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Full Length Research Paper

Management of chickpea wilt caused by *Fusarium oxysporium* f. sp. *ciceri*

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Chemical seed treatment with Thiram (0.15%) + Carbendazim (0.1%) is proved to be the most effective against *Fusarium oxysporium* f. sp. *ciceri*. *In vitro* evaluation of *Trichoderma* sp. against *F. oxysporium* f. sp. *ciceri* revealed the positive cumulative effect of *Trichoderma viride* + *Trichoderma harzianum* + *Trichoderma hamatum* in respect to the percent inhibition of the test fungus. Pot culture studies revealed that the soil application of *T. viride* (@ 25 kg/ha) as the most effective in reducing the incidence of chickpea wilt. Soil amendment with groundnut cake is proved to be effective against *F. oxysporium* f. sp. *ciceri* followed by neem cake. Genetic diversity already existing in pigeon pea germplasm lines can be exploited for breeding wilt resistant chickpea varieties. Thus, chickpea wilt incited by *F. oxysporium* f. sp. *ciceri* being soil borne disease could be managed by the integration of various practices like using resistant varieties, seed treatment with chemicals, seed and soil application of bioagents and amendment of soils with oilseeds cakes.

Key words: Chickpea wilt *Fusarium oxysporium* f.sp. *ciceri*, disease management.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the world's third most important pulse crop, after dry beans (*Phaseolus vulgaris* L.) and dry peas (*Pisum sativum* L.) (Vishwadhar and Gurha, 1998) . Although, chickpea is predominantly consumed as a pulse, dry chickpea is also used in preparing a variety of snack foods, sweets and condiments and green fresh chickpeas are commonly consumed as a vegetable. *Fusarium* wilt caused by *Fusarium oxysporium* Schlecht and Emend Synd. and Hans. is one of the major soil / seed borne disease of chickpea (*C. arietinum* L.). At national level the yield losses encountered due to wilt may vary between five to ten per cent (Singh and Dahiya, 1973). The pathogen is both seed and soil borne; facultative saprophyte and can survive in soil up to six years in the absence of susceptible host (Haware et al. 1978 and 1986). Considering the nature of damage and survival ability of the fungus, use of resistant varieties is the only economical and practical solution. Most of the resistant varieties have been found to be susceptible after some years because of breakdown in their resistance and evolution of variability in the pathogen. There appears to be no apparent reason as to why these already tested wilt resistant material showed such a variable wilt reaction and which creates a doubt about the possibility of existence of physiologic forms of the pathogen. The pathogen with high saprophytic ability can survive in soil for a pretty long period during which it may have to go through different environmental stresses and biological competition which may lead to the existence of physiologic races. Therefore, integrated management strategies are the only solution to maintain plant health. These strategies should includes minimum use of chemicals for checking the pathogen population, encouragement of beneficial biological agents to reduce pathogen inoculum, modification of cultural practices and use of resistant varieties (Bendre and Barhate, 1998). Considering the variable types of wilt reactions of released variety in the farmer's field and sick plot at

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different locations and yield losses caused, the present investigations were undertaken to formulate promising integrated disease management strategies with following objectives, screening of chickpea germplasm lines against major pathogen involved in wilt complex, management of chickpea wilt complex by fungicides, bioagents and organic soil amendments.

MATERIALS AND METHODS

The following materials were used during the present investigations. Biocontrol agents *Trichoderma* species (*T. viride* and *T. harzianum, T. hamatum*) were obtained from Department of Plant Pathology and Agricultural Microbiology, MPKV, Rahuri.

Fungicides

Three fungicides alone and in combination were used *in vitro* and in pot culture studies under glass house conditions against *F. oxysporium* f. sp. *ciceri* viz., Thiram 3.0% (3 g / kg seed), Captan 2.0 (2 g / kg seed), Carbendazim 0.2% (2 g / kg seed)

Organic soil amendments: Various oilseed cakes were obtained from the local market, Latur viz., Groundnut cake: 500 kg/ha, Cotton seed cake: 500 kg/ha, Neem seed cake: 500 kg/ha, Castor seed cake: 500 kg/ha

Methods

***In vitro* assay of fungicides**

Three fungicides were evaluated *in vitro* against *oxysporium* f. sp. *ciceri* by employing "Poisoned food technique" (Nene and Thapliyal, 1993) alone and in combinations. After incubation, radial growth was calculated by applying the formula (Vincent, 1927).

$$\text{Percent inhibition (I)} = \frac{C - T}{C} \times 100$$

Where,
C = Growth of test fungus in control in mm.
T = Growth of test fungus in treatment in mm

***In vitro* assay of *Trichoderma* species**

The studies on antagonism between *F. oxysporium* f. sp. *ciceri* and the fungal antagonists viz., *T. viride*, *T. harzinaum, T. hamatum* were carried out by applying 'Direct Bit Placement Method' (Brodbent et al. 1971). The pure cultures of *Trichoderma* sp. were obtained from Department of Plant Pathology, Agriculture and Microbiology Post Graduate Institute, Rahuri. To study the antagonistic effect, an experiment was laid out in petriplates poured with sterilized PDA. Solidified medium in the plates was inoculated by placing the discs (5 mm diameter) of bioagents culture and exacting opposite to this disc of test fungus (7 days old culture) were placed in such a manner that both organisms would get equal opportunity for their growth. The experiment was conducted with four replications and one control plate containing only test fungus. These plates were then incubated at 27^{o} + 1. Observation were recorded after seven days of inoculation on area covered by the *Trichoderma* sp. and pathogen and percent growth inhibition was calculated as per formula (Arora and Upadhyay, 1978) given below:

$$\text{Percent growth Inhibition} = \frac{\text{Colony growth in control plate} - \text{Colony growth in intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

Evaluation of fungicides, bioagents and organic soil amendments in glass-house (pot culture)

In fungicidal trial, the seeds of the variety JG-62 were treated with Thiram (0.3%) Captan (0.2%) Carbendazim (0.2%) individually (dry seed treatment) and sown in (@10 seeds/pot) earthern pots disinfected with 5% Copper sulphate and containing *Fusarium* sick soil. The pots were watered lightly and kept in glasshouse. For testing of *Trichoderma* sp. against *F. oxysporium* f. sp. *ciceri*, mass culture of *T. viride* was mixed with sterilized soil (ratio 1:3), ten days before the addition of *Fusarium* culture. The inoculum of test pathogen *F. oxysporium* f. sp. *ciceri* mass cultured on sand maize medium (1:1) was added to the soil in pot @ 100 gm/kg of soil. Prior to use, earthern pots were disinfected with 5% copper sulphate. The *Fusarium* culture was also added to sterilized soil in pots (@ 100 g/kg soil) which served as control. The seeds of variety JG-62 were sown (@ 10 seeds/pot) in these pots containing *Fusarium* sick soil and *T. viride*. Seed of variety JG-62 were treated with the culture of *T. viride* and sown in each pot containing only *Fusarium* sick soil. Sterilized earthern pots (25 cm diameter) were filled with autoclaved potting mixture of soil : sand (1:1) and as per treatment details potting mixture was amended with powdered seed cakes (each @ 8 g/pot) before seven days of inoculation with test pathogen and watered lightly. Then these pots were inoculated (@ 80 g/kg soil) with mass culture of test fungus (*F. oxysporium* f. sp. *ciceri*) multiplied on sand: maize medium (1:1), watered lightly and incubated at room temperature for one week. Then to these pots seeds of chickpea variety JG-62 were sown (@ 10 seeds/pot), watered lightly and kept in glass house. Ten seeds of variety JG-62 were sown in earthern pots containing untreated (without cakes) potting mixture but inoculated with test fungus was maintained as control. Observations on per cent seed germination; pre / post emergence wilting were recorded at 15 days interval from germination up to mortality.

Screening

Sick plot technique

Screening of available chickpea germplasm lines, local promising varieties was conducted in *F. oxysporium* f.sp. *ciceri* sick plot developed at ARS, Badnapur (Dist. Jalna). A field screening technique for wilt screening developed at ICRISAT (Nene et al., 1981) was adopted in the present studies. In this screening technique a wilt susceptible check (JG-62) was sown intermittently after every two test entries so as to monitor the disease pressure. A total of 30 genotypes were tested against wilt. Sowing of chickpea genotype was completed on 14.11.2003 with two replications of row length 5 m and 30 x 10 cm row to row and plant to plant spacing, respectively. The seed emergence was recorded 18 days after sowing. Observations on number of plants wilted from each genotype were recorded at 30, 45 and 60 days after sowing. The per cent wilt incidence was calculated on the basis of initial plant count and total number of wilted plants in each genotype and genotypes were graded (Nene et al., 1981) as follows.

Reaction	Percent wilting (mortality)
Resistant (R)	0 - 10 % mortality
Moderately resistant (MR)	10.1 - 20 % mortality
Moderately susceptible (MS)	20.1 - 30 % mortality
Susceptible (S)	30.1 - 50 % mortality
Highly susceptible (HS)	Above 50% mortality

Table 1. Effect of fungicides on growth of *Fusarium oxysporium* f. sp. *ciceri in vitro.*

Sr. No.	Fungicide	Concentration (%)	Mean colony diameter (mm)*	% inhibition
1.	Thiram (T)	0.3	16	82.22
2.	Captan (C)	0.2	24	73.33
3.	Carbendazim (Ca)	0.2	11	87.77
4.	Thiram + Captan (T +C)	0.15 + 0.1	13	85.55
5.	Thiram + Carbendazim (T + Ca)	0.15 + 0.1	9	90.00
6.	Control	-	90	0.00
	S.E. +		0.88	
	C.D. at 5%		2.65	

* = Average of four replications.

Table 2. Antagonistic effect of *Trichoderma sp*. on *Fusarium oxysporium* f. sp. *ciceri in vitro.*

Treatment No.	*Trichoderma sp.*	Mean colony diameter (mm)*	% inhibition
1.	*Trichoderma viride*	21	76.66
2.	*Trichoderma harzianum*	15	83.33
3.	*Trichoderma hamatum*	29	67.77
4.	*Trichoderma viride + Trichoderma harzianum +Trichoderma hamatum*	11	87.77
5.	Control	90	
	S.E. +	0.97	
	C.D. at 5%	2.99	

* = Average of four replications.

RESULTS

Evaluation of fungicides *in vitro*

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. oxysporium* f. sp. *ciceri in vitro* are presented in Table 1. It was observed that chickpea seeds treated with Thiram (0.15%) + Carbendazim (0.1%) effectively inhibited the growth (90%) of pathogen with colony diameter (9 mm) and found significantly superior to rest of fungicide treatments. The next best fungicide observed was Carbendazim (87.77%) followed by Thiram + Captan (85.55%), Captan (73.33%) which produced mean colony diameter of 11, 13 and 24 mm, respectively. Hence, seed treatment with fungicides Thiram + Carbendazim was proved to be most effective in inhibiting the growth of *F. oxysporium* f. sp. *ciceri* compared to other fungicides and untreated control.

In vitro effect of *Trichoderma sp.*

The *Trichoderma* sp. viz., *T. viride*, *T. harzianum, T. hamatum* were tested against the *F. oxysporium* f. sp. *ciceri* in laboratory applying direct bit placement method and results obtained are given in Table 2. The results presented in Table 2 indicated that all *Trichoderma sp.* significantly inhibited the growth of *F. oxysporium* f. sp. *ciceri* as against 90 mm radial growth in control treatment. The combined effect of three *Trichoderma sp.* (*T. viride* + *T. harzianum* + *T. hamatum*) was found to be most effective in checking the growth (11 mm) of *F. oxysporium* f. sp. *ciceri* over control (90 mm). It was also revealed all *Trichoderma* sp. when used individually as biocontrol agents also exhibited antagonistic effect against *F. oxysporium* f. sp. *ciceri* leading to reduced radial growth of the fungus viz., 15, 21 and 29 mm by *T. harzianum, T. viride* and *T. hamatum,* respectively. Cumulative effect of three *Trichoderma sp*. that is *T. viride* + *T. harzianum* + *T. hamatum* was found to be most effective against *F. oxysporium* f. sp. *ciceri* thereby causing maximum inhibition (87.77%) of the fungus *in vitro*. It was further observed that all three *Trichoderma* sp. could also exert antagonistic effect causing 83.33%, 76.66% and 67.77% of *F. oxysporium* f. sp. *ciceri* inhibition by *T. harzianum, T. viride* and *T. hamatum*, respectively. All bioagents were found significantly superior over control in respect to percent inhibition of the test fungus *in vitro*.

Table 3. *In vitro* effect of bioagents, fungicides and organic soil amendments on chickpea wilt incited by *Fusarium oxysporium* f. sp. *ciceri*.

Sr. No.	Treatment	Mean percent wilt incidence*	Percent wilt control
1.	*Trichoderma viride* (Seed treatment)	33.33 (37.25)	66.67
2.	*Trichoderma viride* (Soil application)	19.04 (25.62)	80.86
3.	Thiram	57.14 (49.90)	42.86
4.	Captan	66.66 (48.90)	33.34
5.	Carbendazim	61.90 (51.88)	38.10
6.	Groundnut cake	38.09 (38.11)	61.91
7.	Cotton seed cake	61.90 (51.88)	38.10
8.	Neem seed cake	47.61 (43.63)	52.39
9.	Castor seed cake	52.38 (46.36)	47.62
10.	Control	100 (90.00)	00.00
	S.E. +	1.66	
	C.D. at 5%	4.94	

* = Average of three replications.
Figures in parenthesis are arcsine transformed values.

Evaluation of bioagents, fungicides and soil amendments *in vitro* (pot culture)

With an object of integrated management of chickpea wilt, bioagents, fungicides and oilseeds cakes were evaluated against *F. oxysporium* f. sp. *ciceri* in pot culture under glasshouse condition and results are presented in Table 3. Results revealed that soil and seed application of *T. viride* is found to be effective in controlling the chickpea wilt by 80.86 and 66.67% wilt incidence respectively. Soil application of *T. viride* was found most. The results obtained on the effect of different oilseed cakes as soil amendments indicated that a effective in reducing chickpea wilt than seed treatment. Amongst the fungicidal seed treatments, Thiram followed by Carbendazim and Captan proved to be effective in checking the wilt incidence by 42.46, 38.10 and 33.34%, respectively as against control (100% wilting). The results obtained on the effect of different oilseed cakes as soil amendments indicated that all soil amendments proved to be effective in reducing disease under pot culture. Amongst four oilseed cakes tested groundnut cake followed by neem seed and castor cake were found to be most effective in checking percent wilt incidence by 61.91, 52.39 and 47.62% respectively as against control.

Varital screening of chickpea against *F. oxysporium* f. sp. *ciceri* under field condition

A total of 30 chickpea germplasm lines varieties / cultivars were screened applying the sick plot technique developed by Nene et al. (1981) which was proved to be the most efficient, quick and reproducible. On the basis of disease incidence, germplasm lines, cultivars were categorized as resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible adopting the disease rating scale (Nene et al., 1981). The disease incidence of different germplasm lines evaluated in the *F. oxysporium* f. sp. *ciceri* sick plot development at ARS, Badnapur are tested presented in Table 4. Amongst the 30 germplam lines, 18 lines *viz.*, BCP-3, BCP-8, BCP-17, BCP-36, BCP-57, BCP-73, BCP-91, BCP- 92, BCP-114, BCP-148, BCP-205, BDN-9-3, BDNG-797, PG-9425-5, JAKI-9218, PG-9414-7, BCP-69, PG -96006 were found resistant with an average disease incidence of 5.26 %, six lines viz., BCP-15, BCP-113, BCP- 125, BCP-135, Vishal, BDNG-2001-2-1 as moderately resistant with average incidence (13.85%) two lines (Vijay and PG-9425-9) as moderately susceptible (26.99%), two lines (BCP-54 and BDNG-2025) as susceptible (43.44%) and only one that is, BDNG-2012 as highly susceptible (54.76%). Mean disease incidence reported on the susceptible check JG-62 was 95.00%.

DISCUSSION

Kovacikova (1970) reported that seed treatment with Thiram @ 2 gm per kg seed gave the best protection against the *Fusarium* wilt of chickpea caused by *F. oxysporium* f. sp. *ciceri*. Cother (1977) obtained effective control of gram wilt by seed treatment with Thiram, Benomyl and Captan. Verma and Vyas (1977) reported effective control of chickpea wilt *F. oxysporium* f. sp. *ciceri* with Carbendazim (0.1%) and Chloroneb (0.1%). Haware et al. (1978) reported effective control of chickpea wilt with seed treatment of Thiram + Benomyl. 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. 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 per cent. Gupta et al. (1997) screened 6 fungicides against *F. oxysporium* f. sp. *ciceri* and reported Carbendazim @100 mg/ml as most effective in inhibiting the growth of fungus *in vitro*. Ali (1998) reported that seed treatment with Bavistin + Thiram (2 gm + 1.5

gm/kg of seed) effectively controlled wilt of chickpea caused by *F. oxysporium* f. sp. *ciceri*. Due to synergistic effects of both the chemicals [seed treatment with Thiram (0.15%) + Carbendazim (0.1%)] were found most effective against *F. oxysporium* f. sp. *ciceri*. Biological control may be defined as the reduction in inoculum density or disease 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). Somasekhara et al. (1996) reported that bioagents like *T. viride, T. harzianum* and *T. hamatum* as effective in controlling pigeonpea wilt caused by *F. oxysporium* f. sp. *udum* . Jha and Singh (1997) studied the antagonistic effect of *T. viride* on growth and sporulation of *F. oxysporium* f. sp. *ciceri* and reported maximum fungal growth inhibition by *T. viride.* Kolte et al. (1998) effectively controlled chickpea wilt with seed treatment by *Rhizobium, T. viride, T. harzianum* and *Azotobactor sp*. Prasad et al. (2002) observed two antagonistic fungi viz.,

T. harzianum (PDBCTH-10) and *T. viride* (PDBCTV) against wilt (*Fusarium oxysporium* f. sp. *ciceri*) and wet root rot (*Rhizoctonia solani*) of chickpea in field and reported that soil application of *T. harzianum* and *T. viride* one week before sowing as more effective than seed treatment in reducing wilt and wet root rot of chickpea. These results obtained on the integrated management of chickpea wilt are in conformity with findings of those results reported by Upadhyay et al., (2000) who had reported antagonists *Trichoderma* sp. as inhibitory to *F. oxysporium* f. sp. *ciceri*. Prasad et al. (2002) who had reported soil application of *T. viride* and *T. harzianum* one 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. The results obtained in respect of soil amendments are similar to those reported by Chauhan (1963) and Singh (1973) who had reported significant reduction in the incidence of *F. oxysporium* f. sp. *ciceri* after application of groundnut and sesamum oil cakes as soil amendments. Zakaria (1980) also proved the antifungal properties of oilseed cakes against wilt of chickpea, Pandey et al. (1996) who had reported beneficial role of integrated use of soil solarization + oilseed cakes + fungicides against *Fusarium oxysporium* f. sp. *ciceri* under field conditions. Soil amendment with groundnut cake is proved to be effective against *F. oxysporium* f. sp. *ciceri* followed by neem cake. The chickpea varietal reactions *F. oxysporium* f. sp. *ciceri* observed in present studies are some what similar to those reported earlier by Iqbal et al. (2005), Zote et al. (1983, 1993), Dandnaik and Zote (1988). Zote et al. (1983) tested 42 lines of chickpea against *F. oxysporium* f. sp. *ciceri* in wilt sick plot and reported 4 germplasm lines with 10% wilt incidence as resistant against *F. oxysporum* f. sp. *ciceri*. Sharma et al. (2004) and Dandnaik and Zote (1988) screened 400 germplasm lines of chickpea for resistance against *F. oxysporium* f. sp. *ciceri* in wilt sick plot. Of them ICC-184, ICC-1437, ICC-3099, ICC-3528, ICC-3385 and ICC-11322 were reported as resistant (10% mortality) against chcikpea wilt. Ayyub et al. (2003) and Zote et al. (1993) screened chickpea germplasm lines against wilt and dry root rot and reported BCP- 28, BCP- 29 and BCP-36 lines as resistant to *Fusarium* wilt and lines viz., BCP-15, BCP-23, BCP-28, BCP-29 and BCP-30 were resistant to both wilt and dry root rot. Gurha et al. (2002) screened 570 chickpea genotypes for resistance to Kanpur isolate (Race-2) of *F. oxysporium* f. sp. *ciceri* at Kanpur and reported the cultivars viz., PG-95007, PBG-126, FG-703, ICC-10149, H-82-2, H-92-71, GNG-1000, ICC-11442, BG-372, GL-96004, PBG-1, GL-90236, FG- 702, ICP- 99-1, IPC-99-10, IPC-99-34, IPC-99-38, FG-71, FG-694, GPF -133, H -208 and ICC-2862 to exhibit stable resistance against *Fusarium* wilt.

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