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In vitro efficacy of systemic fungicides against *Fusarium oxysporum* f. sp. *ciceri*, causing chickpea wilt

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Abstract
Chickpea (*Cicer arientum* L.) is the most important pulse crop cultivated in India and affected by many fungal and viral diseases and among all the diseases, wilt caused by *Fusarium oxysporum* f. sp. *ciceri* is one of the most common fungal pathogen which causes ultimate yield loss in chickpea crop. Considering economic importance of the crop as well as destructive nature of the disease, present *in vitro* studies were undertaken to evaluate the efficacy of seven systemic fungicides (each @ 500 and 1000 ppm), to assess their potential against *Fusarium oxysporum* f. sp. *ciceri*. Results revealed that, mean mycelial growth inhibition of test pathogen recorded in all systemic fungicides ranged from 51.66% to 100% as compared with control (00.00%). The highest mycelial growth inhibition was resulted with Carbendazim 50% WP, Tebuconazole 25.9% EC and Thiophanate methyl 70% WP (100%), followed by Propiconazole 25% EC (91.11%), Difenconazole 25% EC (90.00%), Hexaconazole 5% EC (79.44%) and Fosetyl Al 80% WP (51.66%).

Keywords: Chickpea, wilt, *F. oxysporum* f. sp. *ciceri*, systemic, fungicides

Introduction
Chickpea (*Cicer arietinum* L.) is one of the most important *Rabi* pulse crop in India sharing hectarage next to pigeonpea and makes up for 20% of the world pulse production. It belongs to family *Leguminoceae*. Chickpea is commonly known as bengal gram, garbanzo bean, gram, chana, chole and harbhara. It plays a vital role in the diet of poor people which serves as a major source of vegetable protein (21.1%), carbohydrates (61.5%) and fat (4.5%). It does not contain any anti-nutritional factor. It is mainly used for human consumption as well as for animal feeds. It is consumed as whole seed, dal, boiled, fried, salted or more generally which is cooked.

In India, chickpea is an important pulse crop, occupies 9.85 million hectares area, production 11.99 million tonnes and yield 1217 kg/ha. In Maharashtra, area under chickpea cultivation is 2.15 million hectares with production 2.37 million tonnes and yield 1105 Kg/ha. (Agril. Statistics At a Glance, 2021) [1]. In India chickpea accounts for about 45% of total pulses produced in the country. India is the largest producer, with about 10 million tonnes accounting of about 70% of total world production.

Various diseases affect the chickpea *viz*. *Ascochyta* blight, damping off, *Botrytis* grey mold, *Phytophthora* root rot, seed rot / *Pythium* rot and rust but wilt caused by *Fusarium oxysporum* f. sp. *ciceri* is the most serious disease. The chickpea wilt fungus, *Fusarium oxysporum* f. sp. *ciceri* is a vascular pathogen. This pathogen is soil and seed borne (Haware *et al*., 1978) [4]. Under severe conditions, the wilt disease can damage the crop completely and causes losses upto 100% (Navas-cortes *et al.*, 2000) [7]. Considering, the importance of disease in the state, the losses incurred in the farmer's field and the problem has increased in past 6-7 years with heavy economic losses. Therefore, it was felt necessary to investigate on *in vitro* efficacy of systemic fungicides against *Fusarium oxysporum* f. sp. *ciceri*, causing chickpea wilt.

Materials and Methods
The conventional and newer systemic fungicides (each @ 500 and 1000 ppm) were evaluated against the test pathogen by applying poisoned food technique (Nene and Thapliyal. 1993) [8] and using PDA as basal culture medium based on active ingredient, requisite quantity of the test fungicides was calculated, dispensed separately and mixed thoroughly with autoclaved and cooled (40^0 C) PDA medium in glass conical flasks (250 ml capacity) to obtain their desired concentrations. This PDA medium amended separately with the test fungicides was then

poured (20 ml/plate) aseptically in sterile glass petri plates (90 mm dia.) and allowed to solidify at room temperature. For each of the test fungicide and its test concentration, three replications were maintained. After solidification of the PDA medium, all these plates were inoculated aseptically by placing a 5 mm culture disc of the test fungus in the centre, obtained from actively growing 7 days old pure culture of test pathogen. Untreated PDA plates (without fungicide) inoculated separately with pure culture disc of the test fungus per treatment per replication were maintained. Both treated and untreated PDA plates were incubated in an inverted position at 27±2 °C in BOD incubator for a week.

Experimental details: Systemic fungicides (each @ 500 & 1000 ppm)
Design: Completely randomized design (CRD)
Replication: Three
Treatments: Eight

Treatments details

Tr. No	Treatment details	Tr. No	Treatment details
T_1	Carbendazim 50% WP	T_5	Hexaconazole 5% EC
T_2	Fosetyl Al 80% WP	T_6	Propiconazole 25% EC
T_3	Tebuconazole 25.9% EC	T_7	Difenconazole 25% EC
T4	Thiophanate methyl 70% WP	T_8	Control (untreated)

Observations
Observations on radial mycelial growth/colony diameter (mm) of the test fungi at an interval of 24 hrs. was recorded and continued till the untreated control plates were fully covered with mycelial growth of the test fungus. Per cent mycelial growth inhibition of the test fungi with the test fungicides, over untreated control was calculated by applying the formula given by (Arora and Updhyay, 1978) [2].

$$\text{Percent Growth Inhibition} = \frac{C - T}{C} \times 100$$

Where,
C = growth of the test fungus in untreated control plate
T = growth of the test fungus in treated plate

Results and Discussion
Seven systemic fungicides were evaluated (each @ 500 and 1000 ppm) in *in vitro* against *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt by applying poisoned food technique and using PDA as basic culture medium. The results obtained are presented (Plate 1, Table 1 and Fig. 1) and narrated here under.

Table 1: *In vitro* efficacy of systemic fungicides against *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt

Tr. No.	Treatments	Colony Diam.* (mm) At ppm		Av. (mm)	% Inhibition* At ppm		Av. Inhibition (%)
		500	1000		500	1000	
T_1	Carbendazim 50% WP	00.00	00.00	00.00	100.00 (90.00)**	100.00 (90.00)	100.00 (90.00)
T_2	Fosetyl Al 80% WP	54.16	32.83	43.50	39.81 (39.12)	63.51 (52.83)	51.66 (45.95)
T_3	Tebuconazole 25.9% EC	00.00	00.00	00.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_4	Thiophanate methyl 70% WP	00.00	00.00	00.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_5	Hexaconazole 5% EC	24.16	12.83	18.50	73.14 (58.78)	85.74 (67.81)	79.44 (63.04)
T_6	Propiconazole 25% EC	11.66	6.33	8.00	87.03 (68.89)	92.96 (74.61)	91.11 (72.65)
T_7	Difenconazole 25% EC	9.33	8.67	9.00	89.62 (71.20)	90.37 (71.92)	90.00 (71.57)
T_8	Control (untreated)	90.00	90.00	90.0	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)
	S.E. ±	0.71	0.66	-	0.78	0.74	-
	C.D. (P = 0.01)	2.14	2.01	-	2.38	2.23	-

*Mean of three replications **Figure in parenthesis are arcsine transformed values

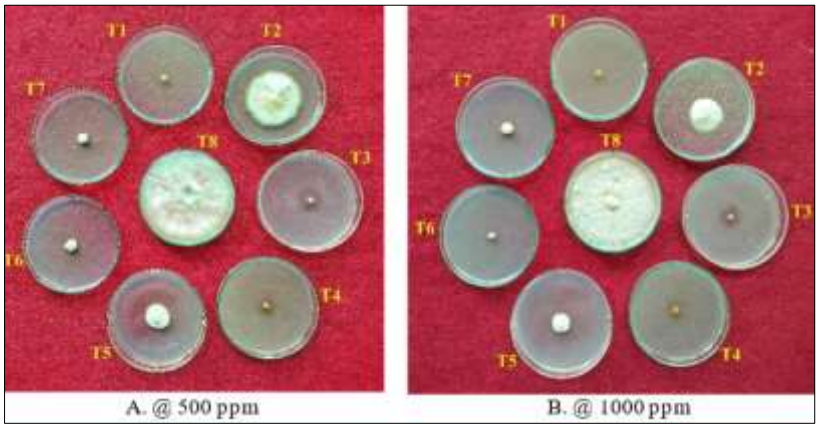

Plate 1: *In vitro* efficacy of systemic fungicides against *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt f. sp. *ciceri* causing chickpea wilt

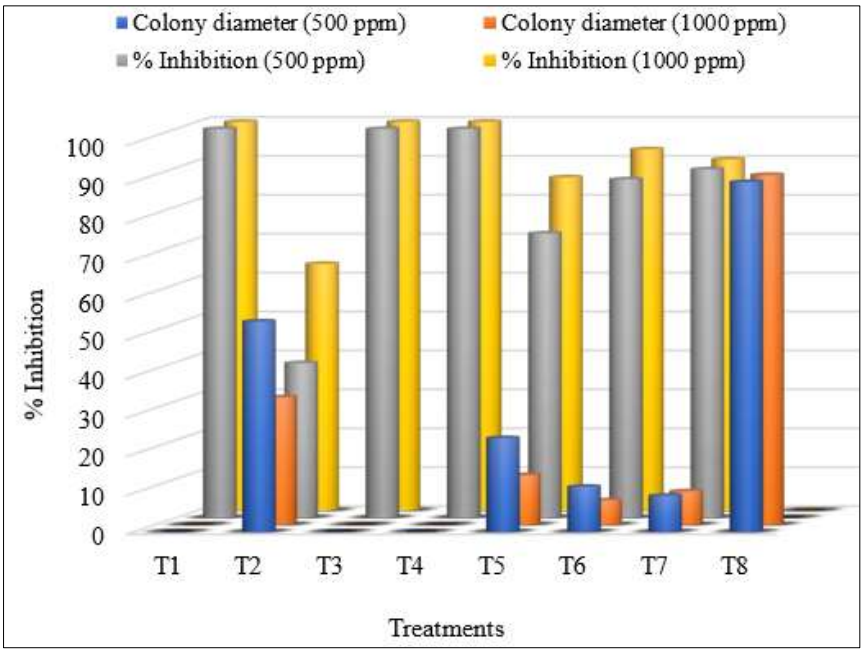

Fig 1: *In vivo* efficacy of systemic fungicides against *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt

Effect on radial mycelial growth

At 500 ppm, the radial mycelial growth of tested pathogen was recorded in the range of 0.00 mm to 54.16 mm as compared with control (90.00 mm). There was none of the mycelial growth with the fungicide tested *viz*. Carbendazim 50% WP, Tebuconazole 25.9% EC and Thiophanate methyl 70% WP. Among rest of the test fungicides Difenconazole 25% EC resulted with least mycelial growth (9.33 mm), followed by Propiconazole 25% EC (11.66 mm), Hexaconazole 5% EC (24.16 mm) and Fosetyl Al 80% WP (54.16 mm).

At 1000 ppm, the radial mycelial growth of tested pathogen was recorded in the range of 0.00 mm to 32.83 mm as compared with control (90.00 mm). There was none of the mycelial growth with the fungicide tested *viz*. Carbendazim 50% WP, Tebuconazole 25.9% EC and Thiophanate methyl 70% WP. Among rest of the test fungicides Propiconazole 25% EC resulted with least mycelial growth (6.33 mm), followed by Difenconazole 25% EC (8.67 mm), Hexaconazole 5% EC (12.83 mm) and Fosetyl Al 80% WP (32.83 mm).

Average radial mycelial growth of tested pathogen was recorded in the range of 0.00 mm to 43.50 mm as compared with control (90.00 mm). There was none of the mycelial growth with the fungicide tested *viz*. Carbendazim 50% WP, Tebuconazole 25.9% EC and Thiophanate methyl 70% WP. Among rest of the test fungicides Propiconazole 25% EC resulted with least mycelial growth (8.00 mm), followed by Difenconazole 25% EC (9.00 mm), Hexaconazole 5% EC (18.50 mm) and Fosetyl Al 80% WP (43.50 mm).

Effect on inhibition of mycelial growth

Results on inhibition of mycelial growth of *Fusarium oxysporum* f. sp. *ciceri* indicated that, all systemic fungicides tested at 500 ppm and 1000 ppm concentrations significantly inhibited mycelial growth of pathogen over control (0.00%). The per cent mycelial growth inhibition increased with increase in the concentration of fungicides.

At 500 ppm, mycelial growth inhibition of the test pathogen was ranged from 39.81% to 100% as compared with control (0.00%). Carbendazim 50% WP, Tebuconazole 25.9% EC and Thiophanate methyl 70% WP resulted with cent per cent (100%) mycelial growth inhibition. This was followed by Difenconazole 25% EC (89.62%), Propiconazole 25% EC (87.03%), Hexaconazole 5% EC (73.14%) and Fosetyl Al 80% WP (39.81%).

At 1000 ppm, all the fungicides shown significantly similar trend of mycelial growth inhibition as that of 500 ppm concentration, but comparatively increased range from 63.51% to 100% as compared with control (0.00%). Carbendazim 50% WP, Tebuconazole 25.9% EC and Thiophanate methyl 70% WP resulted with cent per cent (100%) mycelial growth inhibition. This was followed by Propiconazole 25% EC (92.96%), Difenconazole 25% EC (90.37%), Hexaconazole 5% EC (85.74%) and Fosetyl Al 80% WP (63.51%).

Average mycelial growth inhibition of test pathogen recorded in all systemic fungicides ranged from 51.66% to 100% as compared with control (0.00%). The highest mycelial growth inhibition was resulted with Carbendazim 50% WP, Tebuconazole 25.9% EC and Thiophanate methyl 70% WP (100%), followed by Propiconazole 25% EC (91.11%), Difenconazole 25% EC (90.00%), Hexaconazole 5% EC (79.44%) and Fosetyl Al 80% WP (51.66%).

The results of the present study are found in conformity with the findings of several earlier workers. Systemic fungicides *viz*. Carbendazim 50% WP, Fosetyl Al 80% WP, Tebuconazole 25.9% EC, Thiophanate methyl 70% WP, Hexaconazole 5% EC, Propiconazole 25% EC and Difenconazole 25% EC were reported as potential antifungal compounds with significant maximum mycelial growth inhibition of *Fusarium oxysporum* f. sp. *ciceri* causing wilt of chickpea. Ravichandran and Hegde (2015) [9] reported that Carbendazim 50% WP and Thiophanate methyl 70% WP resulted with cent per cent (100%) mycelial growth inhibition against *F. oxysporum* f. sp. *ciceri* causing chickpea wilt. Golakiya *et al.* (2018) reported that Carbendazim 50% WP and Thiophanate methyl 70% WP resulted with cent per cent mycelial growth inhibition against *F. oxysporum* f. sp. *ciceri* causing chickpea wilt. Magar *et al.*, 2019 [5]; Sanap *et al.*,

2020 [11]; Sahane *et al.*, 2021 [10] and Nandeesha *et al.*, 2021 [6] also reported similar results.

Conclusion

Results indicated that, Carbendazim 50% WP, Tebuconazole 25.9% EC, Thiophanate methyl 70% WP (each @ 500 & 1000 ppm) were proved to be strong fungicides against *F. oxysporum* f. sp. *ciceri*, causing chickpea wilt.

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