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MANAGEMENT STRATEGIES AGAINST CHICKPEA WILT CAUSED BY *FUSARIUM OXYSPORUM* F. SP. *CICERI* THROUGH DIFFERENT PLANT-EXTRACTS, BIO-AGENTS AND FUNGICIDES

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ABSTRACT

The present study was conducted to evaluate the efficacy of various plant extracts, bio-agents and fungicides against *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt under *in vitro* and those plant extracts, bio-agents and fungicides which proved efficacious under *in vitro* were also evaluated by seed treatment (*in vivo*) in earthen pots. The extract of five plants was evaluated against *Fusarium oxysporum* f. sp. *ciceri* by poisoned food technique. Among these, the extract (10% concentration) of garlic and neem were found most promising in inhibiting mycelial growth. In dual culture plate technique, *Trichoderma harzianum* showed the highest inhibition of mycelial growth of the pathogen in comparison to *T. Asperellum, Bacillus subtilis* and *P. fluorescens.* Among six fungicides tested, Carbendazim and Thiram + carboxin were found most effective and at par in inhibiting mycelial growth of fungus followed by Propiconazole. Under pot culture experiment, seed treatment with Carbendazim was recorded minimum disease incidence (13.80%) followed by Thiram + carboxin (15.75%), *Trichoderma harzianum* (18.13%).
***Keywords*:** Bioagents, fungicides, *Fusarium oxysporum* f. sp. *ciceri*, plant extracts.

Introduction

Chickpea (*Cicer arietinum* L.) also known as Bengal gram is one of the most important winter season pulse crops grown in India. It is a member of family *Fabaceae* and believed to be originated in South West Asia. Among the pulse crops, chickpea occupies a prominent place and popular due to high nutritional value, high yield potential and low cost of cultivation. It is rich source of proteins (21.1%), carbohydrates (61.5%) and fats (4.5%).

The production of chickpea in the Indian sub continent and in other Asian countries is severely affected by many plant pathogenic fungi, bacteria, viruses and nematodes which cause diseases such as Fusarium wilt, dry root rot, Ascochyta blight, collar rot, bacterial blight, filiform virusand root nematode. Chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri* [(Padwick) Snyder and Hansen] is most widespread and important disease occurred throughout the world. *Fusarium oxysporum* f. sp. *ciceri* is mainly soil borne and a facultative saprophyte. It can survive in the soil up to six years in the absence of susceptible host. The chickpea wilt plant at seedling stage did not exhibit any rotting on outer surface of the root. However, on splitting vertically from collar region downwards showed brown, black discoloration of internal tissues, while in adult stage the affected plants showed typical wilting *i.e.* drooping of leaflets, rachis and lamina. Affected plants when uprooted and splitted the root of the wilted plants exhibited more pronounced internal brown to black discoloration of the xylem vessels. The highest wilt incidence (45.88%) on chickpea in Mohangarh area of district Jaisalmer and minimum (27.43%) in Ahore area of district Jalore during 2011-12 and 2012-13.

Material and Methods

Laboratory experiment conducted to find out the fungitoxicity of above five plant extracts (Table 1) against the *Fusarium oxysporum* f. sp. *ciceri* by poisoned food technique. One-hundred-gram

leaves/cloves/rhizomes of each were collected and washed 2-3 times with water. Before extraction leaves/cloves/rhizomes of each plant (100 g) were crushed separately with 100 ml sterilized water. The extract was filtered through muslin cloth and centrifuged at 5000 rpm for 30 minute the effect of each plant extract was tested at two concentrations *i.e.* 5 and 10 per cent. The effect of plant extracts against mycelial growth of *Fusarium oxysporum* f. sp. *ciceri* were tested by Poisoned Food Technique. Required quantity of each plant extracts were mixed thoroughly in melted Potato Dextrose Agar, to get desired concentration, just before pouring in sterilized Petri-plates and was allowed to solidify for 2-3 hours. Each plate was inoculated with 5 mm disc of 7 days of old culture of *Fusarium oxysporum* f. sp. *ciceri* with the help of sterilized cork borer. The inoculated Petri-plates were incubated at 30± 1^0 C for 7 days. A control was also maintained where medium was not supplemented with any of the plant extracts. The experiment was conducted in completely randomized design with three replications. Colony diameter was measured on 7^{th} day of incubation.

The mycelia growth of the test fungus was recorded and per cent growth inhibition was calculated by Vincent's (1947) formula given below:

$$\text{Per cent growth inhibition} = \frac{(C - T)}{C} \times 100$$

Whereas,

C=Diameter of the colony in check (Average of both diagonals)

T=Diameter of colony in treatment (Average of both diagonals)

Efficacy of bio-agents against mycelial growth of *Fusarium oxysporum* f. sp. *ciceri* (*in vitro*)

Screening of bio-agents was done by dual culture technique (Dennis and Webstar, 1971). All the bio-agents were obtained from Department of Plant Pathology, S.K.N. College of Agriculture Jobner, Jaipur. *In vitro* efficacy of four bio-agents, *viz. Trichoderma harzianum, T. asperellum, Pseudomonas fluorescens* and *Bacillus subtilis* was tested using dual culture plate method and twenty ml of autoclaved Potato Dextrose Agar was poured into in each sterilized Petri-plates and allowed for solidification after 2-3 hours of pouring, these plates were inoculated with 5 mm diameter mycelial bit taken from 7 day old culture of *Fusarium oxysporum* f. sp. *ciceri* and antagonistic agents both were placed separately at equal distance on the periphery of Petri-plates. Potato Dextrose Agar containing Petri-plates inoculated with pathogen alone served as check. Inoculated Petri-plates were incubated at 30±1ºC in B.O.D. incubator for 7 days. Linear growth of pathogen as well as bio-agent was measured and per cent growth inhibition was recorded on 7^{th} day of incubation.

Efficacy of fungicides against mycelial growth of *Fusarium oxysporum* f.sp. *ciceri* (*in vitro*)

Efficacy of six fungicides (Table 2) were tested against mycelial growth of *Fusarium oxysporum* f. sp. *ciceri* by Poisoned Food Technique. Required quantity of each fungicide was added aseptically to 100 ml of sterilized Potato Dextrose Agar medium in 150 ml flask separately so as to get concentration of 50, 200 and 500 ppm. The flasks were shaken several times to ensure proper and uniform distribution of the fungicides. The medium was poured separately in sterilized Petri-plates and allowed to solidify. For each treatment three replications were taken. Medium without fungicides served as check. Each plate was inoculated with 5 mm diameter mycelial bit of the fungus. Inoculated plates were incubated at 30±1ºC for 7 days. The linear growth of test fungus was recorded and per cent growth inhibition was calculated by Vincent's (1947) formula.

$$\text{Per cent growth inhibition} = \frac{C - T}{C} \times 100$$

Whereas,

C=Diameter of the colony in check (Average of both diagonals)

T=Diameter of colony in treatment (Average of both diagonals)

Efficacy of effective plant extracts, bio-agents and fungicides (*in vivo*)

Plant extracts, bio-agents and fungicides which proved efficacious *in vitro* were also evaluated by seed treatment (*in vivo*) in earthen pots as per following detail (Table 3). Prior to sowing, these pots were sterilized with copper sulphate solution and filled with sterilized soil + FYM (soil : FYM =3:1, sterilized at 1.045 kg/cm^2 for one hour for three consecutive days). These pots were inoculated with fungus inoculum multiplied on sorghum grains @ 20g/ pot. The pots were covered with polythene bags and kept for 24 hours in cage house. The seeds were tested separately with fungicides (1g/kg seed), plant extracts (10%) and bio-agents (4g/kg seeds). These treated seeds were separately sown in pots @ 15 seeds/pot with three replications. Surface sterilized seeds without plant extracts, bio-agents and fungicides sown in inoculated

sterilized soil served as check. The pots were watered as when required. All the pots were maintained under identical conditions. Observations on per cent disease incidence was recorded 45 day after sowing. The per cent disease incidence and per cent disese control were calculated as follows:

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

The per cent disease control (PDC) was also calculated by using the following formula:

$$\text{Per cent disease control} = \frac{\text{Disease in control - Disease in treatment}}{\text{Disease in control}} \times 100$$

Result and Discussion

Efficacy of plant extracts against mycelial growth of *Fusarium oxysporum* f.sp. *ciceri* by Poisoned Food Technique (*in vitro*)

The efficacy of different five plant extracts at two concentrations *viz.*, 5 and 10 per cent were tested against *Fusarium oxysporum* f. sp. *ciceri in vitro* condition by using Poisoned Food Technique. The data present in Table -4 show that all five plant extracts inhibit the fungal growth at two concentrations. Garlic clove gave complete inhibition of growth of *Fusarium oxysporum* f. sp. *ciceri* at 10 per cent concentration, and followed by neem extract at 10 per cent concentration gave (90.00%) inhibition of mycelial growth of pathogen. Ginger and turmeric at 10 per cent, garlic and neem at 5 per cent concentrations were found moderate inhibitor of mycelial growth of fungus. Turmeric at 5 per cent, datura at both concentrations were found least effective against fungus. As the concentration of plant extracts was increase the mycelial growth inhibition of fungus also increase and at 10 per cent maximum inhibition of mycelial growth was observed.

Plant extracts x concentration interaction was also found significant. Complete inhibition of mycelial growth of fungus was observed by garlic clove at 10 per cent concentration followed by neem extract at 10 per cent concentration. Minimum inhibition of mycelial growth of fungus was recorded by datura at 5 per cent (55.50 %) followed by datura at 10 per cent (61.10%) (Table -4, Fig.- 1 and Plate-1).

Our observations are in agreement with the findings of Verma and Dohroo (2003) who showed that garlic cloves extract resulted total inhibition of growth of *Fusarium oxysporum* f. sp *pisi.* Kamdi *et al.* (2012) also reported the effect of aqueous leaf extracts *Azadirachta indica* was found effective against *Fusarium oxysporum* f.sp. *ciceri* followed by *Lantana camara* at 5% concentration.

Efficacy of bio-agents against mycelial growth of *Fusarium oxysporum* f.sp. *ciceri* by dual culture plate method (*in vitro*)

Antagonistic activity of *Trichoderma harzianum, Trichoderma asperellum, Bacillus subtilis and Pseudomonas fluorescence* were investigated in *in vitro* condition by using dual culture plate method on PDA medium. The result presented in Table -5 and plate-2 showed that all bio-agents were antagonistic to the *Fusarium oxysporum* f.sp. *ciceri.* Maximum inhibition of mycelial growth of fungus was recorded by *Trichoderma harzianum* (89.00%) followed by *Trichoderma asperellum* (84.75%). *Trichoderma harzianum* was found at par with *T. asperellum.* Minimum inhibition of mycelial growth of fungus was recorded by *Bacillus subtilis* (68.34%) and Pseudomonas *fluorescence* (65.34%). *Pseudomonas fluorescence* was found least effective against pathogen (Table -5, Fig.- 2 and Plate-2).

Similar results have also been observed by Rani and Mane (2014) who tested the efficacy of two fungal bioagents *viz., T. viride, T. harzianum* and two bacterial bioagents *viz., P. flurescens* and *B. subtilis* against *F. oxysporum* f. sp. *ciceri* under *in vitro* conditions using dual culture technique. The highest per cent inhibition of growth was recorded by *T. harzianum* (76.66 %) followed by *B. subtilis* (63.14%). The lowest growth inhibition was observed in *P. fluorescens* (53.52%). This study is in agreement with the findings of Thaware *et al.* (2016 b) who reported efficacy of six fungal and two bacterial antagonists were evaluated *in vitro* against *F. oxysporum* f. sp. *ciceri* and reported that the *Trichoderma viride* recorded significantly highest mycelial growth inhibition (75.55%) followed by *T. harzianum* (73.77%), *T. koningii* (71.88%) and *P. fluorescens* (43.77%) respectively.

Efficacy of fungicides against mycelial growth of *Fusarium oxysporum* f.sp. *ciceri* by Poisoned Food Technique (*in vitro*)

The efficacy of different six fungicides at three concentrations *viz.* 50, 200 and 500 ppm were tested against *Fusarium oxysporum* f.sp. *ciceri* in *in vitro* condition by using Poisoned Food Technique. The result presented in Table -6, Fig. -3and Plate-3 showed that all six fungicides inhibit the fungal growth at all three concentrations. Carbendazim, Thiram + carboxin both fungicides gave complete inhibition of growth of fungus at 50, 200 and 500 ppm concentrations, and

followed by Propiconazole (98.99%) at 500 ppm and Hexaconazole (95.50%) at 500 ppm concentration. Hexaconazole and Thiophanate methyl were found moderate inhibitor of mycelial growth of fungus at 200 and 500 ppm concentrations. Thiophanate methyl at 50 ppm, Chlorothalonil at 50 and 200 ppm concentration were found least effective against the pathogen. As the concentration of fungicides was increase the mycelial growth inhibition of fungus also increase and at 500 ppm maximum inhibition of mycelial growth was observed.

Fungicide x concentration interaction was also found significant. Complete inhibition of mycelial growth of fungus was observed by Carbendazim and Thiram + carboxin at all concentrations followed by Propiconazole and Hexaconazole at 500 ppm concentration. Minimum inhibition of mycelial growth of fungus was recorded by Chlorothalonil at 50ppm (47.70%) followed by Thiophanate methyl at 50ppm (52.20%) (Table -6, Fig. -3 and Plate-3).

Similar observations were also made by Raju *et al.* (2008) who found that Carbendazim completely inhibited the growth of *Fusarium oxysporum* f. sp. *udum* at all concentrations (100, 250, 500 ppm). Kumari *et al*. (2014) also observed four fungicides (Mancozeb, SAAF, Carbendazim and Cuprozin) in three different concentrations (0.01%, 0.02% and 0.03%) against *F. oxysporum* f. sp. *cubense* by Poison Food Techniques and reported that Carbendazim at all concentrations was found to be the most effective. Our observations are in agreement with the findings of Jat *et al.* (2017) who worked on different fungicides against *Fusarium oxysporum* causing coriander wilt, in which they found that Complete inhibition of the fungal growth *i.e.*100% with Bavistin (Carbendazim) at 200 and 500 ppm followed by Companion (Carbendazim + Mancozeb), Topsin-M (Thiophanate methyl) and Vitavax (Carboxin + Thiram) at 500 ppm.

Efficacy of plant extracts, bio-agents and fungicides againstwilt of chickpea through seed treatment in pot conditions

Plant extracts, bio-agents and fungicides which were found effective in *in vitro* were also tested in pots through seed treatment against *Fusarium oxysporum* f.sp. *ciceri* and these were garlic, neem, *Trichoderma harzianum, Trichoderma asperellum,* Carbendazim and Thiram + carboxin. The results (Table- 7 and Fig.- 4) revealed that the all-plant extracts, bio- agents and fungicides were found significantly superior over control in reducing per cent disease incidence at 40 days after sowing. Minimum per cent disease incidence was recorded with Carbendazim (13.80%) followed by Thiram + carboxin (15.75%), *T. harzianum* (18.13%), garlic clove (20.00%), neem extract (24.80%) and *T. asperellum* (26.75 %) over control (62.25 %). Maximum disease control over check was recorded with Carbendazim (82.18%) followed by Thiram + carboxin (79.74%), *T. harzianum* (62.00%) and garlic clove extract (58.27%) over control at 40 days after sowing.

These observations are in line with those recorded by Kamdi *et al.* (2012) who have been worked with two antagonists, two fungicides and two botanical extracts against *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt in *in vivo* conditions and found that carbendazim seed treatment (@ 2g/ kg seed) gave minimum wilt incidence (26.38%) and maximum yield (13.47 qt/ha) followed by *T. viride* + Carbendanzim, *T. viride* + Thiram and *Trichoderma viride* alone.

Conclusion

Carbendazim and Thiram + carboxin among fungicides, garlic clove extract among plant extracts, *Trichoderma harzianum* among bioagents were proved highly effective in *in vitro* condition. Seed treatment with Carbendazim,Thiram + carboxin, garlic clove extract and *Trichoderma harzianum* were found most effective against wilt of chickpea *in vivo* condition.

Table 1 : List of plant extracts tested against *Fusarium oxysporum* f.sp. *ciceri* (*in vitro*)

S. No.	Name of Plant	Botanical Name	Plant part use	Concentration (per cent)	
				In vitro	*In vivo*
1.	Ginger	*Gingiber officinale*	Rhizomes	5 & 10	10
2.	Datura	*Datura stramonium*	Leaves	5 & 10	10
3.	Garlic	*Allium sativum*	Clove	5 & 10	10
4.	Neem	*Azadirachta indica*	Leaves	5 & 10	10
5.	Turmeric	*Curcuma longa*	Rhizomes	5 & 10	10
6.	Control	-	-		

Table 2 : List of fungicides tested against *Fusarium oxysporum* f.sp. *ciceri* (*in vitro*)

S. No.	Name of fungicide	Concentration (ppm) *in vitro*	Concentration (%) *in vivo*
1	Hexaconazole	50, 200 and 500	0.1
2	Thiophanate methyl	50, 200 and 500	0.2
3	Thiram + Carboxin	50, 200 and 500	0.1
4	Propiconazole	50, 200 and 500	0.1
5	Carbendazim	50, 200 and 500	0.1
6	Chlorothalonil	50, 200 and 500	0.1

Table 3 : List of treatments tested against *Fusarium oxysporum* f. sp. *ciceri* under *in vivo*

Treatment	Dose
Garlic clove	10%
Neem leaves	10%
Trichoderma harzianum	4g/kg
Trichoderma asperellum	4g/kg
Carbendazim	0.1%
Thiram + carboxin	0.1%
Control	---

Table 4 : Efficacy of plant extracts against mycelial growth of *Fusarium oxysporum* f.sp. *ciceri* by poisoned food technique

Common name	Scientific name	Part used	Per cent inhibition of mycelial growth at different concentration*		
			5%	**10%**	**Mean**
Ginger	*Gingiber officinale*	Rhizomes	72.00 (58.05)	78.80 (62.58)	76.90 (61.29)
Datura	*Datura stramonium*	Leaves	55.50 (48.16)	61.10 (51.41)	58.30 (49.79)
Garlic	*Allium sativum*	Clove	78.75 (62.55)	100.00 (90.00)	84.38 (67.06)
Neem	*Azadirachta indica*	Leaves	75.55 (60.37)	90.00 (71.57)	87.78 (75.18)
Turmeric	*Curcuma longa*	Rhizomes	64.40 (53.37)	73.33 (58.91)	68.87 (56.14)
Control	-	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
			SEm ±	**CD (p=0.05)**	
	Plant extracts (P)		**2.00**	**5.54**	
	Concentration (C)		**1.13**	**3.12**	
	P x C		**1.95**	**5.41**	

*Average of three replications Figures given in parentheses are angular transformed values

Table 5 : Efficacy of bio-agents against mycelial growth of *Fusarium oxysporum* f. sp. *ciceri* by dual culture plate method

S. No.	Bio- agents	Inhibition of mycelial growth* (%)
1	*T. harzianum*	89.00 (70.63)
2	*T. asperellum*	84.75 (67.01)
3	*Bacillus subtilis*	68.34 (55.76)
4	*Pseudomonas fluorescens*	65.34 (53.93)
5	Control	0.00 (0.00)
	SEm ±	**1.84**
	CD (p=0.05)	**5.66**

*Average of three replications Figures given in parentheses are angular transformed values

Table 6 : Efficacy of fungicides against mycelial growth of *Fusarium oxysporum* f.sp. *ciceri* by poisoned food technique

Fungicides		**Per cent inhibition of mycelial growth at various concentrations* (ppm)**			
Common name	**Trade name**	**50**	**200**	**500**	**Mean**
Hexaconazole	Sitara	72.00 (58.05)	85.55 (67.66)	95.50 (77.75)	84.35 (67.82)
Thiophanate methyl	Topsin-M	52.20 (46.26)	81.11 (64.24)	90.00 (71.57)	74.44 (60.69)
Thiram+carboxin	Vitavax power	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Propiconazole	Tilt	86.16 (68.16)	89.99 (71.56)	98.99 (84.23)	91.71 (74.65)
Carbendazim	Bavistin	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Chlorothalonil	Kavach	47.70 (43.68)	62.22 (52.07)	80.55 (63.83)	63.49 (53.20)
Control	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
			SEm±	**CD (p=0.05)**	
	Fungicides (F)		**2.00**	**5.60**	
	Concentration (C)		**0.57**	**1.60**	
	F x C		**0.99**	**2.77**	

*Average of three replications Figures given in parentheses are angular transformed values

Table 7 : Efficacy of plant extracts, bio-agents and fungicides against wilt of chickpea through seed treatment in pot conditions

Treatments	**Dose**	**Disease incidence* (%) at 40 DAS**	**Disease control (%)**
Carbendazim	0.1%	13.80 (21.77)	82.18
Thiram+carboxin	0.1%	15.75 (23.35)	79.74
T. harzianum	4g/kg	18.13 (25.18)	62.00
T. asperellum	4g/kg	26.75 (31.13)	51.36
Garlic clove extract	10%	20.00 (27.61)	58.27
Neem leaf extract	10%	24.80 (29.84)	53.33
Control		62.25 (52.07)	0.00 (0.00)
	SEm+	**0.79**	
	CD(p=0.05)	**2.42**	

*Average of three replications Figures given in parentheses are angular transformed values
DAS = days after sowing

PLATE 1 : Efficacy of plant extracts against mycelial growth of *Fusarium oxysporum* f.sp. *ciceri* by Poisoned Food Technique

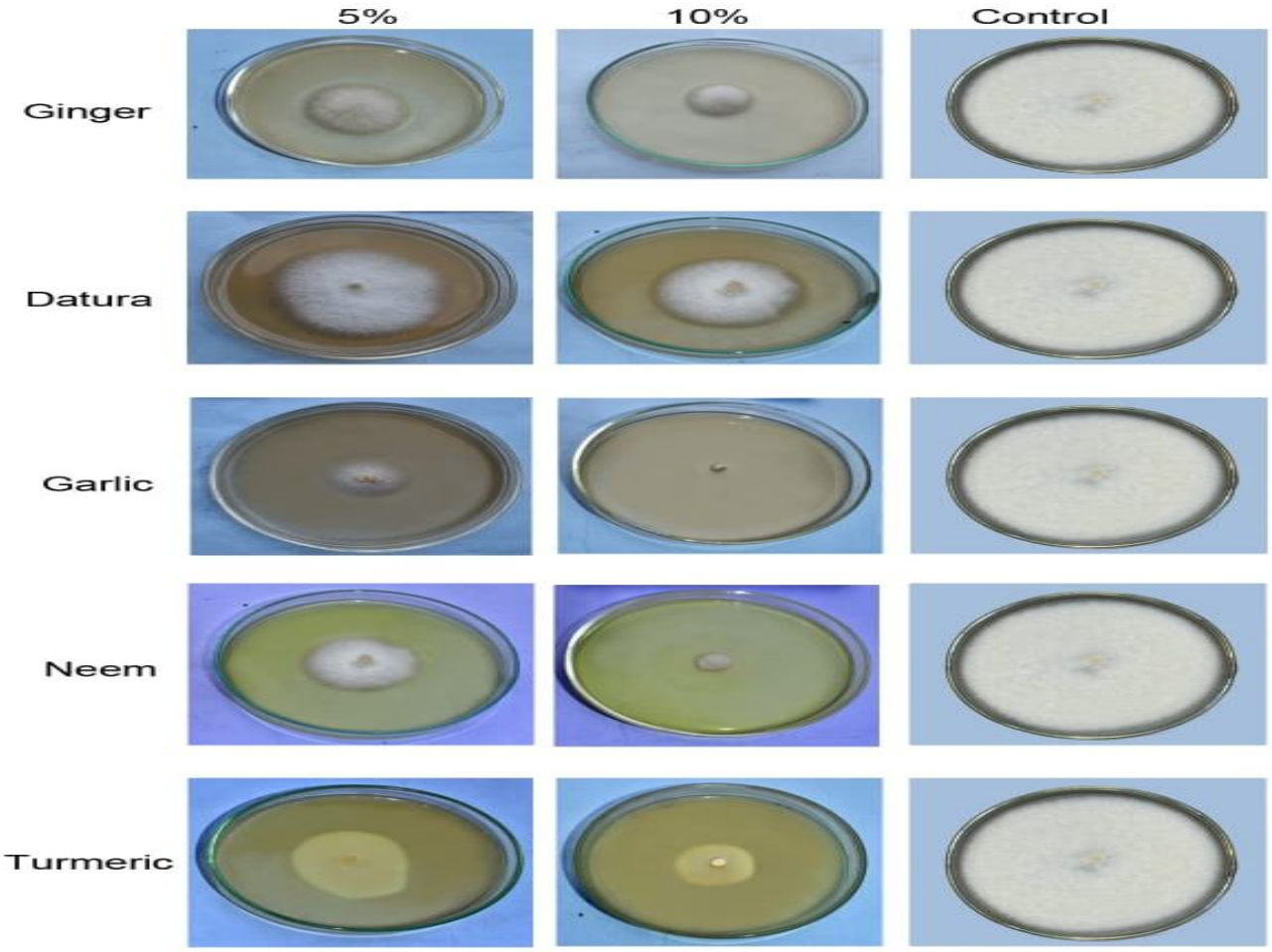


PLATE 2 : per cent inhibition of mycelial growth of *Fusarium oxysporum* f.sp. *ciceri* with biocontrol agents by dual culture plate method

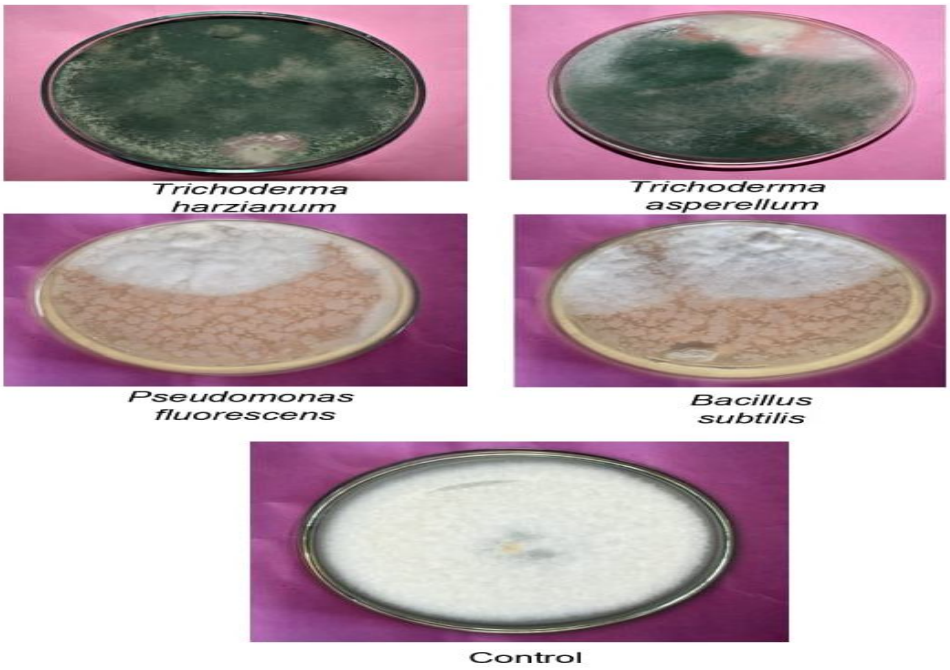
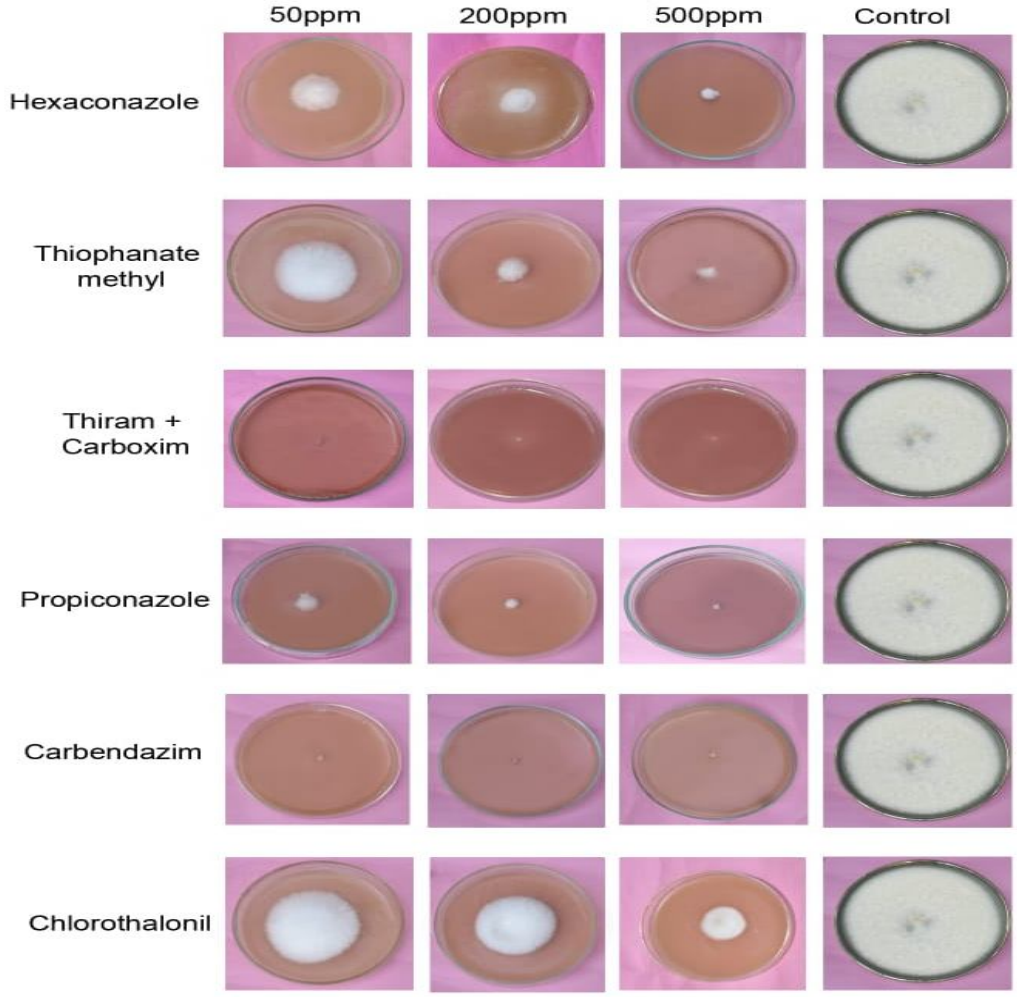


PLATE 3 : Efficacy of fungicides against mycelial growth of *Fusarium oxysporum* f.sp. *ciceri* by poisoned food technique



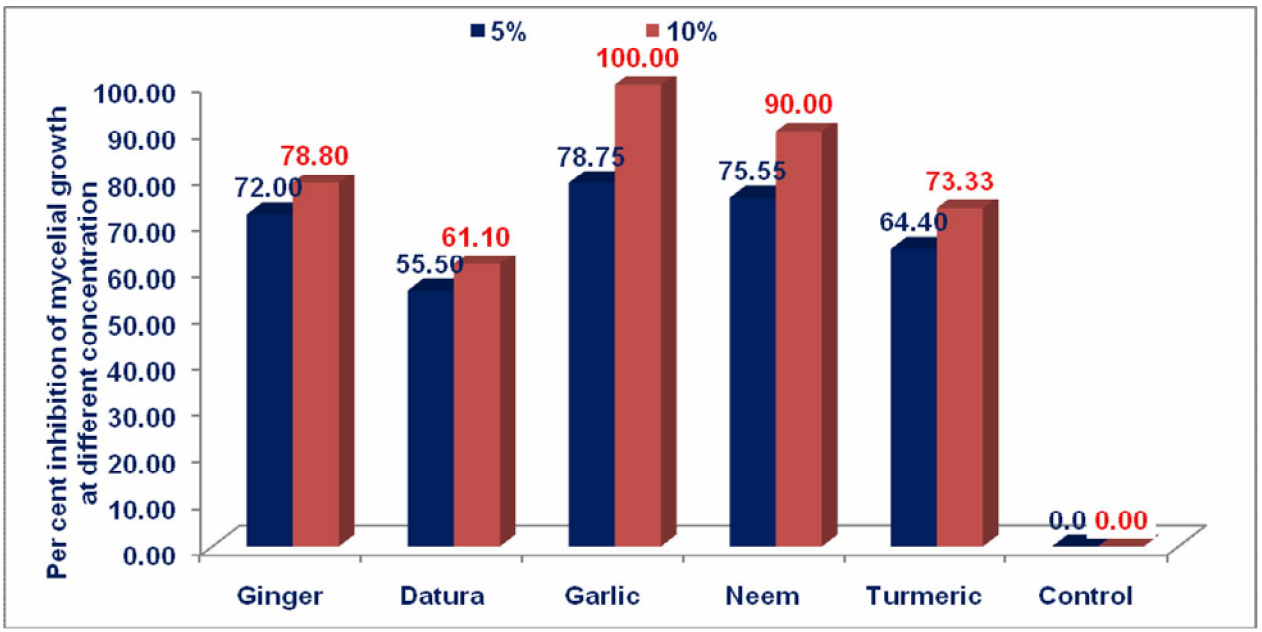


Fig. 1 : Efficacy of plant extracts against mycelial growth of *Fusarium oxysporum* f.sp. *ciceri* by Poisoned Food Technique on 7th day of incubation at 30± 1°C

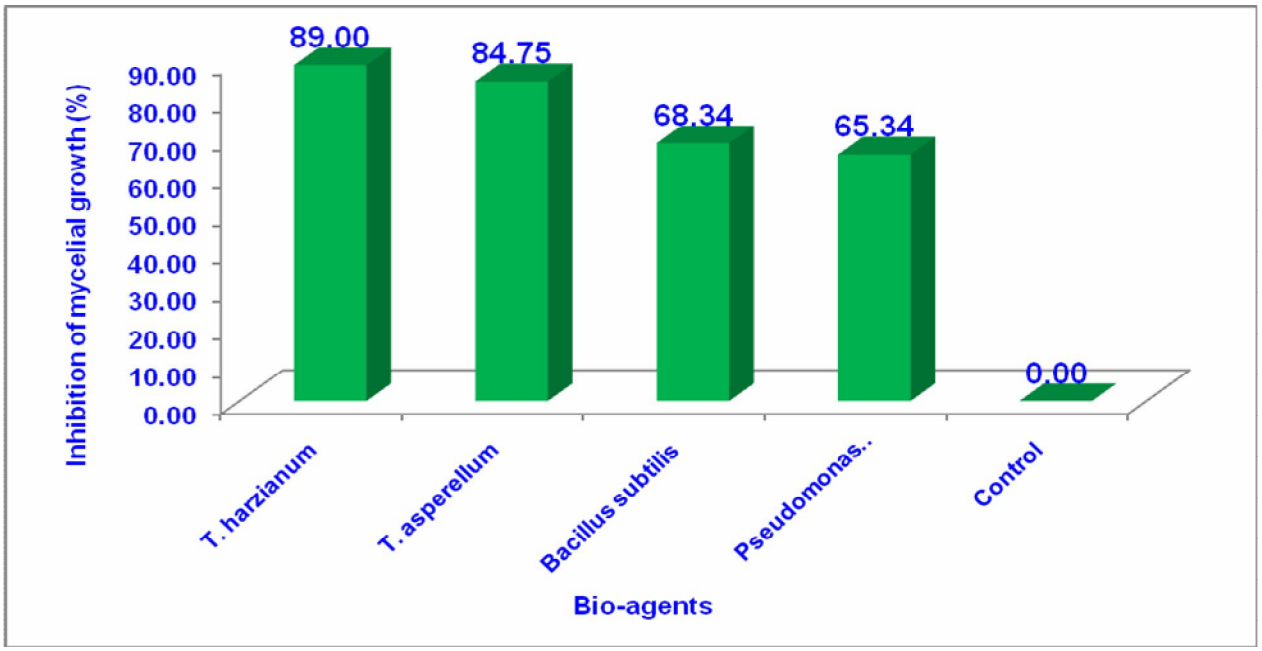


Fig. 2 : Efficacy of bio-agents against mycelial growth of *Fusarium oxysporum* f.sp. *ciceri* by dual culture plate method on 7th day at 30±1°C

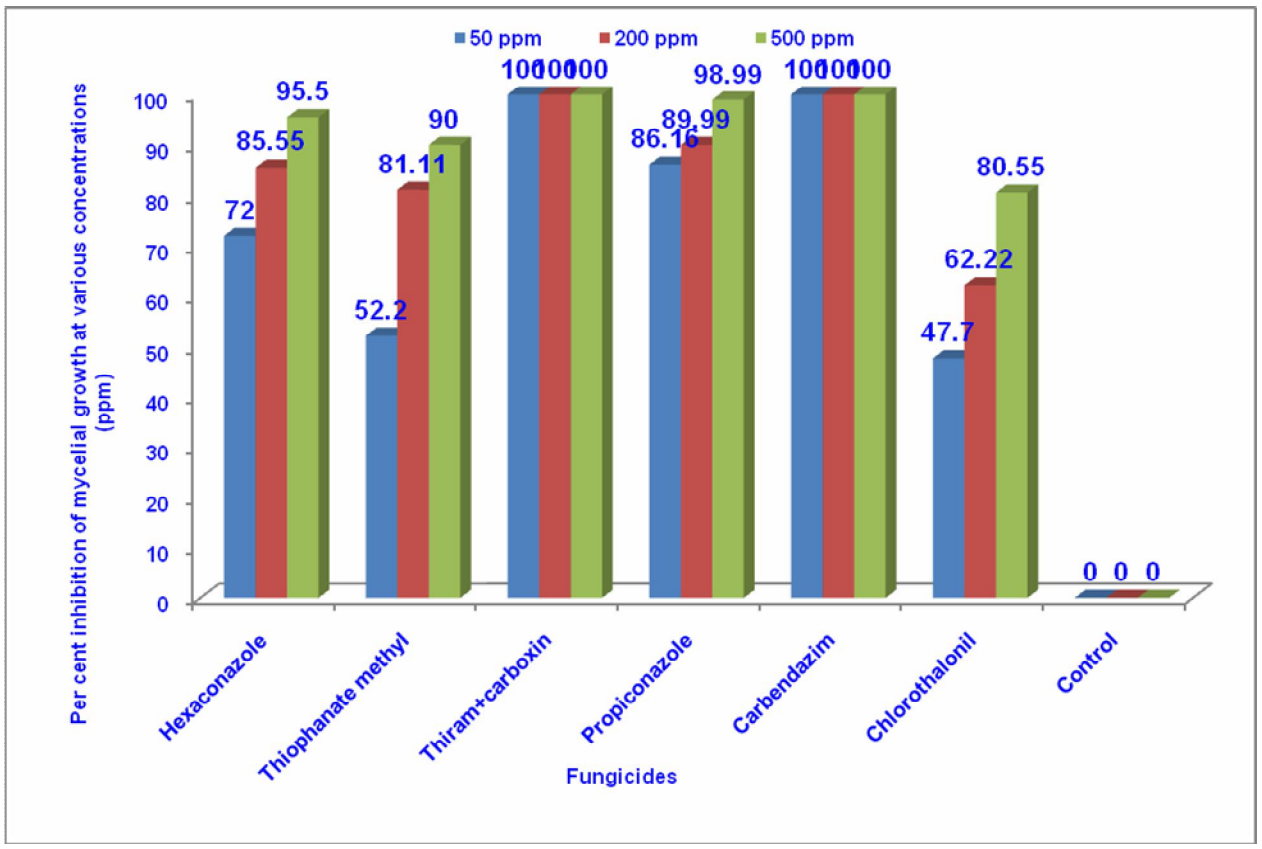


Fig. 3 : Efficacy of fungicides against mycelial growth of *Fusarium oxysporum* f.sp. *ciceri* by Poisoned Food Technique on 7th day of incubation at 30± 1°C

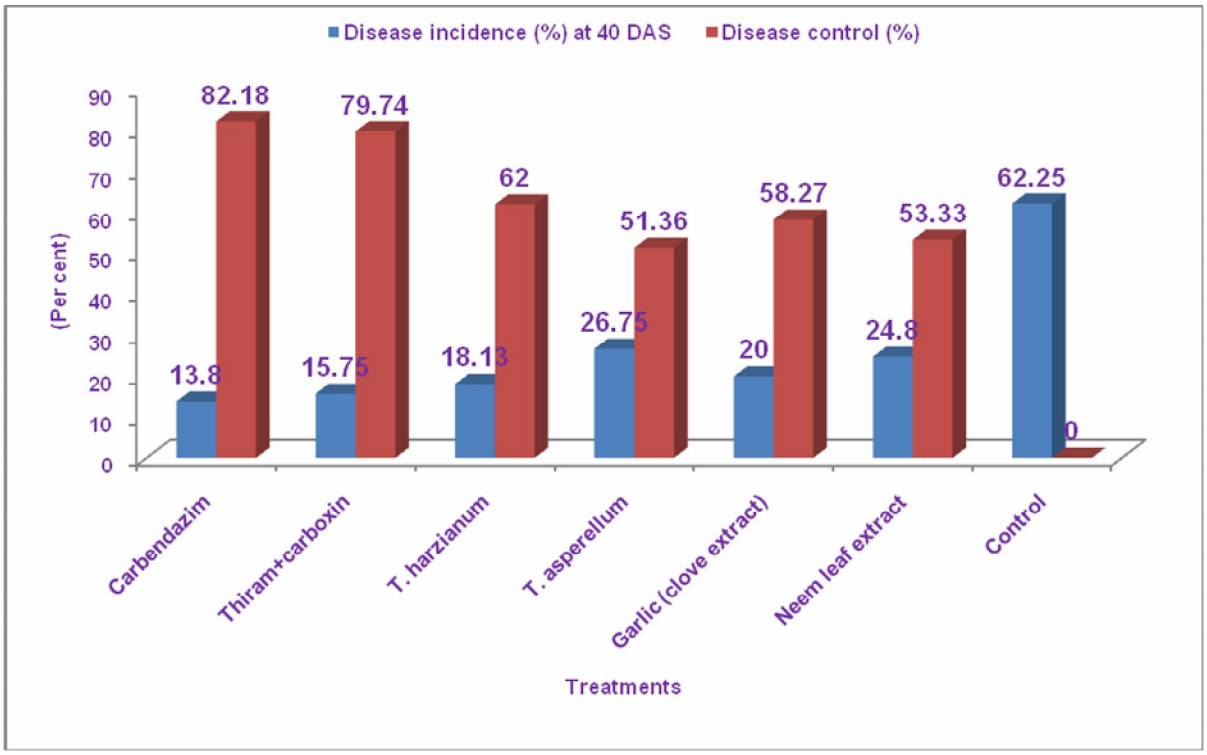


Fig. 4 : Efficacy of plant extracts, bio-agents and fungicides against wilt of chickpea through seed treatment in pot conditions

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