Fungicides combination for management of Chickpea Wilt incited by *Fusarium oxysporum* f.sp. *Ciceri*

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Fungicides combination for management of Chickpea Wilt incited by *Fusarium oxysporum* f.sp. *ciceri*

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The present study was carried out to examine effect of seed treatment with the Fungicides *viz;* Thiram, Carbendazim, Captan, Fosetyl AL, alone and their combination, to control wilt caused by *Fusarium oxysporum* f. sp. *ciceri* in chickpea plants (JG-62) growing in sick soil pots under green house conditions.The studies revealed that the highest germination per cent (83.33%) was recorded in the combined treatmented with Thiram+Carbendazim(0.3%) and also recorded improvement on the growth parameters *viz.*, shoot length, root length, shoot dry weight, root dry weight and plant dry weight with (20.94cm and 24.78cm), (10.96cm and 15.30cm), (3.12g and 4.14g), (0.45g and 0.60g), (3.57g and 4.74g) respectively at 30 DAS and 60 DAS, along with significantly reduced wilting per cent over control (21.86%) and increased Disease reduction per cent over control (78.14%) was noted in treatment of Thiram+Carbendazim (0.3%) compared with untreated control.

Key words: Chickpea, *Fusarium oxysporum* f.sp. *ciceri,* fungicides, combination, management

INTRODUCTION

Pulse crops are an important crop, grown under risk prone marginal lands which is source of proteins, vitamins, lipids and certain minerals and generally. Chickpea provide a high value food and nutritional security of a large fraction of vegetarian people of the world. Pulses being legume crops play a important role in improving soil fertility and conserve natural resources for sustainable agriculture. Chickpea was originated from West Asia and now cultivated in more than 56 countries of the world. Worldwide it is grown on an area of 13.5 million hectares with a production of more than 13 million tons. India ranks first in the world in terms of the acreage cultivated and the annual yield. In India, chickpea is grown in 8.82 Mha with a total production of 8.35 M tonnes and an average productivity of 947 kg/ha (Agriculture Statistics at a Glance, 2015).

Worldwide annual yield losses were estimated to be 4.8 million tones, due to biotic stresses, including infectious plant diseases (Ryan, 1997). The chickpea is widely attacked by soil-borne diseases cause severe yield losses. Among the soil-borne diseases affecting chickpea, fusarium wilt caused by *Fusarium oxysporum* f.sp*. ciceri* is the major one(Merkuz *et al*. 2011). This pathogen is soil borne (Singh *et al*.2009) and seed borne (Haware *et al.*1978). It can survive in soil, even in the absence of a host for 3-6 years (Ayyub *et al.* 2003; Haware *et al.* 1996). The wilt infection can damage the crop completely and cause 100% yield loss under severe conditions (Navas-Cortes *et al*. 2000).

The chickpea wilt fungus *Fusarium oxysporum* f. sp. *ciceris* is a vascular pathogen. The spores of

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fungus enter in the plants passing through the roots. When the spores reach in vascular system they produce certain cell wall digestive enzymes and block the plants transport system. Discoloration occurs inside vascular tissues from the roots to the aerial parts. Yellowing and wilting of the foliage occurs and finally there is necrosis (Leslie and Summerell,2006). Relatively high temperature with drought may cause upto 80% plant mortality (Govil and Rana, 1994). As a result of wilt infection, the complete plant or plant parts may die within few weeks of infection. In field conditions, the typical wilting can be appeared within 3-4 weeks after sowing, if the variety is susceptible.

Several measures are taken by growers but chemical control based on the use of fungicides is most effective and reliable method. Chemical control is widely being used in past and present to cope with Fusarium wilt disease.The main objective of presented study was to evaluate the possibility of controlling Fusarium wilt of chickpea with the use of fungicides under the glasshouse conditions.

MATERIALS AND METHODS

Chickpea seeds of JG-62 variety were obtained from the Pulse Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (M.S). Materials such as Fungicides viz., Thiram, carbendazim, captan, fosetyl AL and Bioagents *viz.*, *Trichoderma harzianum, T. viride, Pseudomonas fleorescens*, glasswares and plastic pots had obtained from Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

All the isolation and inoculation work were carried out in laminar air flow under aseptic condition. The platform of laminar air flow was sterilized by glowing ultraviolet light for half an hour prior to commencement of work. The working surface of laminar flow and side glasses were surface sterilized with denatured spirit. Moreover, other such necessary care was taken to maintain and carryout work under aseptic condition.The glasswares such as Petri plates, pipettes, beakers and test tubes were sterilized in hot air oven at 180^{0}C for 1 hour and media were sterilized in autoclave at 121.6^{0}C, 15 lbs/inch^2 for 15 minutes

Isolation of Fusarium oxysporum f.sp. *ciceri*

Chickpea plant showing typical wilt symptoms were collected from the field of Pulses Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (MS). The repeated isolations were made to isolate pathogen from wilted plants showing browning of vascular tissue. The roots and stem of infected plants were washed in running tap water to remove soil before isolation to avoid contamination. The roots were cut into small bits of the size 2.5 mm, with sterilized blade. These bits were then surface sterilized with 0.1 per cent mercury chloride for two minutes and washed with three changes of sterilized water to remove traces of mercury chloride. Each bit was blot dried and four bits placed on the each prepoured solidified potato dextrose agar (PDA) plates. These plates were then incubated at 27±2^{0}C for seven days. The fungal growth was transferred to the plates of PDA.

Purification, Identification and Maintanance of pathogen

Fusarium oxysporum f.sp. *ciceri* culture isolated from wilted chickpea plant were purified from single spore method and identified by the colony characteristics of the isolated fungi appeared as white cottony growth on PDA medium which became felted and wrinkled in old culture colonies. Microscopically by abundance of micro and fewer macro conidia. Microconidia were oval to cylindrical, straight to curved and measured 2.5-3.5 x 5-11 μm and were produced on short, unbranched monophialides. Macroconidia borne on branched conidiophores, were thin walled, 3-5 septate, fusoid and pointed at both ends and 3.5-4.5 x 25-65 μm (Trivedi and Rathi, 2015). The pathogen was subcultured on PDA slants and allowed to grow at 27 ± 2^{0}C temperature for 10 days. The culture obtained was stored in refrigerator at 4^{0}C and were sub cultured periodically once in a month.

Mass Multiplication of culture

The sorghum grains were soaked for one hour in warm water (40^{0}C to 45^{0}C) and then spread on the clean blotting paper for air drying. About 30^{0}g moistened grains were filled in each 1000 ml flask with 10 ml water and autoclaved for 30 minute at 15 lbs psi pressure. The mycelium bit of pure culture of *Fusarium oxysporum* f. sp. *ciceri* were inoculated under aseptic condition in those flask containing grains and incubated at 28± 2^{0}C for 10 days. Meanwhile flasks were shaken to avoid clumping of grains and to facilitate early growth of the fungus.

The grains turn whitish due to mycelial growth of the test fungus (Kamdi *et al.* 2012).

Preparation of sick soil

Soil were put in gunny bags and sterilized in autoclave at 30 lbs/inch$_2$ for 2 hours consequently for 3 days.The mass multiplied inoculum was added to sterilized soil at 1:10 proportion and thoroughly mixed thus the soil was made sick. The sick soil was filled in sterilized pots 1/4th of its capacity. The pots were watered lightly and incubated for 4 days.

The experiments were conducted in a CRD with 3 replications to evaluate the four fungicides *viz.,* Thiram, Carbendazim, Captan, Fosetyl AL and their combination. Seeds of susceptible chickpea cultivar JG-62 were treated with fungicides. Fungicides treated chickpea seeds were sown (@ 10 seeds per pot) in each pot. The pots sown with untreated seeds were also maintained as controls.

RESULTS AND DISCUSSION

Based on present study,four fungicides alone and their combination were tested under green house condition. The results indicated that the effect of all treatments found significant.Data on per cent seed germination are given in Table 1. Among the fungicides, highest per cent seed germination (83.33%) was observed in treatment Thiram+Carbendazim which was followed by treatment Fosetyl AL+Carbendazim (73.33%). Where minimum germination per cent was recorded in the control treatment (36.67%).The data from Table 2 regarding effect on chickpea shoot length was observed that highest shoot length was 20.94 cm and 24.78 cm in Thiram+Carbendazim at 30 DAS and 60 DAS, followed by 19.04 cm and 24.10 cm in Fosetyl AL+Carbendazim. Whereas in control it was 13.78 cm and 17.98 cm at 30 and 60 DAS respectively.The data from Table 3 revealed that, significantly maximum root length was observed under the treatment Thiram + Carbendazim (10.96 cm and 15.30 cm) followed by 10.50 cm and 14.70 cm in treatment Fosetyl AL+Carbendazim. The lowest in control it was 6.84 cm and 11.96 cm at 30 and 60 DAS respectively.Table 4 regarding effect of treatment on shoot dry weight revealed that highest shoot dry weight was 3.12 g and 4.14 g in Thiram + Carbendazim which was followed by 2.84 g and 3.84 g in Fosetyl AL+Carbendazim. The lowest was 1.08 g and 1.88 g in control.Table 5 regarding effect of treatment on root dry weight revealed that highest root dry weight was 0.45 g and 0.60 g in Thiram + Carbendazim which was followed by 0.42 g and 0.54 g in Fosetyl AL+Carbendazim. In control it was 0.19 g and 0.28 g.The data from Table 6 revealed that, significantly maximum plant dry weight was observed under the treatment Thiram + Carbendazim (3.57 g and 4.74 g) followed by 3.26 g and 4.36g in treatment Fosetyl AL+Carbendazim. The lowest in control it was 1.27g and 2.16 g at 30 and 60 DAS respectively.The data from Table 7 revealed that treatment Thiram+Carbendazim show lowest wilting per cent over control (21.86%) and highest disease reduction per cent over control (78.14%) followed by 27.56% and 72.44 % respectively wilting per cent and disease reduction per cent over control in Fosetyl AL+Carbendazim.

Chickpea wilt pathogen *Fusarium oxysporum* f. sp. *ciceri* is seed-borne and soil-borne nature. Therefore, highly effective approach will needed. Application of chemicals for management is successful in the presence of high level of inoculum and favourable weather conditions. Continuous application of one chemical required more amount and induce resistance in pathogen. The present study was focused on the development of an combination of fungicides for chickpea wilt. Appropriate combination of fungicide will highly effective than individual one. Defferent chemical synergistically work to better perform.Above findings of the present investigation revealed that Thiram+Carbendazim(0.3%) combination enhance seed germination, improve growth parameters and reduce per cent wilting over control significantly than individual of these fungicide.The present findings are supported by earliar experiment. Dubey *et al.* (2015) found the most effective treatment was Bavistin+Thiram for enhancing the seed germination and grain yield. Shivankar *et al.* (2000) found Carbendazim 50 WP (0.1 %) recorded highest root length and shoot length followed by Thiram 75 WP (0.25%). Sindhan *et al*. (2002) observed Carbendazim 0.2% was most effective with increased shoot length, shoot dry and root dry weight in comparision to control. Abed *et al.* (2013) found thatCarbendazim all were significantly increased growth parameter, fresh and dry shoot weight of plants in pot condition. Yadav *et al*. (2006), Saxena *et al.* (1994) showed that Bavistin and Thiram increased the total dry matter per plant.

Table 1 : Effect of seed treatments on seed germination

Treatments	Fungicide	Concentration	Percent seed germination
T_1	Thiram	0.3%	63.33 (52.73)*
T_2	Carbendazim 50% WP	0.1%	70 (56.79)*
T_3	Captan 50% WP	0.3%	56.67 (48.83)*
T_4	Fosetyl AL 80% WP	0.2%	66.67 (54.74)*
T_5	Thiram + Captan	0.3% (1:1)	63.33 (52.73)*
T_6	Thiram + Carbendazim	0.3% (2:1)	83.33 (65.91)*
T_7	Fosetyl AL + Carbendazim	0.3% (1:1)	73.33 (58.91)*
T_8	Control		36.67 (37.27)*
F test			Sig
SE(m)±			0.630
CD (P=0.01)			2.450

(*=Figures in parentheses indicates arc sin transformed value)

Table 2 : Effect of seed treatments on shoot length (cm)

Treatments	Fungicide	Concentration	Shoot length (cm)*	
			30 DAS	60 DAS
T_1	Thiram	0.3%	15.30	19.06
T_2	Carbendazim 50% WP	0.1%	18.76	23.60
T_3	Captan 50% WP	0.3%	14.86	18.98
T_4	Fosetyl AL 80% WP	0.2%	17.98	22.96
T_5	Thiram + Captan	0.3% (1:1)	18.38	22.90
T_6	Thiram + Carbendazim	0.3% (2:1)	20.94	24.78
T_7	Fosetyl AL + Carbendazim	0.3% (1:1)	19.04	24.10
T_8	Control		13.78	17.98
F test			Sig	Sig
SE(m)±			0.418	0.246
CD (P=0.01)			1.857	1.092

(*=Average of five)

Table 3 : Effect of seed treatments on root length (cm)

Treatments	Fungicide	Concentration	Root length (cm)*	
			30 DAS	60 DAS
T_1	Thiram	0.3%	8.66	14.54
T_2	Carbendazim 50% WP	0.1%	9.46	13.92
T_3	Captan 50% WP	0.3%	8.50	13.26
T_4	Fosetyl AL 80% WP	0.2%	9.38	14.40
T_5	Thiram + Captan	0.3% (1:1)	8.12	14.06
T_6	Thiram + Carbendazim	0.3% (2:1)	10.96	15.30
T_7	Fosetyl AL + Carbendazim	0.3% (1:1)	10.50	14.70
T_8	Control		6.84	11.96
F test			Sig	Sig
SE(m)±			0.240	0.236
CD (P=0.01)			1.067	1.047

(*=Average of five)

Table 4: Effect of seed treatments on shoot dry weight (g)

Treatments	Fungicide	Concentration	Shoot dry weight (g)*	
			30 DAS	60 DAS
T_1	Thiram	0.3%	2.16	3.02
T_2	Carbendazim 50% WP	0.1%	2.68	3.56
T_3	Captan 50% WP	0.3%	2.08	3.14
T_4	Fosetyl AL 80% WP	0.2%	2.58	3.42
T_5	Thiram + Captan	0.3% (1:1)	2.26	3.18
T_6	Thiram + Carbendazim	0.3% (2:1)	3.12	4.14
T_7	Fosetyl AL + Carbendazim	0.3% (1:1)	2.84	3.84
T_8	Control		1.08	1.88
F test			sig	Sig
SE(m)±			0.126	0.140
CD (P=0.01)			0.561	0.623

(*=Average of five)

Table 5 : Effect of seed treatments on root dry weight (g)

Treatments	Fungicide	Concentration	Root dry weight (g)*	
			30 DAS	60 DAS
T_1	Thiram	0.3%	0.36	0.43
T_2	Carbendazim 50% WP	0.1%	0.41	0.53
T_3	Captan 50% WP	0.3%	0.34	0.42
T_4	Fosetyl AL 80% WP	0.2%	0.40	0.52
T_5	Thiram + Captan	0.3% (1:1)	0.39	0.48
T_6	Thiram + Carbendazim	0.3% (2:1)	0.45	0.60
T_7	Fosetyl AL + Carbendazim	0.3% (1:1)	0.42	0.54
T_8	Control		0.19	0.28
F test			Sig	Sig
SE(m)±			0.013	0.014
CD (P=0.01)			0.059	0.064

(*=Average of five)

Table 6 : Effect of seed treatments on plant dry weight

Treatments	Fungicide	Concentration	Plant dry weight (g)*	
			30 DAS	60 DAS
T_1	Thiram	0.3%	2.52	3.45
T_2	Carbendazim 50% WP	0.1%	3.09	4.09
T_3	Captan 50% WP	0.3%	2.42	3.56
T_4	Fosetyl AL 80% WP	0.2%	2.98	3.96
T_5	Thiram + Captan	0.3% (1:1)	2.65	3.65
T_6	Thiram + Carbendazim	0.3% (2:1)	3.57	4.74
T_7	Fosetyl AL + Carbendazim	0.3% (1:1)	3.26	4.36
T_8	Control		1.27	2.16
F test			Sig	Sig
SE(m)±			0.122	0.140
CD (P=0.01)			0.542	0.624

(*=Average of five)

Table 7 : Effect of seed treatments on wilting percent at 30 DAS

Treatments	Fungicide	Concentration	Percent Wilting Over control	Disease reduction per cent over control
T_1	Thiram	0.3%	39.83 (39.13)*	60.17
T_2	Carbendazim 50% WP	0.1%	32.25 (34.60)*	67.75
T_3	Captan 50% WP	0.3%	44.85 (42.04)*	55.15
T_4	Fosetyl AL 80% WP	0.2%	31.82 (34.34)*	68.18
T_5	Thiram + Captan	0.3% (1:1)	33.25 (35.21)*	66.75
T_6	Thiram + Carbendazim	0.3% (2:1)	21.86 (27.88)*	78.14
T_7	Fosetyl AL + Carbendazim	0.3% (1:1)	27.56 (31.67)*	72.44
T_8	Control		100 (90)*	0.00
F test			Sig	
SE(m)±			7.147	
CD (P=0.01)			27.790	

(*=Figures in parentheses indicates arc sin transformed value)

Nikam *et al.* (2007) proved that chemical fungicidal seed treatments Thiram followed by Carbendazim and Captan were most effective in checking the wilt incidence by 42.46, 38.10 and 33.34%, respectively as against control (100% wilting).

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