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Ashutosh Tiwari
Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture & Technology, Kanpur, Uttar Pradesh, India

Ramesh Singh
Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture & Technology, Kanpur, Uttar Pradesh, India

Vallabhaneni
Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture & Technology, Kanpur, Uttar Pradesh, India

Tilak Chowdary
Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture & Technology, Kanpur, Uttar Pradesh, India

Somesh
Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture & Technology, Kanpur, Uttar Pradesh, India

Correspondence
Ashutosh Tiwari
Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture & Technology, Kanpur, Uttar Pradesh, India

In vitro evaluation of Systemic and Non-systemic fungicides against Fusarium wilt of Chickpea caused by *Fusarium oxysporum* F. sp. *ciceri* (Padw.) Snyd. and Hans

Ashutosh Tiwari, Ramesh Singh, Vallabhaneni Tilak Chowdary and Somesh

Abstract
Chickpea (*Cicer arietinum* L.) is one of the most important pulse crop, in concern of it's acreage and production and serve as a best source for protein where majority of the population depend upon it for meeting their dietary requirements.

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceri* is one of the major constraints in chickpea production causing considerable yield losses. In order to find the best chemical for management of Fusarium wilt different Systemic and Non-systemic Fungicides have been evaluated by using food poison technique in which Carbendazim 50 % WP is highly effective with an inhibition of 86.76 % at 1500 ppm concentration with radial mycelial growth of 10.16 mm when compared to the control followed by Difenconazole 25 % EC with an inhibition of 86.36 % at 1500 ppm concentration with a radial mycelial growth of 16.57 mm. Among the Non-systemic fungicide Copper oxy chloride 50 % WP was found effective in inhibiting the growth of the pathogen representing the values 77.88 %, 76.54 %, 75.80% at 2500 ppm, 2000 ppm, 1500 ppm respectively followed by Mancozeb 75% WP with a radial mycelial growth of 30.50 mm at 2500 ppm with an inhibition of 66.11 % when compared to control.in the present study Systemic fungicides were found effective as compared to Non-systemic fungicides.

Keywords: systemic, Non-systemic fungicides, fusarium, *Fusarium oxysporum*

1. Introduction
Chickpea (*Cicer arietinum* L.) commonly known as gram or Bengal gram belongs to the family Leguminosae. It is one of the third most important pulse crops in the world after dry beans (*Phaseolus vulgaris* L.) and dry peas (*Pisum sativum* L.) but of first importance in the Mediterranean basin, South Asia and India accounting 60 to 75 % of the world's Chickpea production. Chickpea is a vital source of plant derived edible protein in many countries. Chickpea also has an advantage in the management of soil fertility, particularly in dry lands and the semiarid tropics. In India it is grown as *Rabi* season pulse crop. Chickpea is contributing nearly 42-47 % of total pulse production. It is susceptible to several diseases like wilt [*Fusarium oxysporum* f. sp. *ciceri* (Padw.) Snyd. and Hans.], black root rot (*F. solani*), wet root rot (*Rhizoctonia solani*), dry root rot (*R. bataticola*), Aschochyta blight (*Aschochyta rabie)*, and color rot (*Sclerotium rolfsii*) which are of considerable importance among which Fusarium wilt is one of the major constraint contributing yield losses up to 60 per cent (Singh *et al*., 2007) [7]. Whereas, Kumar and Bourai (2012) [12] reported 72.16 per cent yield losses due to Fusarium wilt of chickpea. Fusarium wilt caused by *F. oxysporum* f. sp. *ciceri* is a soil borne (Jimenez-Fernandez *et al*., 2011) [3] and seed borne (Pande *et al*., 2007) [7] disease. Wilt pathogen colonizing the xylem vessels and blocking them completely results wilting. The fungus survives in soil in the form of Chlamydospores and mycelia (Singh *et al*., 2007) [7]. Various major which are generally used to manage the disease includes cultural, biological, chemical and use of resistant varieties. Cultural practices can minimise the severity of disease but can't completely manage the disease in standing crop. Another alternative is biological control in this context different bio-agents were used, it is noticed that due to alteration in temperature and pH bio-agents were not as effective as expected. Another alternative, use of resistant varieties become susceptible.

Hence the use of fungicides was the most prominent method of plant disease management.

2. Materials and Methods

All the experiments were conducted in the laboratory of Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture & Technology, Kanpur during 2017-2018. The statistical design used was CRD.

2.1 Collection, isolation, purification, identification and maintenance of *Fusarium oxysporum* f. sp. *ciceri*

Plants showing typical wilt symptoms were collected from IIPR (Indian Institute of Pulse Research, Kanpur). The collected samples were washed in sterilized water and the samples were then placed in a humidity chamber and were incubated at 25±2ºC in BOD, to use for further investigations. Isolation of the fungus was made by tissue isolation technique on potato dextrose agar (PDA) and incubated at 25±2ºC. The resulting fungal culture was purified by hyphal tip method. The fungus was isolated, purified and sub cultured in aseptic condition. The pathogen was identified based on colony characters and spores morphology (Booth, 1971) [2]. The pathogen was stored and maintained in PDA slants inoculated through single spore technique for further experiments.

2.2 Collection of Fungicides

Total ten fungicides (Systemic and Non-systemic) *viz.*, Carbendazim 50% WP, Hexaconazole 5% EC, Difenconazole 25 % EC, Propiconazole 25%EC, Thiophanate methyl 70 % WP, Copper oxychloride 50% WP, Zineb 75% WP, Mancozeb 75% WP, Propineb 70% WP and Chlorothalonil 75% WP were collected from Departmental store of Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture & Technology, Kanpur used for *in vitro* evaluation.

2.3 Laboratory (*in-vitro*) screening of systemic and non-systemic fungicides against the pathogen

Five Systemic fungicides *viz.* Carbendazim 50% WP, Hexaconazole 5% EC, Difenconazole 25 % EC, Propiconazole 25%EC, Thiophanate methyl 70 % WP at 500, 1000 and 1500 ppm concentration and five Non-systemic fungicide viz. Copper oxychloride 50% WP, Zineb 75% WP Mancozeb 75% WP, Propineb 70% WP, Chlorothalonil 75% WP at 1500, 2000 and 2500 ppm concentration, by employing poisoned food technique developed by Nene and Thapliyal (1993) [6], to find the inhibitory effect of different fungicides on the growth of target pathogen. The basic concept of this method is to Poisoned the medium after sterilization with fungicides to be tested at given concentration. Three replications were kept for each treatment. One set of control was maintained in which the medium was not mixed with any fungicide but simply inoculated with the pathogen. The data on radial growth of fungal colony was measured in mm. after every 24 hours till the control petri plates were not filled up. The per cent inhibition over control was calculated by the following formula as given by Schimtz (1930) [9].

$$I = \frac{C-T}{C} \times 100.$$

Where,
I= Per cent inhibition in mycelia growth
C = Growth of pathogen in control plates.
T = Growth of pathogen in dual culture plates.

3. Results and discussion

The average diameters of the fungal colonies were noted in the poured plates containing different fungicides and inhibition % was recorded in Table-1 & Table-2.

3.1 Inhibitory effect of Systemic fungicides on the growth of *Fusarium oxysporum* f. sp. *ciceri in vitro* condition.

Five Systemic fungicides viz. Carbendazim 50% WP, Hexaconazole 5% EC, Difenconazole 25 % EC, Propiconazole 25%EC, Thiophanate methyl 70 % WP were tested for their efficacy against *F. oxysporum* f. sp. *ciceri* at 500, 1000 and 1500 ppm concentration, as shown in Table-1 at 500 ppm, radial mycelial growth of the test pathogen ranged from 13.21 mm (Carbendazim) to 22.57 mm (Thiophanate methyl). Significantly least mycelial growth was recorded with the Carbendazim, (13.21 mm) followed by Difenconazole (16.57 mm), Hexaconazole (17.52 mm), Propiconazole (18.37 mm) and Thiophanate methyl (22.57 mm). At 1000 ppm Significantly least mycelial growth was recorded with the Treatment Carbendazim, (12.37 mm) followed by Difenconazole (14.50 mm), Hexaconazole (15.19 mm), Propiconazole (16.77 mm) and Thiophanate methyl (20.55 mm) and at 1500 ppm, all the Systemic fungicides tested exhibited somewhat similar trend of mycelial growth as that of at 500 ppm and 1000 ppm and ranged from 10.16 mm (Carbendazim) to 18.25 mm (Thiophanate methyl). All of them were statistically significant. Maximum inhibition of the test pathogen was recorded with Carbendazim, 86.76% at 1500 ppm followed by Difenconazole at 1500 ppm then Carbendazim at 1000 and 500 ppm Hexaconazole at 1500 ppm with 86.36%, 86.25%, 85.32% and 85.02% respectively as compared to untreated control. Andrabi *et al.* (2011) [8] also found that Carbendazim at different concentrations can completely inhibit the growth of wilt causing pathogen in chickpea. (Korde, 2011 and Ravichandran & Hedge 2015) [4, 8] also observed Carbendazim and Difenconazole as most effective fungicides for the management of Fusarium wilt of chickpea.

3.2 Inhibitory effect of Non-systemic fungicides on the growth of *Fusarium oxysporum* F. sp. *ciceri in vitro* condition

Five Non-systemic fungicides *viz*. Copper oxychloride 50% WP, Zineb 75% WP Mancozeb 75% WP, Propineb 70% WP, Chlorothalonil 75% WP at 1500, 2000 and 2500 ppm concentration were tested for their efficacy against *F. oxysporum* f. sp. *ciceri.* As shown inTable-2 at 1500 ppm, % mycelial growth inhibition ranged from 36.04 per cent (Zineb) to 75.80 per cent (Copper oxychloride). Significantly highest mycelial inhibition was recorded with the Copper oxychloride (75.80%) followed by Mancozeb (57.60%), Propineb (56.68%), and Chlorothalonil (38.93%). The fungicide Zineb was found least effective with 36.04 per cent inhibition when compared to control. At 2000 and 2500 ppm, similar trend of mycelial growth inhibition as that of at 1500 ppm was observed and it was ranged from 39.73 per cent (Zineb at 2000 ppm) to 76.54 per cent (Copper oxychloride at 2000 ppm) and 39.87 per cent (Zineb at 2500 ppm) to 77.88 per cent (Copper oxychloride at 2500 ppm), except Mancozeb (61.06%) and Propineb (61.07%) which were statistically at par with each other at 2000 ppm concentration. Among all the non- Systemic fungicides least mycelial growth was recorded in copper oxy chloride treated media with 19.90 mm, 21.11 mm, 21.78 mm at 2500 ppm, 2000 ppm, 1500 ppm respectively followed by Mancozeb 75 % WP with 30.50 mm

growh at 2500 ppm and Propineb 70% WP with 32.93 mm at 2500 ppm concentration ppm. Ravichandran and Hedge (2015) [8] also reported that Copper oxychloride has given best results at 0.3% concentration in inhibiting the growth of *F. oxysporum* f. sp. *ciceri.*

Table 1: Inhibitory effect of systemic fungicides on the growth of *Fusarium oxysporum* f. sp. *ciceri* in *in vitro* condition.

S.No.	Treatment no.	Treatments	Colony diameter at different concentration (mm)*			Inhibition %		
			500ppm Ppm	1000ppm	1500ppm	500ppm	1000ppm ppm	1500 ppm
1.	T1	Carbendazim 50% WP	13.21	12.37	10.16	85.32	86.25	86.76
2.	T2	Hexaconazole 5% EC	17.52	15.19	13.48	80.53	83.12	85.02
3.	T3	Difenconazole 25 % EC	16.57	14.50	12.27	81.58	83.88	86.36
4.	T4	Propiconazole 25% EC	18.37	16.77	14.92	79.58	81.36	83.42
5.	T5	Thiophanate methyl 70 % WP	22.57	20.55	18.25	74.92	77.16	79.72
6	Control		90	90	90	00	00	00

Table 2: Inhibitory effect of Non-systemic fungicides on the growth of *Fusarium oxysporum* F. sp. *ciceri* in *in vitro* condition.

S.no.	Treatment No.	Treatment	Colony diameter at different concentration (mm)*			Inhibition %		
			1500 ppm	2000 ppm	2500 ppm	1500 ppm	2000 ppm	2500 ppm ppm
1.	T6	Copper oxychloride 50% WP	21.78	21.11	19.90	75.80	76.54	77.88
2.	T7	Zineb 75% WP	57.56	54.24	54.10	36.04	39.73	39.87
3.	T8	Mancozeb 75% WP	38.16	35.04	30.50	57.60	61.06	66.11
4.	T9	Propineb 70% WP	38.98	35.03	32.93	56.68	61.07	63.41
5.	T10	Chlorothalonil 75% WP	54.96	47.32	39.89	38.93	47.42	55.67
6.	Control		90	90	90	-	-	-

4. Conclusion

It is evident that among Systemic and Non-systemic fungicides Systemic fungicides were found effective in inhibiting the growth of *Fusarium oxysporum* F. *sp. ciceri*, among them Carbendazim 50 % WP is highly effective with an inhibition of 86.76 % at 1500 ppm concentration when compared to the control and among the Non-systemic fungicide Copper oxy chloride 50 % WP was found effective in inhibiting the growth of the pathogen representing the values 77.88 %, 76.54 %, 75.80% at 2500 ppm, 2000 ppm, 1500 ppm respectively.

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