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Efficacy of Fungicides and Biocontrol Agents against *Fusarium oxysporum* f.sp. *ciceri*

Praful Kumar* and S.S. Mane

Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, MS 444104, India
**Corresponding author*

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A B S T R A C T

The present study was conducted under In vitro condition to examine effectiveness of four fungicides (*viz.* Thiram @0.3%, Carbendazim @0.1%, Captan @0.3%, fosetyl AL @0.2%) alone and in combination of these fungicides and three Biocontrol agents (*viz. Trichoderma harzianum, T. viride* and *Pseudomonas fluorescens)* against *Fusarium oxysporum* f. sp. *ciceri*. Among these fungicides Carbendazim @0.1%, Fosetyl AL @0.2%, Thiram+Carbendazim @0.3% and Fosetyl AL+Carbendazim @0.3% was found the most effective with absolute (100%) inhibition of mycelial growth of *Fusarium oxysporum* f.sp. *ciceri*. Whereas, Captan @0.3% was less effective with 80.82% and 82.59% inhibition of mycelial growth respectively at 5 DAI and 7 DAI. Among three biocontrol agents, *Trichoderma harzianum* show highest per cent inhibition of mycelial growth (66.67% and 72.96%) respectively at 5 DAI and 7 DAI.

Introduction

Chickpea (*Cicer arietinum* L.) is an important pulse crop and it is used as a big source of protein in the human diet. Chickpea was originated from West Asia and now cultivated in 55 countries of the world. 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).

Annual yield losses in chickpea were estimated to be 4.8 million tones worldwide due to biotic stresses, including infectious plant diseases (Ryan, 1997).

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceri* (Padwick) Matuo and K. Sato, is considered most serious and widespread disease of chickpea throughout the chickpea growing areas of the world (Jalali and Chand, 1992). The wilt infection can damage the crop completely and cause 100% yield loss under severe conditions (Navas-Cortes *et al.*, 2000; Halila and Strange, 1996).

The chickpea wilt fungus *Fusarium oxysporum* f. sp. *ciceris* is a vascular pathogen. 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 spores of fungus enter in the plants passing through the roots. When the spores reach in vascular system they produce certain enzymes that digest the cell walls and obstruct 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 (Brayford, 1998; 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 (Haware, 1990).

Several measures such as use of resistant cultivars, cultural practices, use of chemicals and bio-control agents, are taken by growers but control is not satisfactory. Although each of these methods of disease management practices has their own importance, yet none is completely successful when applied alone for disease control (Chandel and Deepika, 2010). Despite many attempts to control chickpea wilt fungus, the problem is still important throughout the world. The chemical control based on the use of fungicides is most effective and reliable method. Biological control is a potential alternative to chemical fungicides (Parker *et al.,* 1985). The uses of *Trichoderma* as a bioagent have attracted attention because of its effectiveness against various plant pathogens and for its growth promoting action (Harman *et al.,* 2004). The *Trichoderma* species evaluated against the wilt pathogen exhibited great potential in managing chickpea wilt under glass house and field conditions (Dubey *et al.,* 2007). Selected isolates of Pseudomonas fluorescens were found to be effective in reducing the wilt incidence and increasing the plant growth as well as grain yield of chickpea (Liu *et al.,* 2007).

Materials and Methods

The studies were carried out under In vitro conditions. 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 glass wares such as Petri plates, pipettes, beakers and test tubes were sterilized in hot air oven at 180^0C 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 (M.S). 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 maintenance 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 appeared as white cottony growth on PDA medium which became felted and wrinkled in old culture colonies. By microscopically their morphological characteristics such as abundance of micro and fewer macro conidia were analysed. Microconidia were oval to cylindrical, straight to curved and measured 2.5-3.5 x 5-11µm 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. Obtained culture was stored in refrigerator at 4^{0}C and were sub cultured periodically once in a month.

Evaluation of fungicides against *Fusarium oxysporum* f.sp. *ciceri*

To evaluate the effect of fungicides against *Fusarium oxysporum* f.sp *ciceri* "Poison Food Technique" was used. The requisite amount of each fungicide based on active ingradient was added to an autoclaved potato dextrose agar to obtain the desired concentrations of all fungicides. The same medium without the fungicide served as control. The medium was poured into 90mm Petri plates in 3 replicates and after solidification, each plate was inoculated with a 5mm mycelial disc of test fungus. The inoculated Petri plates were incubated at 27±2^{0}C. After incubation, radial growth was measured at 5 DAI and 7 DAI. Per cent growth inhibition was calculated by applying the following formula (Vincent, 1947).

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

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

Evaluation of biocontrol agents against *Fusarium oxysporum* f. sp. *ciceri*

The studies on antagonism between *F. oxysporum* f. sp. *ciceri* and the fungal antagonists *viz*., *Trichoderma viride*, *T. harzinaum* were carried out by applying 'Direct Bit Placement Method' (Brodbent *et al.*, 1971). Solidified PDA medium in the plates was inoculated by placing the discs (5 mm diameter) of biocontrol agents 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. Antagonism between *F. oxysporum* f. sp. *ciceri* and *Pseudomonas fluorescens was* carried out by dual culture method (Dubey *et al.*, 2015). Nutrient agar medium (15 ml) was poured in each Petri dish (90 mm). 7 days old inoculum (5 mm disc) of *F. oxysporum* f. sp. *ciceri* was placed in the centre and 3 days old inoculum of bacteria were streaks at both sides of the inoculum of the pathogen. The control Petri dishes were inoculated only with the pathogen (Dubey *et al.*, 2015). Plates were incubated at 27±2^{0}C. The growth of *Fusarium oxysporum* f.sp *ciceri* was measured and the per cent growth inhibition was calculated as per following the formula (Vincent, 1947).

$$\text{Per cent growth Inhibition} = \frac{X - Y}{X} \times 100$$

Where,
X= the mycelial growth of the pathogen (*F. oxysporum f. sp. ciceri*) in the absence of antagonist.

Y = the mycelial growth of the pathogen (*F.*

oxysporum f. sp. ciceri) in the presence of the antagonist.

Results and Discussion

Evaluation of fungicides against *Fusarium oxysporum* f.sp. *ciceri*

Based on *in vitro* efficacy against *Fusarium oxysporum* f. sp. *ciceri*, the result (Table 1) concluded that among all the 4 fungicides alone and their combination, Carbendazim, Fosetyl AL, Thiram+Carbendazim and Fosetyl AL+Carbendazim at given concentration were found to be significantly superior over control in inhibition (100%) the radial growth and sporulation of *Fusarium oxysporum* f. sp. *ciceri* at 5 DAI and 7 DAI. This results are in conformity with Dubey *et al.,* (2015) found the fungicide Bavistin (Carbendazim) inhibited 100% mycelium growth of *Fusarium oxysporum* f.sp *ciceri*, Maitlo *et al.,* (2014) reported Aliette (Fosetyl AL) fungicide was effective against Foc, Poddar *et al.,* (2004) reported that the use of systemic fungicide Carbendazim was effective against *Fusarium oxysporum* in chickpea.

Table.1 Effect of fungicides on growth of *Fusarium oxysporum* f.sp. *ciceri*

Treatments	Fungicides	Concentration	5 Days after incubation		7 Days after incubation	
			Mean colony diameter (mm)	Per cent growth inhibition	Mean colony diameter (mm)	Per cent growth inhibition
T_1	Thiram	0.3%	11.17	83.92 (66.36)*	12.83	85.74 (67.81)*
T_2	Carbendazim 50% WP	0.1%	0.00	100 (90.00)*	0.00	100 (90.00)*
T_3	Captan 50% WP	0.3%	13.33	80.82 (64.03)*	15.67	82.59 (65.34)*
T_4	Fosetyl AL 80% WP	0.2%	0.00	100 (90.00)*	0.00	100 (90.00)*
T_5	Thiram + Captan	0.3% (1:1)	11.00	84.17 (66.55)*	11.67	87.03 (68.89)*
T_6	Thiram + Carbendazim	0.3% (2:1)	0.00	100 (90.00)*	0.00	100 (90.00)*
T_7	Fosetyl AL + Carbendazim	0.3% (1:1)	0.00	100 (90.00)*	0.00	100 (90.00)*
T_8	Control		69.50	0.00	90.00	0.00
F test				Sig		Sig
SE(m)±				**0.134**		**0.086**
CD (P=0.01)				**0.705**		**0.452**

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

Table.2 Antagonistic effect of bioagents on growth of *Fusarium oxysporum* f.sp. *ciceri*

Treatments	Bioagents	5 Days after incubation		7 Days after incubation	
		Mean colony diameter (mm)	Per cent growth inhibition	Mean colony diameter (mm)	Per cent growth inhibition
T_1	*Trichoderma viride*	24.67	64.50 (53.43)*	25.33	71.85 (57.96)*
T_2	*Trichoderma harzianum*	23.17	66.60 (54.70)*	24.33	72.96 (58.67)*
T_3	*Pseudomonas fluorescens*	31.33	54.90 (47.81)*	37.67	58.15 (49.69)*
T_4	Control	69.50	0.00	90	0.00
F test			Sig		Sig
SE(m)±			**0.130**		**0.092**
CD (P=0.01)			**1.398**		**0.992**

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

Evaluation of biological agents against *Fusarium oxysporum* f. sp. *ciceri*

Based on *In vitro* efficacy, the result (Table 2) indicated that among the 3 biocontrol agents, *Trichoderma harzianum* show highest per cent mycelial growth inhibition (66.67% and 72.96%) respectively at 5 DAI and 7 DAI.

The antagonistic effect can be attributed to diffusible substances (antibiosis) secreted by the antagonist or due to their direct effect on the target pathogen. Effectiveness of *Trihoderma harzianium* against *Fusarium oxysporum* f. sp. *ciceri* has also been reported by Yadav *et al.,* (2012) who established that *T. harzianum* was most effective against *Fusarium oxysporum* f. sp. *ciceri* (71.36%).

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