IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 03, 2022

In vitro efficacy of fungicide, botanicals and biocontrol agents against *Fusarium oxysporum* f. sp. *Ciceris*

Kartikay Bisen, Anuj Rana, Udai Bhan Singh, Rahul Singh Rajput

Faculty of Agricultural Sciences and Allied Industries, Rama University, Kanpur

Corresponding author email: kartikaybisen.fas@ramauniversity.ac.in

Abstract

The current study, which was conducted at the Plant pathology laboratory, Rama University in Kanpur (2020), examined the effects of varying concentrations of fungicides, botanical and biocontrol agents on radcal growth and per cent inhibition of *Fusarium oxysporum* f. sp. *ciceris in vitro.*Among the fungicides; carbendazim and mancozeb were selected. For botanical extract of neem, garlic and onion were studies. Biocontrol agents *viz*. *Trichoderma viride*, *Pseudomonas fluorescence* and *Bacillus subtilis* were selected. The minimum radial growth was obtained at 500 ppm concentration of Mancozeb (0.79 mm) followed by Carbendazim (1.07 mm), at 5 % per cent concentration of Garlic (25.00 mm), Neem (34.00 mm) and Onion (36.00 mm) while the maximum radial growth was observed in control (Table no. 8). Among the treatments Garlic, Neem and Onion were found significantly differed to each other but Carbendazim and Mancozeb were found at par with each other.

Introduction

Chickpea (*Cicer arietinum* L.), 2n=2x=14, which is a member of the Papilionaceae subfamily of the Leguminoceae family. Bengal gram, or King of Pulse Crop, is another name for chickpea. One of the main pulse crops grown during the Rabi season is the chestnut bean. Pulses continue to play a significant role in human diets, particularly among the vast majority of vegetarians in the nation. India, Pakistan, Turkey, Iran, Myanmar, Ethiopia, Mexico, Australia, Syria, Spain, Canada, United States, Bangladesh, Algeria, Malawi, Sudan, and Portugal are among the major producers of chickpeas in the world. With a production of around 12 million tonnes, India is the world's top producer in 2021, contributing to about 75% of global output.

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India is also the world's biggest consumer of chickpeas. As a result, despite accounting for over 75% of global production, the nation came in second.

Amongst all the biotic and abiotic constrains of chickpea production, wilt disease caused by *Fusarium oxysporum* f. sp. *ciceris* is very significance. All phases of plant development can be affected by the pathogen, however flowering and pod filling are when infections are most common. If the variety is sensitive, the wilt manifested itself in the field three to four weeks after sowing **(Haware, 1990).** While seeds from late-wilted plants are lighter, rougher, and duller than those from healthy plants, early wilting results in more loss than late wilting. Drought combined with relatively high temperatures might result in up to 80% plant death **(Govil and Rana, 1994).** The disease is distributed throughout the world and prevalent particularly in warm dry climates. The pathogen (*F. oxysporum* f. sp. *ciceris*) is a soil invading or root inhabiting, polyphagous obligate fungal parasite. *F. oxysporum* attack different crops like + chickpea, linseed, banana, tomato, and gladiolus *etc*. The pathogen (*Fusarium oxysporum* f. sp. *ciceris*) is predominantly distributed in tropical and subtropical countries. The fungus is a primarily soil borne pathogen, however, few reports indicated that it can be transmitted through seeds **(Haware *et al.*, 1978).**

Material and methods

Efficacy of Plant extracts against Fusarium wilt of chickpea *in vitro.*

To find out the efficacy of four plants extract against the Fusarium wilt *viz.*, Garlic, Neem leaves and Onion were used. A detailed description of the plant and their part used are given in Table 1.

Table 1 List of plants with English name, botanical name and their part used

S. No.	English name	Botanical name	Part used
1.	Neem	*Azadirachta indica*	Leaves
2.	Onion	*Allium cepa*	Bulb

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3.	Garlic	*Allium sativum*	Bulb

For the preparation of neem leaf extract, 200g of fresh leaves were washed with sterilized distilled water and grinded in a mortar and pestle using 200 ml of sterilized distilled water (Bhat and Sivaprakasan, 1994). The resultant mixture was placed in a beaker covered with sterile aluminium foil and allowed to stand on a laboratory bench for four hours. The neem mixture was filtered through a fine muslin cloth to remove fibrous and suspended material and utilised as crude aqueous extracts in experiments. Same process was used for making onion and garlic extracts. All of the plant extracts were tested at 5 and 10% concentration under *in vitro* conditions by using the food poison technique to study the inhibitory effect of these botanicals on the radial growth of *F. oxysporum* f. sp. *ciceris.*

Per cent growth inhibition was calculated using the formula (Vincent, 1947):

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

I = Per cent inhibition of fungal growth

C = Radial growth of control

T = Radial growth in treated Petri plates.

Efficacy of Biocontrol agents against Fusarium wilt of Chickpea *in vitro*

Sl. No.	**Name of bio-agents**
1.	*Trichoderma viride*
2.	*Bacillus subtilis*
3.	*Pseudomonas fluorescens*

Culture of these three bio-agents were obtained from the Rama University, Kanpur (U.P.) and used for experiment. The antagonistic potential of *Trichoderma viride, Pseudomonas fluorescence* and *Bacillus subtilis* against Foc *was* assessed through dual culture technique by measuring radial growth of the pathogen as well as that of bio-agents. The per cent inhibition of the interacting fungi was calculated as follows:

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$$I = \frac{A^1 - A^2}{A^1} \times 100$$

Where,

I = Per cent inhibition of fungal growth

A^1 = Area covered by *Fusarium oxysporum*f. sp. *ciceris*in the control.

A^2 = Area covered by *Fusarium oxysporum*f. sp. *ciceris*in the dual culture

***In vivo* study**

The field area of Rama University falls humid subtropical climate and located between 18.16 and 20.20 North latitude 76.00 and 78.00 East longitude on an elevation of about 111 meters from sea level in the genetic alluvial plains of eastern Kanpur, Uttar Pradesh during *Rabi* season 2022-23.

Design and Layout

The experiment was conducted in randomized block design (RBD) with nine treatments and three replications.

Spacing and Plot Size

Plot size: (4.0 X 3.0) m^2

Spacing: Row to row spacing –30 cm

Plant to plant spacing – 10 cm

Variety sown

A susceptible variety Ujjwal was used in this experiment.

Land preparation

The land was prepared through ploughing, harrowing, irrigation and soil was brought to a fine tilth.

Treatments Details

a. T1 : Trichoderma viride @ 500 ppm and 1000 ppm
b. T2 : Bacillus subtilis @ 500 ppm and 1000 ppm

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c. T3 : Pseudomonas fluorescens @ 500 ppm and 1000 ppm
d. T4 : Garlic bulb @ 5 % and 10 % concentration
e. T5 : Neem Leaf extract @ 5 % and 10 % concentration
f. T6 : Onion @ 5 % and 10 % concentration
g. T7 : Mancozeb @ 500 ppm and 1000 ppm
h. T8 : Carbendazim @ 500 ppm and 1000 ppm
i. T9 : Untreated Control

Efficacy of bio-agents against Fusarium wilt of chickpea *in vivo*

For better mixing, water was poured during seed dressing. During this process hand gloves were used. After the treatment seeds were allowed to dry in shade. Then, in each wilt sick plot, treated seeds were planted. At 30, 45 and 60 days following sowing, the incidence of wilt was measured in percent. Per cent disease incidence and per cent disease control were calculated by using the following formula.

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

$$\text{Per cent disease control} = \frac{C-T}{C} \times 100$$

Where,

C = Per cent disease incidence of control plot

T = Per cent disease incidence in treated plot

Efficacy of plant extracts against Fusarium wilt *in vivo.*

Per cent disease incidence and per cent disease control were calculated by using the following formula.

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

$$\text{Per cent disease control} = \frac{C-T}{C} \times 100$$

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Statistical analysis

To get to a conclusion, the data was statistically evaluated. The data obtained in all the present investigations for various parameters were subjected to ANOVA for a completely randomized design for *in vitro* studies. The significance of treatment difference was tested by variance ratio test at 5% level of probability.

Results

Efficacy of botanicals and chemicals against *Fusarium oxysporum* f. sp. *ciceris* on radial growth and per cent inhibition *in vitro*

The fungicides Mancozeb and Carbendazim (500 ppm), as well as plant extracts from neem leaf (*Azadirachta indica*), garlic bulbs (*Allium sativum*), and onion bulbs (*Allium cepa*) at a concentration of 5%, were evaluated for effectiveness in vitro using the food poisoning technique. Similar in vitro tests using 10% concentrations of plant extracts, including neem leaf, garlic, and onion bulb extracts, were done with 1000 ppm concentrations of Mancozeb and Carbendazim.

Evaluation of 5% of botanical extract and 500 ppm chemical against *Fusarium oxysporum* f. sp. *ciceris* on radial growth *in vitro*

Radial growth and Growth inhibition after 4 days of incubation

The minimum radial growth was obtained at 500 ppm concentration of Mancozeb (0.79 mm) followed by Carbendazim (1.07 mm), at 5 % per cent concentration of Garlic (25.00 mm), Neem (34.00 mm) and Onion (36.00 mm) while the maximum radial growth was observed in control (Table no. 8). Among the treatments Garlic, Neem and Onion were found significantly differed to each other but Carbendazim and Mancozeb were found at par with each other. The growth inhibition percentage was computed, and the results show that Mancozeb (500 ppm concentration) has the highest growth inhibition (98.38%), followed by Carbendazim (97.81%), and 5% concentrations of garlic (51.02%), neem (32.65%), and onion (26.53%) (Table no. 8). At 5% concentration, it was discovered that the treatments garlic, neem, and onion considerably

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differed from one another. At four days, Carbendazim and Mancozeb were shown to be equivalent.

Radial growth and Growth inhibition after 7 days of incubation

Garlic, Neem, and onion treatments were shown to significantly differ from one another at 5% concentration, although carbendazim and mancozeb treatments were found to be equivalent. Similar results were obtained in the study conducted by Patra and Biswas (2016), who observed that the percentages of Mancozeb and Carbendazim that inhibited growth at 500 ppm were 92.22% and 78.89%, respectively.

Table 2: Effect of different concentration of plant extracts (5%) and fungicide against *Fusarium oxysporum* f. sp. *ciceris* on radial growth and per cent inhibition *in vitro* at 4 days and 7 days

Sl. No.	Treatments	Dose (%/ppm)	4 days after inoculation		7 days after inoculation	
			Radial growth (mm)	Percent inhibition (%)	Radial growth (mm)	Percent inhibition (%)
1.	Garlic bulb extract	5%	24.00	51.02	49.60	40.24
2.	Neem leaf extract	5%	33.00	32.65	59.70	28.07
3.	Onion bulb extract	5%	36.00	26.53	62.40	24.81
4.	Mancozeb	500 ppm	0.79	98.38	1.40	98.31
5.	Carbendazim	500 ppm	1.07	97.81	1.69	97.96
6.	**control**	**-**	**49.00**	**0.00**	**83 .00**	**0.00**
	SEm±		**0.3788**		**0.7046**	
	CD at 1%		**1.6365**		**3.0438**	

Evaluation of @ 10% of botanical extract and @ 1000 ppm fungicide against *Fusarium oxysporum* f. sp. *ciceris* on radial growth *in vitro*

Radial growth and Growth inhibition after 4 days of incubation

Neem, Garlic, and Onion treatments were found to significantly differ from one another. Maximum growth inhibition was obtained at 1000 ppm of Mancozeb (100.00%) and Carbendazim (100.00%), according to the growth inhibition percent calculation. Both were the

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same, followed by 10% extracts of onion (50.62%), neem leaf (69.08%), and garlic bulb (81.22%) (Table No. 3). Neem, Garlic, and Onion treatments were found to differ significantly from one another.

Radial growth and Growth inhibition after 7 days of incubation

At 1000 ppm of Mancozeb and Carbendazim, the smallest amount of radial growth was recorded (0.00 mm). The largest radial development was seen in the control, which was followed by 10%concentrations of garlic (29.70 mm), neem (37.60 mm), and onion (42.50 mm). Garlic, Neem, and onion treatments were shown to significantly differ from one another.

Table no. 3: Effect of different concentration of plant extracts (10%) and fungicide against *Fusarium oxysporum* f. sp. *ciceris* on Radial growth and per cent inhibition *in vitro* at 4 days and 7 days.

Sl. No.	Treatments	Dose (%/ppm)	4 days after inoculation		7 days after inoculation	
			Radial growth (mm)	Percent inhibition (%)	Radial growth (mm)	Percent inhibition (%)
1.	Garlic bulb extract	10%	9.05	81.22	29.70	64.43
2.	Neem leaf extract	10%	14.90	69.08	37.60	54.97
3.	Onion bulb extract	10%	23.80	50.62	42.50	49.10
4.	Mancozeb	1000ppm	0.00	100	0.00	100
5.	Carbendazim	1000ppm	0.00	100	0.00	100
6.	**control**	-	48.20	0.00	83.50	0.00
	SEm±		0.260		0.4468	
	CD at 1%		1.1231		1.9301	

Efficacy of bio-control against *F. oxysporum* f. sp. *ciceris*on radial growth and growth inhibition using dual culture technique after 4 days and 7 days incubation.

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The efficacy of three bio-agents *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* were tested for radial growth and per cent growth inhibition of *Fusarium oxysporum* f. sp. *ciceris* by using dual culture technique.

After 4 days of incubation

The results were obtained after 4 days of incubation, however *Trichoderma viride* (9.80 mm) had the smallest amount of radial growth, followed by Bacillus subtilis (11.90 mm) and Pseudomonas fluorescens (13.40 mm). In this case, all the treatments significantly varied from one another.

After 7 days of incubation

Similar findings were made in the experiment conducted by Rani and Mane (2014), who showed that *Trichoderma viride* (72%) and *Bacillus subtilis* (63.14%) and *Pseudomonas fluorescens* (53.52%) had the highest percentages of growth inhibition.

Table 4: Efficacy of bio-control against *F. oxysporum* f. sp. *ciceris* on radial growth and growth inhibition using dual culture technique after 7 days incubation

Sl. No.	Treatments	4 days after inoculation		7 days after inoculation	
		Radial growth (mm)	Percent inhibition (%)	Radial growth (mm)	Percent inhibition (%)
1.	*Trichoderma viride*	9.80	79.45	21.00	72.83
2.	*Bacillus subtilis*	11.90	75.05	24.90	70.12
3.	*Pseudomonas fluorescens*	13.40	71.90	26.60	67.16
4.	Control	47.70	0.00	81.00	0.00
	SEm±	**0.4306**		**0.5827**	
	CD at 1%	**1.8601**		**2.5173**	

Discussion

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According to the study, the minimum radial growth was obtained at 500 ppm of Mancozeb (0.79 mm), followed by Carbendazim (1.07 mm), at 5% of concentration of Garlic (25.00 mm), Neem (34.00 mm), and Onion (36.00 mm), while the maximum radial growth was seen in the control. Mancozeb (500 ppm concentration) had the highest growth inhibition (98.38%), followed by carbendazim (97.81%), garlic (51.02%), neem (32.65%), and onion (26.53%) at 5% concentration.

After 7 days of incubation, radial growth and growth inhibition Maximum radial growth was seen in the control, while minimum radial growth was obtained at 500 ppm of Mancozeb (1.40 mm), followed by Carbendazim (1.69 mm), at 5% per cent of Garlic (49.60 mm).

The present finding supported by Calistru *et al.* (1997) they used culture filtrates of *T. Viride* T5 and *T. harzianum*T2 were generally more effective in inhibiting *A. flavus*, *F. moniliforme* and *F. oxysporium ciceris* growth than those of the other two aggressive strains. *T. harzianum* T1 and *T. viride* T6 had little influence on *A. flavus*. After four days of inoculation, Mancozeb (0.00 mm) and Carbendazim (0.00 mm) yielded the lowest radial growth, followed by garlic (9.05 mm), neem (14.90 mm), and onion (23.80 mm) at 10% concentration, whereas control showed the highest radial growth. The highest level of growth inhibition was attained at 1000 ppm of both carbendazim (100.00%) and mancozeb (100.00%). garlic bulbs (64.43%), neem leaves (54.97%), and onion bulbs (49.10%).. 10% concentrations of onion, neem, and garlic were shown to be significantly different from one another among the treatments. Similar findings were made in the experiment conducted by Ghante et al. (2018), who showed that the concentration of carbendazim and mancozeb at which the greatest growth inhibition was observed was 1000 ppm. *Trichoderma viride* (9.80 mm) recorded the lowest radial growth of the pathogen's mycelium, followed by *Bacillus subtilis* (11.90 mm) and *Pseudomonas fluorescens* (13.40 mm). Here, all the treatments considerably varied from one another. In the control plate, *Fusarium oxysporum* f. sp. *ciceris* growth was not inhibited (0.00%). There, *Trichoderma viride* showed the highest level of bioagent growth inhibition of the test fungus, at 79.45%. The performance of the other bio-agents, such as *Bacillus subtilis* (75.05%) and *Pseudomonas fluorescens* (71.90%), was excellent in terms of fungus growth inhibition.

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Conclusion

It is possible to control the chickpea wilt disease caused by *F. oxysporum* f. sp. *ciceris* by employing an effective integrated disease management strategy. The seeds treated with the fungicide Mancozeb 0.5%, which completely controls disease incidence and severity, yielded the greatest results in the current investigation, followed by carbendazim. The most effective biocontrol was *T. viride*, while the most effective botanical was 10% concentration of garlic extract.

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