated (4.62) and control (3.95) and the differences in these were significant. This indicates a significant effect of treatments in dry shoot weight. *T. viride* was significantly effective but was at par with carbendazim Similar findings have been reported by Poddar *et al.* (2004) on fresh shoot weight in respect to various treatments on chickpea.

Regarding effect of treatments on dry root weight of chickpea, it was observed that highest mean dry root weight in neem cake (1.9 g) followed by carbendazim (1.38 g), carbendazim+*T. viride* (1.31 g), carbendazim+neem cake (1.30 g), neem cake+*T. viride* (1.15 g), carbendazim+neem cake +*T. viride* (1.13 g), uninoculated control (1.08 g), control (0.90 g) and carbendazim (0.09 g) and

Table 5: Effect of carbendazim, neem cake and *Trichoderma viride* on dry root weight of chickpea at different intervals

	Dry root weight (g)		
	----------DAS----------		
Treatments	30	60	90
T_0: Control (*Fusarium* inoculated)	0.140	0.267	0.900
T_1: Carbendazim	0.250	0.647	0.090
T_2: *Trichoderma viride*	0.400	0.813	1.380
T_3: Neem cake	0.277	0.477	1.907
T_4: Carbendazim+neem cake	0.163	0.560	1.300
T_5: Carbendazim+*Trichoderma viride*	0.207	0.607	1.313
T_6: Neem cake+*Trichoderma viride*	0.210	0.840	1.153
T_7: Carbendazim+neem cake+*Trichoderma viride*	0.293	0.587	1.133
T_8: Control (*Fusarium* uninoculated)	0.237	0.403	1.080
SE±	0.016	0.040	0.062

Table 6: Effect of carbendazim, neem cake and *Trichoderma viride* on yield per plot of chickpea

	Yield (g plot^{-1})
T_0: Control (*Fusarium* inoculated)	286.667
T_1: Carbendazim	566.667
T_2: *Trichoderma viride*	601.667
T_3: Neem cake	560.000
T_4: Carbendazim+neem cake	483.333
T_5: Carbendazim+*Trichoderma viride*	400.000
T_6: Neem cake+*Trichoderma viride*	460.000
T_7: Carbendazim+neem cake+*Trichoderma viride*	336.667
T_8: Control (*Fusarium* uninoculated)	323.333
SE±	29.690

the differences in these were significant. All treatments were significantly effective compared to control except carbendazim (Table 5). Similar findings have been reported by Saralamma and Reddy (2005). Karthikeyan and Karunanithi (1996) who found neem cake effective for control of *F. oxysporum*.

Highest mean yield of chickpea per plot was recorded in *T. viride* (601.66 g) followed by carbendazim (566.66 g), neem cake (560 g) and carbendazim+neem cake (483.33 g). However, lowest yield was observed with control (286.66 g) followed by neem cake+*T. viride* (460 g), carbendazim+neem cake+*T. viride* (336.66 g) and un-inoculated control (323.33 g). Thus, it can be concluded that *T. viride* was the most effective treatment under field condition (Table 6). Similar findings have been reported by Jha and Jalali (2006). However, effect of these treatments on disease intensity revealed that the lowest percentage of incidence` of wilt (19.0%) was found with *T. viride* (T_2) followed by carbendazim (21.0%), neem cake (42.6%), carbendazim+neem cake (45.2%), carbendazim+*T. viride* (47.2%), neem cake+*T. viride* (48.2%), carbendazim+neem cake+ *T. viride* (49.8%), uninoculated control (51.3%) and control (61.3%) and differences in these were significant *T. viride* was found most effective which is in accordant with reports of Pandey and Upadhyay (1999). De *et al.* (1996) found that coating of chickpea seeds with Carbendazim (0.2%) was more effective in reducing wilt and increasing seed yield by 25.9 to 42.6%. Gupta *et al.* (1997) screened 6 fungicides against *F. oxysporum* f. sp. *ciceris* and reported Carbendazim at the rate of 100 mg mL^{-1} as most effective in inhibiting the growth of fungus *in vitro*. Due to synergistic effects

of both the chemicals seed treatment with Thiram (0.15%)+Carbendazim (0.1%) were found most effective against *F. oxysporum* f. sp. *ciceris*. Somasekhara *et al.* (1996) reported that bioagents like *T. viride, Trichoderma harzianum* and *Trichoderma hamatum* as effective in controlling pigeon pea wilt caused by *F. oxysporum* f. sp. *udum*. Kolte *et al.* (1998) effectively controlled chickpea wilt with seed treatment by *Rhizobium, T. viride, T. harzianum* and *Azotobacter* sp. Prasad *et al.* (2002) who had reported soil application of *T. viride* and *T. harzianum* 1 week before sowing as more effective in reducing wilt and wet root rot of chickpea. The consortium (*T. viride*+ *T. harzianum* +*T. hamatum*) found very effective for control of chickpea wilt due to synergistic effect.

CONCLUSION

Minimizing biotic stresses can only be accomplished by careful crop management. Biological control is now being considered for an increasing number of crops and managed ecosystems as the primary method of pest control. The present study revealed that carbendazim and neem cake in poisoned food technique inhibited highest growth of test pathogen at the rate of 0.3% followed by 0.2, 0.1 and 7% under field condition. *T. viride* at the rate of 4 g kg^{-1} was most effective treatment for minimizing wilt of chickpea. Thus, chickpea wilt could be managed by the integration of various practices like, seed treatment with chemicals, seed and soil application of biological agents and amendment of soils.

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