



Evaluation of Promising Fungicides, Bio-control Agents and Neem Cake *in vivo* against *Fusarium oxysporum* f.sp. *ciceri* causing Wilt Disease in Chickpea (*Cicer arietinum* L.)

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ABSTRACT: Chick pea wilt is considered as one of the most important disease of tomato in field. In present study fungicides and bio-agent and neem cake were combined evaluated for their efficacy against the *Fusarium wilt* of chickpea. Under *in vivo* it was observed that Carbendazim 50% WP @ 0.1% (ST) + Neem cake @ 500gm /sqm (SA) + *T. viride*@ 2% (SA) was found best effective compare to other treatments at 75 days percent plant mortality (12.90), percent wilt control (80.31) whereas followed by Carbendazim 50% WP @ 0.1% (ST) +*T. viride*-5@ 2% (SA) at 75 days percent plant mortality (16.00), percent wilt control (75.57). Application of bio control agents and neem oil cake will be an alternative to synthetic chemicals to control wilt of chickpea.

Keywords: Fusarium wilt, Chickpea, *T. viride*, Bio-gents, neem cake.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the world's third most important pulse belongs to family *Fabaceae* and grown in tropical, subtropical and temperate regions of the world. Chickpea is an important source of dietary protein that meets the protein requirement of vegetarian people of our country as it contains 20-22 per cent protein. It also improves soil fertility by fixing atmospheric nitrogen into available form in the rhizosphere which is very useful for the succeeding crop (Singh and Saxena 1996).

Chickpea cultivation is often subjected to several biotic stresses of which diseases like *Fusarium* wilt caused by *F. oxysporum* f.sp. *ciceri*, *Ascochyta* blight, *Botrytis* grey mould, *Alternaria* blight, Powdery mildew and dry root rot are important.

Among them, *Fusarium* wilt in chickpea caused by *F. oxysporum* f.sp. *ciceri* has assumed serious proportions in the recent years (Nene *et al.*, 1984). Since, *F. oxysporum* f.sp. *ciceri* is a noxious soil and seed borne pathogen that can survive through chlamydo spores and mycelia for several years in soil or on plant debris. However, due to its soil borne nature and long survival of pathogen, it is difficult to manage through conventional method. The application of fungicides though effective, but is un-economical. They not only affect associated beneficial micro-biota in soil but also are main source contributing towards environmental pollution. As such, use of alternative methods like eco-friendly neem cake and bio-agents integration with fungicides seems to be more appropriate to manage such soil borne diseases.

MATERIALS AND METHODS

Isolation, Purification and Identification of Pathogen

Fresh chickpea wilted plants root showing typical wilt symptoms were used for isolation of the pathogen. The infected portion of the plant was cut into small bits in such a way that each bit consists of infected as well as healthy tissues. The bits were surface sterilized with 0.1 per cent HgCl₂ (mercuric chloride) solution for 30-60 seconds followed by three washing with sterilized distilled water and then placed aseptically on sterilized Petri plates containing 20 ml PDA medium and incubated at (27±2°C). When the fungal growth develops from the infected tissues, it was sub-cultured aseptically and purified by using single hyphal tip culture technique. The pure culture of different locations samples thus obtained was microscopically examined for identification of the pathogen and made confirmation based on the morphological characters *viz.*, mycelia growth pattern, color and formation of macro & micro-conidia, its size and shape.

Preparation of mass inoculums of *F. oxysporum*.

Culture of *F. oxysporum* was separately multiplied on the autoclaved sorghum grains for preparation of mass inoculum in the laboratory for soil inoculation. Sorghum grains were soaked overnight in the water and on next day drained excess water from grains and dried for fifteen minutes. About 150-gram sorghum grains were filled in each 500 ml conical flask. Then it was sterilized in autoclave at 15 lb. (1.036 kg/cm²) for 60 minutes. These flasks were separately inoculated with actively growing fungal culture of each isolate under

aseptic condition in laminar air flow cabinet and incubated at 27±2°C in an incubator (BOD) for ten days. After ten days, the mycelium covered entire surface of sorghum grains. The flasks were shaken regularly during incubation for even colonization of seeds and avoid clumping of seeds. The developed mass cultures of each isolate were checked by microscopic observation for sporulation and were used for soil inoculation to make sick soil and obtain the epiphytotic condition.

Pathogenicity Test. Pathogenicity of culture of *F. oxysporum* was tested by growing chickpea plants in pots containing pathogen-infested soil as per Koch's postulates. Firstly, the required quantity of soil was sterilized by using formalin solution (1.5–2.0%) and filled in earthen pots. The pathogen (inoculums) of each isolate developed on sorghum grains was separately mixed in sterilized soil @ 10g/kg. Then the inoculated soil was filled in sterilized pots. The pots filled with inoculated soil were kept in the cage house for 7 days and were irrigated with sterile water to allow the establishment of pathogen. The seeds of susceptible cultivar of chickpea 'GNG 1958' were sown in the inoculated pots @ 10 seeds/pot. Three replications of each isolate were maintained along with un-inoculated were kept as control in Complete randomized design (CRD). The pots were irrigated on alternate days to provide good moisture. The initial symptoms of wilt started as mortality (Damping off) of seedling on 15th day and fully wilting symptoms were manifested within 35 days. The affected plants produced symptoms yellowing and drying of leaves from base to upward, drooping of petioles and rachis, roots showed browning at the soil surface. Eventually the diseased plants wilted and died prematurely. Re-isolation of the pathogen was attempted to prove the Koch's postulates. Whereas in control healthy plants continued to grow without any wilting symptoms.

Integrated management of chickpea wilt under field conditions

Soil application and seed treatment with bio-control agents, neem cake and fungicides

Based up on the *in vitro* studies, one fungicide Carbendazim 50 WP 0.1%, one bio-agents *T. viride* that was found most effective further evaluated in field condition alone as well as their integration with the neem cake for the management of chickpea wilt. Required quantity of Carbendazim (0.1%), *T. viride* and neem cake were used alone and in various combination. The fungicide Carbendazim 50 WP @ 0.1% was applied as seed treatment while *T. viride* @ 2% and neem cake 500g/sqm were applied as soil application before seed sowing.

Fungicidal seed treatment. Since, as only small quantity of fungicide to be used for seed treatment, with best fungicide (Carbendazim 50 WP) found most effective in *in-vitro*. Required quantity of chickpea seeds were soaked in Carbendazim 50 WP @ 0.1 % solution for 30 minutes. The treated seeds were air dried in shade and then used for sowing.

Sowing and inter-culture operations. The treated seeds of chickpea cultivar "GNG 1058" were sown in micro plots (sized 1.5m × 1.5m) at spacing (30x10cm) keeping three replications in Randomized block design. Agronomic practices or inter-culture operations (*viz.*, recommended dose of NPK, weeding, hoeing) was followed as prescribed package and practices.

Per cent wilt control was also calculated to find out the best treatment for the controlling chickpea wilt by using standard formula given by Wheeler (1969).

$$\text{Per cent wilt incidence} = \frac{\text{Number of wilted plants per plot}}{\text{Total number of plants per plot}} \times 100$$

$$\text{Per cent wilt control} = \frac{\text{PWI (C)} - \text{PWI (T)}}{\text{PWI (C)}} \times 100$$

Where, PWI_(C) - per cent wilt incidence in control plot
PWI_(T) - per cent wilt incidence in treated plot

Population load (c.f.u./g soil) of *T. viride* and *F. oxysporum f.sp. ciceri* in the rhizosphere soil of different treatments after 85 days of sowing.

The augmentation/population load of bio-agent (c.f.u./g soil) in the chickpea rhizosphere and its effects on inoculum density of *F. oxysporum f.sp. ciceri* was studied in laboratory. For this study, rhizosphere soil samples from each treatment were collected at 85 days after sowing and analyzed in the laboratory for enumerating population of *T. viride* and *F. oxysporum f.sp. ciceri*. The inoculum density of the bio-control agents was studied by determining the colony forming units (c.f.u./g soil) by serial dilution plating technique.

The constituents were added to 950 ml of distilled water, made up to 1000 ml and autoclaved at 121°C for 15 min.

Soil dilution of 10⁴ was prepared taking Rhizosphere soil from each treatment separately using serial dilution technique. One ml dilution was poured in each Petri-dish, keeping 3 plates as three replications. Then 15 ml medium was poured in each plate; Petri-plates were incubated for 5 days at 26±2 °C. The discrete colonies of *Trichoderma* spp. in these plates were counted with the help of a "QUEBEC" colony counter. Random mounts from these *Trichoderma* colonies were prepared on microscopic slides and checked for their identity at species level.

Number of conidia of *F. oxysporum f.sp. ciceri* in the rhizosphere soil of healthy and diseased plants, were counted by a simple method. 250 mg from well mixed rhizosphere soil was added in 2.5 ml sterilized water. The mixture was shaken thoroughly and poured over the water agar medium in Petri-plates, maintaining 3 replications of each treatment. These plates were incubated for 2-3 days, and then the number of colonies of *F. oxysporum f.sp. ciceri* in each plate was counted under a digital colony counter by marking segments on Petri-dish back side.

RESULTS AND DISCUSSION

Survey for Chickpea Wilt Incidence and Distribution of Pathogen.

To record wilt incidence and distribution of chickpea wilt pathogen at farmer's field of Udaipur and Pratapgarh districts, a survey was carried out during *rabi* 2019-20. Randomly nine

chickpea growing villages viz., Dangiyo Ki Hundar, Barodia, Vishma, Badrana, Gogunda of Udaipur and Sevana, Kalwani, Rampuria and Kherot of Pratapgarh district were surveyed for recording the incidence of wilt on respective chickpea cultivars. In all the surveyed, maximum areas none of the farmer's field remained free from the wilt incidence. The minimum and maximum wilt incidence in these villages ranged from 18.94 to 48.76 per cent, which indicates its distribution and wilt incidence in large areas. The chickpea wilt samples were collected and pathogen were isolated and coded. In surveyed areas the maximum (48.76%) incidence was observed in Badrana village of Udaipur district on "JG 62" chickpea cultivar caused by *Fusarium* isolate (BDN Foc-04) followed by Rampuria- RMP Foc-08 (43.11%), Vishma- VSM Foc-03 (35.92%), Sevana- SVN Foc-07 (38.83%) and Dangiyo Ki Hundar- DKH Foc-01 (31.76%), followed by Gogunda- GND Foc-05 (23.51%), Kalwani- KLV Foc-07 (28.48%), Kherot- KHR Foc-09 (27.58%). The minimum (18.94%) incidence of wilt was recorded in the village Barodia of Udaipur district in PGC-1 chickpea cultivar by *Fusarium* isolate BRD Foc-02.

Similar, studies carried out by Ghosh *et al.* (2013) on distribution and incidence of chickpea wilt (*F. oxysporum* f.sp. *ciceri*) in central and southern parts of India and reported 25-48 per cent chickpea wilt incidence on local cultivars that predominate in most of farmer's fields.

Isolation, Purification and Identification of the Pathogen. The pathogen responsible for causing wilt of chickpea, *F. oxysporum* f.sp. *ciceri* was isolated from wilted plants which were collected from different villages of Udaipur and Pratapgarh districts. The wilted stem and root parts cut into 2 to 5 mm size bits were surface sterilized, washed thrice with sterile distilled water and then placed aseptically potato dextrose agar medium poured Petri plates and incubated at 27±2°C.

To purify fungal isolates which were recovered from the collected samples of each village, single hyphal tip isolation technique was used. When the fungal growth develops from the infected tissues within 2-3 days of incubation, it was observed under microscope and carefully single hyphal tips of each fungal isolates were aseptically transferred on potato dextrose agar (PDA) slants. The pure cultures of different isolates were identified as *F. oxysporum* f.sp. *ciceri* on the basis of cultural and morphological characters of the fungus and confirmed by comparing with the standard description (Burnett and Hunter 2003; Leslie and Summerell 2006).

Integrated management of chickpea wilt caused by *F. oxysporum* f.sp. *ciceri* under field conditions. The fungicide and bio-control agents which were found most effective in *in vitro* study were further tested in the field for the suppression of wilt of chickpea in alone as well as in various combinations. The results thus obtained are presented in Table 1.

The data revealed that minimum per cent wilt incidence 6.00, 8.33, 11.50 and 12.90% with highest wilt control 75.51, 77.32, 79.09 and 80.31 per cent recorded at 30, 45, 60 and 75 days respectively, in the treatment combination where Carbendazim was applied as seed treatment (ST) and neem cake + *T. viride* were applied as soil application (SA), followed by next effective treatment combination [Carbendazim (ST) + *T. viride* (SA)] that were showed 7.50, 10.50, 14.67 and 16.00 per cent wilt incidence with 69.38, 71.41, 73.33 and 75.57 per cent wilt control at 30, 45, 60 and 75 days. The treatment combination Carbendazim (ST) + neem cake (SA) showed 8.90, 12.39, 16.30 and 18.00 per cent wilt incidence with 63.67, 66.27, 70.36 and 72.52 per cent wilt control at 30, 45, 60 and 75 days. The solely organic based treatment combination Neem cake (SA) + *T. viride* (SA) that showed 10.80, 15.67, 22.00 and 25.00 per cent wilt incidence with 55.92, 57.34, 60.00 and 61.83 per cent wilt control at 30, 45, 60 and 75 days. In individual application of different treatments, Carbendazim (ST) showed 14.50, 20.67, 29.00 and 33.00 per cent wilt incidence with 40.82, 43.72, 47.27 and 49.62 per cent wilt control at 30, 45, 60 and 75 days. Alone application of *T. viride* as soil application showed 18.65, 26.33, 35.87 and 38.22 per cent wilt incidence with 23.88, 28.31, 34.78 and 41.65 per cent wilt control at 30, 45, 60 and 75 days. Whereas higher wilt incidence 22.05, 30.88, 42.33 and 46.33 per cent with 10.00, 15.92, 23.03 and 29.26 per cent wilt control was recorded at 30, 45, 60 and 75 days respectively, by soil application of neem cake over control plot, where wilt incidence of 24.5, 36.73, 55.0 and 65.50 per cent was recorded (Table 1).

Similar results were found by Thaware and Kohire (2015) applied combined fungicides, bio-agents and neem cake formulation in the combined form (Carbendazim + Thiram) (ST) + (*T. viride* + *P. fluorescens*) (SA) + Neem cake seed powder (SA) and obtained maximum seed germination (92.12%) with minimum wilt incidence (12.77%) along with higher plant populations and other growth parameters of chickpea plants. The reports of Animisha *et al.* (2012) also indicate that application of *T. viride* @ 4g/kg was found most effective in suppression of wilt incidence as compared to other bio agents applied under pot culture condition. Similar studies were also conducted by Abhinav *et al.* (2023); Gahlot *et al.* (2022); Anita and Ratnoo (2015) for the management root rot of pea caused by *Fusarium solani*. The results indicated that most effective treatment was Bavistin + Neem oil (ST) + *T. harzianum* + soil application of neem cake (SA) in control of root rot of pea caused by *Fusarium solani* compared to other treatments. Similar studies were also conducted and similar results were obtained by Rehman *et al.* (2013).

Table 1: Evaluation of promising fungicide, bio-agent and neem cake for the management of chickpea wilt in micro plots population of *T. viride* and *Fusarium oxysporium* f.sp. *ciceri* in chickpea plant rhizosphere.

Sr. No.	Treatments	Per cent plant mortality*				Per cent wilt control*				Population load at 85* DAS	
		At 30 days	At 45 days	At 60 days	At 75 days	At 30 days	At 45 days	At 60 days	At 75 days	<i>Trichoderma viride</i> -5 × 10 ⁴ cfu/g soil	<i>F. oxysporum</i> f. sp. <i>ciceri</i> × 10 ⁴ cfu/g soil
1.	Carbendazim 50% WP @ 0.1% (ST)	14.50 (22.34)	20.67 (27.02)	29.00 (32.57)	33.00 (35.03)	40.82	43.72	47.27	49.62	0.0	12.5
2.	<i>Trichoderma viride</i> -5 @ 2% (SA)	18.65 (25.54)	26.33 (30.86)	35.87 (36.77)	38.22 (38.16)	23.88	28.31	34.78	41.65	11.5	14.0
3.	Neem cake @ 500gm /sqm (SA)	22.05 (27.98)	30.88 (33.74)	42.33 (40.57)	46.33 (42.88)	10.00	15.92	23.03	29.26	0.0	15.5
4.	Carbendazim 50% WP @ 0.1% (ST) + <i>T. viride</i> -5 @ 2% (SA)	7.50 (15.88)	10.50 (18.88)	14.67 (22.48)	16.00 (23.54)	69.38	71.41	73.33	75.57	10.0	7.0
5.	Carbendazim @ 0.1% (ST) + Neem cake @ 500gm/sqm(SA)	8.90 (17.27)	12.39 (20.58)	16.30 (23.76)	18.00 (25.09)	63.67	66.27	70.36	72.52	0.0	11.5
6.	Carbendazim 50% WP @ 0.1% (ST) + Neem cake @ 500gm/sqm (SA) + <i>T. viride</i> -5 @ 2% (SA)	6.00 (14.04)	8.33 (16.73)	11.50 (19.77)	12.90 (20.93)	75.51	77.32	79.09	80.31	12.0	5.5
7.	Neem cake @ 500gm /sqm (SA) + <i>T. viride</i> -5 @ 2% (SA)	10.80 (19.16)	15.67 (23.29)	22.00 (27.93)	25.00 (29.95)	55.92	57.34	60.00	61.83	13.0	10.5
8.	Control	24.50 (29.64)	36.73 (37.29)	55.00 (47.85)	65.50 (54.01)	0.00	0.00	0.00	0.00	0.0	21.5
	SEM ± =	0.963	0.538	0.779	0.946	--	--	--	--	0.462	0.847
	CD (P=0.05) =	2.950	1.649	2.385	2.898	--	--	--	--	1.397	2.561

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

*ST- Seed treatment and *SA- Soil application

Inoculum density of *T. viride* and *F. oxysporum* f.sp. *ciceri*. The augmentation of bio-agent (*Trichoderma viride* and pathogen (*F. oxysporum* f.sp. *ciceri*) with plant rhizosphere soil was also studied and determined the population of both fungi on their respective treatments on selective media.

Among the different treatments, maximum colony of *T. viride* 12.0 × 10⁴ c.f.u./g with minimum population (5.5 × 10⁴ c.f.u./g) of *F. oxysporum* f.sp. *ciceri* was determined in the treatment where seed treatment with Carbendazim 50% WP @ 0.1% + soil application of Neem cake @ 500g/sqm + *T. viride* @ 2% was applied compared to other treatments over control. Among the different treatments, maximum population count (13.0 × 10⁴ c.f.u./g soil) was determined in the treatment of soil application of neem cake + *T. viride* followed by (12.0 × 10⁴ c.f.u./g soil) was enumerated in the treatment where, Carbendazim + neem cake + *T. viride* was applied. Followed by 11.5 × 10⁴ c.f.u./g soil population of *T. viride* was enumerated in the treatment where only *T. viride* @ 2% (SA) was applied and 10.0 × 10⁴ c.f.u./gm soil was enumerated in the treatment where Carbendazim 50% WP @ 0.1% (ST) + *T. viride* @ 2% (SA) were applied.

The results of data revealed that all the treatments effectively suppressed the population of *F. oxysporum* f.sp. *ciceri* over the un-treated control. The least population of *F. oxysporum* (5.5 × 10⁴ c.f.u./g soil) and maximum population of *T. viride* was enumerated in treatment where Carbendazim + neem cake + *T. viride* was applied in integration and was found best for suppressing the chickpea wilt in the field compared to other treatments. The maximum population of *T. viride* (15.5 × 10⁴ c.f.u./g soil) was enumerated in the

treatment where neem cake @ 500g/sqm was applied as soil application (SA) followed by 14.0 × 10⁴ c.f.u./g soil in treatment where *T. viride* @ 2% applied alone as (SA) and 12.5 × 10⁴ c.f.u./g soil was counted in seed treatment with Carbendazim 50% WP @ 0.1% and 11.5 × 10⁴ c.f.u./g soil of *F. oxysporum* f.sp. *ciceri* was counted in seed treatment with Carbendazim @ 0.1% + soil application of Neem cake @ 500 g/sqm was applied. The population of *F. oxysporum* f.sp. *ciceri* (10.5 × 10⁴ c.f.u./g soil) was recorded in treatment where soil application of Neem cake @ 500g/sqm + *T. viride* @ 2% was used and 7.0 × 10⁴ c.f.u./g soil was recorded in seed treatment with Carbendazim 50% WP @ 0.1% + *T. viride* @ 2% was applied as soil application. The highest population of *F. oxysporum* f.sp. *ciceri* (21.5 × 10⁴ c.f.u./g soil) was enumerated in the control plot where only pathogen was applied in the soil. These results indicate that application of bio-agent (*T. viride*) in the soil with integration of other components is more beneficial in relation to decrease the pathogen population and increase the population of bio-agent in the applied soil. However, control of plant diseases through biological aids is not sufficient alone to suppress the disease completely under field condition and therefore, the strategies of combined use of additive or synergistic combinations of biotic, cultural and chemical control measures are needed to manage soil borne pathogens.

CONCLUSIONS

In present study the antagonism activity of *Trichoderma viride* with neem cake found to be highly effective against *F. oxysporum* f.sp. *ciceri* under *in vivo* conditions. In the integrated disease management under

field conditions, treatment combination of Carbendazim 50 WP (ST) + *T. viride* (SA) + neem cake (SA) recorded maximum per cent wilt control. Therefore, management of Fusarium wilt we should follow strategies that combine the use of additive or synergistic combinations of chemical, cultural and biotic control measures.

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Conflict of Interest. None.

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