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Evaluation of fungicides and biocontrol agents *in vitro* against *Fusarium oxysporum* f. sp. *ciceri* scausing wilt in Chickpea

Kamlesh Charpota, RN Bunker, Abhinav and Nitisha Gahlot

Abstract
Seven fungicides and four biocontrol agents were evaluated *In vitro* for their efficacy against virulent isolate (BDN Foc-04) of *Fusarium oxysporum* f. sp. *ciceris* causing wilt in chickpea. Carbendazim 50% wp was found the most effective with completely (100 per cent) inhibition the mycelial growth of *F. oxysporum* at all concentrations (100, 500 and 1000 ppm) followed by Saaf (Carbendazim 12% + Mancozeb 63% WP) was found effective with 70.00, 78.67 and 84.89 percent mycelial growth inhibition at 100, 500 and 1000 ppm respectively. Among all tested antagonist *viz Trichoderma viride* (*T.v.-5*), *T. harzianum* (JH. h. 89-2), *T. aureoviride* (DG.a.91-5), and *T. viride* (PCI- Banglore) by dule culture method *Trichoderma viride* (*T.v.-5*), found most effective with 73.6 percent mycelia growth inhibition.

Keywords: BDN Foc-4, Bio-control agents, Chickpea wilt, Fungicides and *Fusarium oxysporum* f. sp. *ciceri*

Introduction
Chickpea is an important winter legume crop of Rajasthan, grown principally both in irrigated and rain-fed areas. Its cultivation is often subjected to several biotic stresses of which diseases like Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceris*, Ascochyta blight, Botrytis grey mould, Alternaria blight, Powdery mildew and Dry root rot. *Fusarium oxysporum* f. sp. *ciceris* has assumed as one of the most important limiting factors in successful cultivation of chickpea (Nene *et al*., 1984) [9]. The pathogen causes a significant reduction in yield 10 -60 per cent every year in different regions (Singh and Dahiya, 1973; Jalali and Chand**,** 1992) [11, 5]. Since, *F. oxysporum* f. sp. *ciceris* become a noxious soil borne pathogen and it is difficult to manage through conventional method. The application of fungicides though effective but is un-economical and unfriendly for environment. So, use of alternative methods like bio-agents and organic amendments integration with fungicides may be more appropriate to manage such soil borne diseases.

Materials and Methods
Isolation and Purification of chickpea wilt pathogen
Fresh chickpea wilted plants root showing wilt symptoms were collected from various locations of southern Rajasthan and use for isolation the pathogen. The diseased portion of the plant was cut into small bits. The bits were superficial sterilized with 0.1 per cent $HgCl_2$ (mercuric chloride) solution for 30 to 45 seconds followed by three washing with sterilized distilled water and then transported aseptically under laminar air flow cabinet on sterilized Petri plates comprising 20 ml PDA (potato dextrose agar) medium and incubated at (27±2ºC). When the fungal growths progression from the diseased tissues, it was sub-cultured aseptically on potato dextrose slants. The pure culture was identified by their morphological and cultural characteristics such as myceliam growth, size and shape ofmacro & micro-conidia were studied under low (10X) and higher (40X) power amplification from 10 days old culture of *Fusarium oxysporum* f. sp. *ciceris* and were acknowledged by using the key of (Burnett and Hunter, 2003; Leslie and Summerell, 2006) [3, 8].

Evaluation of fungicides against *Fusarium oxysporum* f. sp. *ciceris*
Seven fungicides *viz*., Thiram 75 WP, Carbendazim 12% WP + Mancozeb 63% WP, Copper oxychloride 50 WP, Tebuconazole 25 EC, Azoxistrobin 23% WP, Carbendazim 50 WP, and Hexaconazole 5 SC were tested at three concentrations *i.e.* 100, 500 and 1000 ppm for their

efficacy against the most virulent isolate of *F. oxysporum* f. sp. *ciceris* (BDN Foc-04) using poisoned food technique.

Twenty (20) ml of warm melted potato dextrose medium was poured aseptically in sterilized Petri plates and allowed to solidify. The Petri plates were inoculated with 5 mm diameter mycelial disc, cut from the periphery of 10 days old (*F. oxysporum*) fungus cultures. The mycelial disc was placed in the center of the plates in an inverted portion to make a direct contact with the poisoned medium and incubated at 27±2°C for seven days. In each treatment four replications were maintained in Complete Randomize Design. Same time control Petri plate was also maintained by growing the fungus on chemical free PDA. Observations on linear growth were recorded after seven day of inoculation.

The per cent growth inhibition of the fungus in each treatment was calculated by using the following formula given by Vincent (1947) [13].

$$I = [(C - T)/C] \times 100$$

Where,
I = Per cent inhibition
C = Growth of test fungus in control (mm)
T = Growth of test fungus in respective treatment (mm)

Evaluation of bio-agents against *Fusarium oxysporum* f. sp. *ciceris*

The dual culture test was carried out to determine the antagonistic activity of known four species of bio-agent Trichoderma *viz. Trichoderma viride-T.v.5* procured from (Biopesticide laboratory, RCA, MPUAT, Udaipur), *T. harzianum* (Tabiji- Ajmer), *T. aureoviride* (DG. a 91-5), and *T. viride* (PCI- Bangalore) against wilt pathogen *F oxysporum* f. sp. *ciceris.*

Twenty (20) ml PDA media was poured aseptically in each petri plates and allowed to solidify. Five mm diameter mycelial disc of each antagonist and test isolate **(**BDN Foc-04) of *F oxysporum* f. sp. *ciceris* were placed on solid media in the same Petri plates approximately four cm away from each other. Simultaneously a suitable control was also maintained by growing the pathogenon antagonist free PDA. In each treatment four replications were maintained. All the inoculated and uninoculated plates were incubated at 27±2°C and observed after seven days. Antagonistic activity of bio-control agent was calculated by measuring the growth of test pathogenin dual culture and in control plate.

Per cent growth inhibition of pathogen and index of antagonism were determined in each treatment by following standard formula (Vincent, 1947) [13].

$$I = [(C - T)/C] \times 100$$

Where,
I = Per cent growth inhibition zone of pathogen
C = Growth of test fungus in control (mm)
T = Growth of test fungus in respective treatment (mm)

Results and Discussion

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

The results of in *In-vitro* study revealed that all the tested fungicides significantly inhibited the mycelial growth of *F. oxysporum* f. sp. *ciceris* at all concentration. Among tested fungicides Carbendazim 50 WP was found the most effective with completely (100%) inhibition the mycelial growth of *F. oxysporum* f.sp. *ciceris* at all concentrations 100, 500 and 1000 PPM, respectively. Followed by Saaf (Carbendazim 12% + (Mancozeb 63% WP) was found effective with percent mycelial growth inhibition of 70.0%, 78.67% and 84.89%at 100, 500 and 1000 PPM concentrations, respectively.

However, Tebuconazole 25 EC showed Per cent mycelial growth inhibition 61.78, 72.22 and 77.33, respectively which followed by Hexaconazole 5 SC showed 54.67, 64.00 and 72.00 per cent growth inhibition at 100, 500 and 1000 PPM concentration, respectively.

Thiram 75 WP showed 46.67, 54.89 and 63.56 per cent growth inhibition at 100, 500 and 1000 PPM concentration, respectively and Per cent growth inhibition 40.89, 47.11, 54.67 at 100, 500 and 1000 PPM was recorded in Azoxystrobin 23 WP. Whereas, least Per cent mycelial growth inhibition 26.44, 39.11 and 55.33 was recorded in Copper oxychloride 50 WP (table1 and plate1). Gadhave *et al*. (2020) [4] conducted *in-vitro* study and concluded that Carbendazim 50% WP, Copper oxychloride 50WP and Carbendazim 25% + Mancozeb 50% WS were found most effective with maximum growth inhibition 100%, 65.22% and 100%, respectively. Patra and Biswas (2017) [10] reported Carbendazim was the most superior with 100% fungal growth inhibition at 1000 and 1500 ppm out of 10 fungicides in *In-vitro* as well as in field condition exhibited minimum 9.66% wilt incidence of chickpea compare combination of fungicides (Tebuconazole + Trifloxystrobin) with 10.12% disease incidence for the management of chickpea wilt pathogen *F. oxysporum* f. sp. *ciceris.*

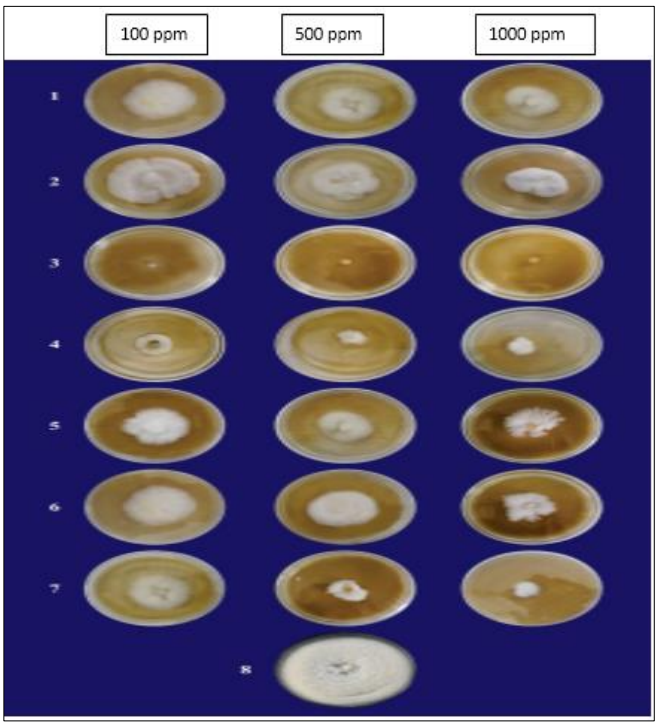

Plate 1: *In-vitro* evaluation of selected fungicides at different concentration against *Fusarium oxysporum* f. sp. *ciceris* isolate BDNFoc-04

1. Thiram 75 WP
2. Copper oxychloride 50 WP
3. Carbendazim 50 WP
4. SAAF 75WP
5. Hexaconazole 5 SC
6. Azoxistrobin 23 WP
7. Tebuconazole 25 EC
8. Control

Evaluation of bio-agents against *Fusarium oxysporum* f.sp. *ciceris*: Efficacy of four bio-control agents *viz*; *Trichoderma viride (T.v-5), Trichoderma harzianum, Trichoderma aureoviride* and *Trichoderma viride* were studied *in-vitro* against *Fusarium oxysporum* f. sp. *ciceris* isolate (BDNFoc-04). All the four bio-agents were significantly inhibited the mycelial growth of *F. oxysporum* f. sp. *ciceris* isolate. Severe antagonism and significant higher Percent mycelium growth inhibition of *F. oxysporum* f.sp. *ciceris*73.56% was recorded in *T. viride-T.v.5* in dual culture method which was followed by *T. harzianum* and *T. viride* showed moderate antagonism with 62.22 and 58.22 per cent growth inhibition, respectively. The least mycelial growth inhibition was recorded in *T. aureoviride* which was 41.11 per cent (table 2, plate 2).

Plate 2: *In-vitro* evaluation of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *ciceris*

Rehman *et al.* (2013) recorded maximum Per cent growth inhibition was recorded against *F. oxysporum* f.sp. *ciceris* in *T. harzianum* and *T. viride* 81.00 and 83.33, respectively. Jambhulkar *et al.* (2011) [6] studied efficacy of bio-control agents against chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceris* and concluded that *Trichoderma viride* reduced disease incidence between 56 to 61 per cent. Keote *et al.* (2019) [7] conducted an experiment by using bio-agents against chick pea wilt under *in-vitro* conditions. Under Dual culture, bio-agents like *Trichoderma viride*, *Bacillus subtilis* and *Pseudomonas fluorescens* showed significant results to suppress the pathogen.

Table 1: Comparative efficacy of different fungicides on the growth of *Fusarium oxysporum* f.sp. *ciceris* isolate (BDN Foc-04) under *in-vitro.*

Treatments	Name of the Fungicide	Different concentrations(ppm)					
		100		500		1000	
		Colony Diameter (mm)*	Per Cent Growth inhibition*	Colony Diameter (mm)*	Per Cent Growth inhibition*	Colony Diameter (mm)*	Per Cent Growth inhibition*
T_1	Thiram 75 WP	48.00 (43.83)	46.67 (43.07)	40.60 (39.56)	54.89 (47.79)	32.80 (34.91)	63.56 (52.85)
T_2	Copper oxychloride 50 WP	66.20 (54.44)	26.44 (30.90)	54.80 (47.74)	39.11 (38.69)	40.20 (39.33)	55.33 (48.04)
T_3	Carbendazim 50 WP	5.0 (12.92)	94.44 (76.33)	5.0 (12.92)	94.44 (76.33)	5.0 (12.92)	94.44 (76.33)
T_4	SAAF 75WP (Carbendazim 12% + (Mancozeb 63% WP)	27.00 (31.28)	70.00 (56.78)	19.20 (25.97)	78.67 (62.47)	13.60 (21.62)	84.89 (67.11)
T_5	Hexaconazole 5 SC	40.80 (39.68)	54.67 (47.66)	32.40 (34.67)	64.00 (53.12)	25.20 (30.11)	72.00 (58.04)
T_6	Azoxistrobin 23 WP	48.00 (43.83)	46.67 (43.07)	40.60 (39.56)	54.89 (47.79)	32.80 (34.91)	63.56 (52.86)
T_7	Tebuconazole 25 EC	34.40 (35.89)	61.78 (51.79)	25.00 (29.98)	72.22 (58.18)	20.40 (26.83)	77.33 (61.55)

T_8	Control	90.00 (71.54)	0.00	90.00 (71.54)	0.00	90.00 (71.54)	0.00
S.Em±		0.452	0.509	0.423	0.463	0.445	0.480
CD (P=0.05)		1.317	1.484	1.232	1.349	1.294	1.399

*Mean of four replications; Figures in parentheses are arcsine √ per cent angular transformed values.

Table 2: Per cent growth inhibition of *Fusarium oxysporum* f. sp. *ciceris* isolate BDNFoc-04 by *Trichoderma* sp. under *in-vitro*

Treatments	Bio-control agent	Mycelial growth (mm) *	Per cent growth Inhibition *	Antagonism Index**
T1	*Trichoderma viride-5*	23.8 (29.14)	73.56 (59.07)	+ + + +
T2	*Trichoderma harzianum*	34.0 (35.65)	62.22 (52.05)	+ + +
T3	*Trichoderma aureoviride*	53.0 (46.70)	41.11 (39.86)	+ +
T4	*Trichoderma viride*	37.6 (37.80)	58.22 (49.71)	+ + +
T5	Control	90.0 (71.5)	0.00	--
S.Em±		0.561	0.607	
CD (p=0.05)		1.667	1.803	

*Average of four replications. Values in the parentheses are arcsine √ per cent angular transformed values.
** Antagonism index (Bell *et al*., 1982)
+ + + + = Severe antagonism
+++ = Moderate antagonism
++ = Weak antagonism
-- = No antagonism

Conclusion

In view of rising wilt incidence in chickpea growing areas, efforts were made to evaluate seven (systemic and non-systemic) fungicides *viz.*, Thiram 75WP,Carbendazim50 WP, SAAF 50WP, Hexaconazole 5 SC, Azoxystrobin 23 WP and Tebuconazole 25 EC and Copper oxychloride 50 WPat 100, 500 and 1000 ppm concentrations against *F. oxysporum* f. sp. *ciceris* under *in-vitro.* Current experiment was conducted to unveil the efficacy of fungicides and bio-control agents against growth inhibition of pathogen. The findings and conclusions resulted from the study are here as follows. Efficiency of Carbendazim 50 WP, Saaf50 WP and Tebuconazole 25 EC was found more pronounced against *F. oxysporum* f.sp. *ciceris*, whereas Carbendazim50 WP was highly effective in controlling the mycelial growth of *F. oxysporum* f.sp. *ciceris* at all the concentrations tested.
Comparative efficacy of four bio-control agents was tested against *F. oxysporum* f.sp. *ciceris.* The antagonism activity of *Trichoderma viride (T.v.-5)* found highly effective against *F. oxysporum* f.sp. *ciceris* under *in-vitro*.

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