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Evaluation of Fungicides, Botanicals and *Trichoderma* spp. against Wilt of Chickpea Caused by *Fusarium oxysporum* f. sp. *ciceri*

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A B S T R A C T

All the fungicides at different concentrations were significantly inhibitory to the fungal growth as compared to control. The cent percent growth inhibition was recorded in carbendazim and Propiconazole at 100 to 1000 ppm). The next effective fungicide was Metalaxyl (500 to1000ppm), benlate (750 and 1000ppm) which were also inhibited cent percent growth of fungus. *Trichoderma harzianum* isolate 1 (Jammu) showed strong antagonistic effect, followed by *T. harzianum* isolate 3 (Kathua). The phytoextracts screened *in vitro* by poisoned food technique against *Fusarium oxysporum* f. sp. *ciceri* revealed that *Azadirachta indica* leaves extract showed maximum growth inhibition of fungus followed by *Allium sativum*.

Introduction

Chickpea (*Cicer arietinum*) is one of the most important pulse crop cultivated and consumed in India. In India chickpea accounts for about 45% of total pulses produced in the country. Crop duration is about 90 to 120 days. Chickpea is the third most important pulse crop after dry bean and peas produced in the world. India is the largest producer, with about 8 million tonnes accounting of about 70% of total world production (AICRP chickpea). Among the soil borne diseases of chickpea, wilt diseased caused by *Fusarium oxysporum* f. sp. *ciceri* is an important soil borne disease in Jammu division. Considering seriousness of the disease, the present investigation was carried out.

Materials and Methods

The required quantities of each test fungicides were put in the conical flask containing 100ml molten PDA medium so as to get required concentration in ppm. The flask containing poisoned medium was well shaken to

facilitate uniform mixture of fungicides and 20ml was poured in each sterilized petriplates. Further standard procedure was adopted on % growth inhibition of pathogen. Similar procedure was adopted for testing bioagents. Healthy fresh plant parts i.e., leaves/ bulbs/rhizomes were taken washed thoroughly with fresh water and finally rinsed with sterilized distilled water. Fifty gram of plant parts was grinded in a mixture by adding 100ml acetone, filtered through double-layers muslin cloth and tested as per the above techniques. The studies were carried out under *in vitro* conditions. All the isolation and inoculation work was 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/conditions. The glass wares such as petriplates, beakers and test tubes were sterilized in hot air oven at 180^0C for 1 hour and media were sterilized in autoclave at 121.6lbs/inch^2 for 15 minutes.

Isolation of *Fusarium oxysporum* f. sp. *ciceri*

Chickpea plant showing typical wilt symptoms were collected from the farmer's field of Rayean village from Samba district. 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 2.5mm with sterilized blade. These bits were then surface sterilized with 0.1 percent mercury chloride. Each bit was blot dried and four bits placed on the each prepoured solidified potato dextrose agar (PDA) plated. These plates were then incubated at 27^0C for seven days. The fungal growth was transferred to the plates of PDA

Purification, identification and maintenance of pathogen

F. o. f. sp. *ciceri* culture isolated from the 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 characters such as abundance of micro and fewer macro conidia were analysed. Microconidia were oval to cylindrical, straight to curved and measured 2.5-3.5x 5-11um and were poured on short, unbranched monophialides. Macroconidia borne on branched conidiophores, were thin walled, 3.5-4.5 x 2.5- 6.5 um (Trivedi and Rathi, 2015).

The pathogen was subculture on PDA slants and allowed to grow at 27^0C temperature for 10 days. Obtained culture was stored in refrigerator at 40C and were sub cultured periodically once in a month.

Results and Discussion

Results revealed that all the systemic fungicides were capable of inhibiting the growth of the test fungus at different concentrations as compared to check. Carbendazim and Propiconazole proved be the most effective inhibiting cent percent growth of the test fungus at all the concentrations (100, 250, 500, 750 and 1000ppm followed by Metalaxyl (500, 750 and 1000ppm) and benlate (750 and 1000ppm).

Table.1 Evaluation of fungicides against *Fusarium oxysporum* f. sp. ciceri at different concentrations

Systemic Fungicides (PPM)	**Growth Inhibition (%)**	**Non systemic Fungicides (PPM)**	**Growth Inhibition (%)**	**Mix fungicides (PPM)**	**Growth inhibition (%)**
Metalaxyl		**Mancozeb (Diathane M-45 75WP)**		**Carboxin + Thiram (Vitavax power 75WP)**	
100	92.1(74.24)	500	32.1 (34.85)	100	52.2 (46.55)
250	93.3 (78.21)	1000	59.4(50.71)	250	88.3 (70.46)
500	100.0 (88.15)	1500	65.5 (54.39)	500	91.7 (73.80)
750	100.0 (88.15)	2000	77.2 (61.84)	1000	100.0 (88.15)
1000	100.0 (88.15)	2500	82.7 (69.81)	1500	100.0 (88.15)
Carbendazim (Bavistin 50WP)		**Carboxin (Kavach 75 WP)**		**Carbendazim(12WP) +Mancozeb (63WP)**	
100	100.0 (88.15)	500	33.2 (35.51)	100	32.5 (35.08)
250	100.0 (88.15)	1000	46.5 (43.27)	250	100.0 (88.15)
500	100.0 (88.15)	1500	52.4 (46.67)	500	100.0 (88.15)
750	100.0 (88.15)	2000	53.3 (47.18)	1000	100.0 (88.15)
1000	100.0 (88.15)	2500	52.7(46.86)	1500	100.0 (88.15)
Propiconazole (Tilt)		**Thiram**		**Metalaxyl (8WP) + Mancozeb (64WP)**	
100	100.0 (88.15)	500	66.0 (54.61)	100	21.3 (27.82)
250	100.0 (88.15)	1000	80.1 (63.88)	250	31.4 (34.40)
500	100.0 (88.15)	1500	88.2 (70.35)	500	38.6 (38.71)
750	100.0 (88.15)	2000	88.4 (70.52)	1000	65.0 (53.01)
1000	100.0 (88.15)	2500	88.70 (70.79)	1500	66.4 (54.88)
Benlate		**Captan (Captaf 75WP)**		Thiram +Mancozeb	
250	90.6 (72.68)	500	66.0 (54.66)	250	93.0 (75.20)
500	82.7 (65.78)	700	94.3 (76.92)	1500	100.0 (88.15)
750	100.0 (88.15)	1000	100.0 (88.15)	1000	100.0 (88.15)
1000	100.0 (88.15)				
Treatment	Conc. TXC	Treatment	Conc. T&C	Treatment	Conc. TxC
S.E.± 0.08	0.09 0.17	0.08	0.09 0.19	0.20	0.26 0.45
C.D.(=0.05) 0.21	0.24 0.49	0.24	0.27 0.53	0.58	0.75 1.30

*Average of three replication; Figures in parenthesis are arc sine transformed values.

Table.2 *In vitro* efficacy of plant extracts against *Fusarium oxysporum* f. sp. *cicero*

Plant extracts	Percent inhibition of mycelial growth at different concentration (percent)			Mean
	5%	10%	15%	
Azadirachta indica (Neem)	35.64 (36.65)	46.90 (43.22)	58.15 (49.69)	56.33
Daturastramonium(Daturas)	25.40 (30.26)	33.51 (33.37)	41.61 (40.17)	42.22
*Allium sativum (*Garlic)	30.17 (33.32)	40.21 (39.35)	50.24 (45.14)	48.18
*Ocimumtenuiflorum*Tulsi)	21.54 (27.65)	25.90 (30.59)	30.25 (33.37)	35.16
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	
SEm±	0.59	0.56	0.66	
CD(P=0.05)	1.83	1.73	2.04	

*Average of four replications**Figures given in parenthesis are angular transformed values.

Table.3 Antagonistic efficacy of *Trichoderma* spp. against wilt of chickpea under *in vitro* condition

Antagonists	Growth inhibition (%)
Trichodermaharzianum isolate 1(Jammu)	81.42 (64.82)
Trichodermaharzianum isolate 2 (Samba)	72.3 (58.56)
Trichodermaharzianum isolate 3 (Kathua)	77.4 (61.94)
Trichodermaviride isolate 1 (Jammu)	74.0 (59.64)
Trichodermaviride isolate 2 (Samba)	67.6 (55.65)
Trichodermaviride isolate 3 (Kathua)	63.8 (53.31)
Trichodermavirens isolate 1 (Jammu)	66.0 (54.66)
Trichodermavirens isolate 2 (Samba)	70.7(57.53)
Trichodermavirens isolate 3 (Kathua)	71.1 (57.78)
Control	0.0 (4.05)
S.E.m±	0.29
C.D. (P=0.05)	0.83

*Average of three replication ** Figures in parenthesis are arc sine transformed values.

Carbendazim and Mancozeb at 250, 500, 1000 and 1500ppm concentrations were found most effective. Gupta *et al.*, (2014) found that carbendazim were most effective at higher doses against fungus *in vitro*. Singh and Singh (2006) found that carbendazim and mancozeb completely inhibited the fungal growth *in vitro* at higher. Carboxin and thiram at 1000, 1500ppm were cent percent effective. Thiram + Mancozeb at 1500 and 1000ppm showed effective inhibitory effects. Among the non-systemic fungicides, Captan proved to be the most effective in inhibiting cent percentmycelial growth at 1000, 2000 and 2500ppm concentrations followed by Thiram at 2500ppm (88.70%) inhibition. Similarly Nikam *et al.*, (2007) reported that Thiram (0.15%) + carbendazim (0.1%) is proved to be the most effective against *Fusarium oxysporium* f. sp. *ciceri*as shown in table 1.

In vitro effect of Plant extract against *Fusarium oxysporum* f. sp. ciceri

Effect of plant extracts was tested at 5, 10, and 15 percent concentration against inhibition of mycelial growth of *Fusarium oxysporum* f. sp. *ciceri* by poison food technique. The *Azadirachta indica* leaf extract was found significantly superior in inhibiting the mycelial growth (56.33%), followed by *Allium sativum* love extract (48.18%) and

Daturastra monium (42.22). *Ocimum tenuiflorum* leaf extract was found least effective (35.16%) against inhibition of mycelial growth of the fungus. As the concentration of plant extracts increased, the inhibition of test fungus decreases (Table 2). Similarly Kumar *et al.*, (2012) evaluated 17 plant extracts and 7 completely inhibited the mycelial growth in vitro. Similar results were found by B. D. S. Nathawat and Mahindra (Pratap (2014). Sahani and Saxena (2008) reported that the seeds treated or soaked in *Azadirachta indica* (seed) extracts were the most effective for *Fusarium oxysporum* and significantly increased seed germination.

In vitro efficacy of *Trichoderma* spp against wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri*

Out of nine antagonists tested, *Trichoderma harzianum* isolate 1 (Jammu) showed significantly maximum growth inhibition 81.42% followed by *T. harzianum* isolate 3 (Kathua) as 77.4% growth inhibition as compared to control (Table 3). Mishra *et al.*, 2012 also reported almost similar result on groundnut. *Trichoderma viride* isolate 1 (Jammu) showed 74.0% inhibition, *Trichoderma virens* isolate 3 (Kathua) inhibited 71.1% test fungus.

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