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In-vitro* evaluation of different fungicides and botanicals against *Fusarium oxysporum* f. sp. *ciceris

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Abstract

Fusarium wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceris* has been emerging as one of the biotic threats in profitable cultivation of chickpea crop. The fungicides evaluated *in vitro* against *Fusarium oxysporum* f. sp. *ciceris* were significantly reduced the mycelial growth. Five chemicals viz. Mancozeb 63% WP, Thiophanate methyl 70WP, Carbendazim 12% WP + Mancozeb 63% WP, Carboxin 37.5% DS + Thiram 37.5% DS and Tebuconazole 25.9% SC at 100, 250 and 500 ppm concentration evaluated by poison food technique. Among that Carboxin 37.5% DS + Thiram 37.5% DS were found most effective with maximum growth inhibition (100%) while Mancozeb 63% WP was found least effective at 100, 250 and 500ppm concentration. Among the botanicals tested against *Fusarium oxysporum* f. sp. *ciceris*, the garlic bulbs extract (*Allium sativum*) was found most effective with minimum mycelial growth (30.42 mm) and (27.30 mm) at 5% and 10% concentration respectively with (65.23) and (68.80) growth inhibition. while maximum mycelial growth was found in ashwagandha (48.55 mm) with 44.51 percent growth inhibition at 5% concentration and (42.74 mm) with 51.15 percent growth inhibition at 10% concentration.

Keywords: Chickpea, *Fusarium oxysporum* f. sp. *ciceris*, Wilt, Fungicides, Botanicals

Introduction

Pulse crops are an important source of food proteins, vitamins, lipids and certain minerals and generally grown under risk prone marginal lands. They are important crops for providing a high value food and nutritional security of a large fraction of vegetarian people of the world and are generally known as poor man's meat (Singh and Singh, 1992) [13]. Pulses being legume crops plays vital role in improving soil fertility and conserve natural resources which are essential for sustainable agriculture. Chickpeas are cultivated across India, chickpeas are grown on 10.91 million hectares, producing 130.75 million tonnes (Anonymous, 2021-22) [1]. In Madhya Pradesh (M.P.) over an area of 26.60 lakh hectares, yielding 19.88 lakh tonnes. Northern India contributes approximately 90 percent of the chickpea-growing area and 95 percent of the total production. The average yield in M.P. is about 908 kg/ha, which is relatively low. Many factors contribute to the low yield of chickpeas, with pathological constraints being the most significant. Chickpea wilt, caused by *Fusarium oxysporum* Schlechtend Fr. f. sp. *ciceris* (Padwick) Matuo & K. Sato, is the most critical soil-borne disease affecting chickpeas globally, particularly in the Indian Subcontinent, the Mediterranean Basin, and California (Haware, 1990; Jalali and Chand, 1992; Nene & Reddy, 1987) [6, 8, 11]. In India, the disease was first reported by Butler in 1918. The pathogen, *Fusarium oxysporum* f.sp. *ciceris*, can survive in soil and crop residues as chlamydospores for up to six years without a susceptible host and spreads through both soil and infected seeds (Haware *et al.*, 1978) [5]. *Fusarium* wilt can completely destroy chickpea crops or cause significant annual yield losses, especially in low-rainfall areas, posing a continuous threat to chickpea cultivation. The pathogen also produces mycotoxins (Gopalakrishnan and Strange, 2005) [4]. *Fusarium* wilt is widespread in nearly all chickpea-growing regions globally, with its incidence in India ranging from 14 to 32% across different states (Dubey *et al.*, 2010) [3]. Under favorable conditions, this disease can lead to yield losses of up to 100% (Anjaiah *et al.*, 2003; Landa *et al.*, 2004) [2, 9].

Managing this pathogen cannot be effectively achieved through a single method, such as cultural practices, fungicides, host plant resistance, or bio-agents alone, highlighting the need for an integrated management approach. Although fungicides have shown promise in controlling the pathogen, their widespread use is limited due to concerns about phytotoxicity, fungicidal residues, environmental contamination, and human health risks. As a result, there is growing interest in replacing fungicides with botanicals and/or organic amendments products, which are seen as more sustainable, economical, and environmentally friendly alternatives for profitable agriculture.

Materials and Methods

The experiment was conducted in the central laboratory and the research field, faculty of Agriculture RNTU, Raisen (M.P.).

Chemicals and media

The chemicals have been used in the study were, Sodium chloride, D glucose, Formalin, Ethyl alcohol (90%) etc. and Potato Dextrose Agar, maize extract agar, water agar media were used.

Preparation of culture media

The pathogen that causes Fusarium wilt was isolated and purified *in-vitro* by the use of Potato Dextrose Agar (PDA) media.

Composition of Potato Dextrose Agar medium

Ingredients	Quantity (g)
Peeled potato	200.0
Dextrose	20.0
Agar	20.0
Distilled Water	1000 ml

Evaluation of fungicides

"Poisoned Food Technique," was employed to check the efficacy of five fungicides against *F. oxysporum* f. sp. *ciceris* was assessed in *in-vitro*. An autoclaved potato dextrose agar medium was filled with the necessary amount of each fungicide based on active ingredient and heated to 45°C. This resulted in the appropriate concentrations of 100, 250, and 500 ppm for all fungicides. The check was performed using the same media without the fungicide. Four replicates of the media were added to 90 mm petri plates and each plate was then inoculated with a 5 mm mycelial disc of the test fungus after it had solidified. The inoculated petri plates were incubated for 7 days at 27°C. After incubation, radial growth was measured. Percent inhibition in growth was calculated from the mean diameter after the days when petri plates in control was fully covered with mycelial growth of pathogen as per following formula. Completely randomized design was used for statistical analysis.

Evaluation of Botanicals

The effectiveness of seven plant extracts, including Neem, Tulsi, Garlic, Ashwagandha, Onion, Turmeric and Ginger against Fusarium wilt was investigated. To prepare neem leaf extract 200g of fresh leaves were cleaned with sterilized distilled water and 200 ml of sterilized distilled water was used to ground the leaves in a mortar and pestle. The final

mixture was put in a beaker and left on a lab bench for four hours, covered with sterile aluminum foil. The neem mixture was used as crude aqueous extracts in studies after being filtered through a fine muslin cloth to eliminate fibrous and suspended debris. Other extracts were made using the same procedure. Using the food poisoning technique, all plant extracts were evaluated at concentrations of 5 and 10% in *in-vitro* to examine their potential to suppress the radial growth of *F. oxysporum* f. sp. *ciceris*. For the purpose to get a 5% concentration in aseptic conditions, 95 ml of cooled and sterilized PDA medium were filled with 5 ml of plant extracts from the stock solution and placed into sterilized flasks. To 90 ml of sterilized, cool PDA medium in aseptic flasks, 10 ml of plant extracts of the stock solution were added in the case of a 10% concentration. Before adding the extracts to the petri dishes, the flasks were carefully shaken to ensure an even distribution of the extracts under aseptic conditions. Each petri dish was filled with twenty-five milliliters of medium. Three replications of each treatment were kept up, while a control treatment was kept up by just adding PDA media without any plant extracts. Using a sterilized cork borer, 5 mm mycelia discs of a 7-day-old pure culture of *F. oxysporum* f. sp. *ciceris* were cut and positioned in the middle of plant extracts amended petri plates. In B.O.D., these petri plates were incubated at 25±2 °C. Observation was recorded on radial growth and percent inhibition at 7 days of incubation in plant extracts amended petri plates as well as in control. CRD design was applied in this case. Per cent growth inhibition was calculated using the formula (Vincent, 1947):

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

where,

C = Growth of test fungus in control in mm,

T = Growth of test fungus in treatment in mm

Results and Discussion

In-vitro testing of fungicides against *F. oxysporum* f. sp. *ciceris*

Five chemicals viz. Mancozeb 63% WP, Thiophanate methyl 70% WP, Carbendazim 12% WP + Mancozeb 63% WP, Carboxin 37.5% DS + Thiram 37.5% DS and Tebuconazole 25.9% SC at 100, 250 and 500 ppm concentration evaluated by poison food technique under *in vitro* condition. The effect of treatments increased with an increase in concentration.

Based on the (Table 1), In 100 ppm concentration among all the treatment most effective fungicide with minimum mycelial growth was observed in combination of fungicides Carbendazim 12% WP + Mancozeb 63% WP (0.0 mm) with 100% growth inhibition. Second minimum mycelial growth recorded in Thiophanate methyl 70% WP (5.9 mm) which is at par with Carboxin 37.5% DS + Thiram 37.5% DS (6.5 mm) with 92.44 and 91.67 percent growth inhibition respectively while maximum mycelial growth recorded in Mancozeb 63% WP (25.6 mm) with 67.18 percent growth inhibition. The radial growth in control was 78 mm.

In 250 ppm concentration among all the treatment most effective treatment was Carbendazim 12% WP + Mancozeb 63% WP and Carboxin 37.5% DS + Thiram 37.5% DS with minimum mycelial growth (0.0 mm) and 100% growth inhibition. Second minimum mycelial growth was found in Tebuconazole 25.9% SC (2.1 mm) and 97.31 percent

growth inhibition at par with Thiophanate methyl 70% WP (3.5 mm) and 95.6 percent growth inhibition. Least effective fungicide among all treatment was Mancozeb 63% WP (21.4 mm) with 72.56 percent growth inhibition.

Among all the treatment in 500 ppm concentration Carbendazim 12% WP + Mancozeb 63% WP, Carboxin 37.5% DS + Thiram 37.5% DS, Tebuconazole 25.9% SC and Thiophanate methyl 70% WP were most effective and at par with each other with (0.0 mm) mycelial growth and

100 percent growth inhibition. Mancozeb 63% WP was found least effective fungicide among all the treatments with maximum mycelial growth (13.3 mm) and minimum percent growth inhibition (82.95%). The radial growth in control was 78 mm. Similarly Sahane *et al* (2021) reported that combination product of fungicide viz, Carbendazim + Mancozeb, Tebuconazole + Trifloxystrobin and carboxin + Thiram inhibited the complete growth of pathogen at all tested concentration of 1000, 1500 and 2000 ppm.

Table 1: Efficacy of different fungicides against *Fusarium oxysporum* f. sp. *ciceris* in- vitro

S No.	Treatments	Concentrations (ppm)					
		100		250		500	
		Mycelial Growth (mm)	Percent inhibition (%)	Mycelial Growth (mm)	Percent inhibition (%)	Mycelial Growth (mm)	Percent inhibition (%)
1.	Mancozeb 63% WP	25.6	67.18	21.4	72.56	13.3	82.95
2.	Thiophanate methyl 70% WP	5.9	92.44	3.2	95.90	0.0	100.
3.	Carbendazim 12% WP + Mancozeb 63% WP	0.0	100	0.0	100	0.0	100
4.	Carboxin 37.5% DS + Thiram 37.5% DS	6.5	91.67	0.0	100	0.0	100
5.	Tebuconazole 25.9% SC	7.4	90.51	2.1	97.31	0.0	100
6.	Control	78.0	0.0	78.0	0.0	78.0	0.0
	C.D. at 5%	1.12		1.36		1.22	
	SE (m)	0.38		0.46		0.41	

In-vitro testing of botanicals against *Fusarium oxysporum* f. sp. *ciceris*

In present studies eight plant extract (Table 2) were used to evaluate under *in-vitro* against *F. oxysporum* f.sp. *ciceris* by Poisoned food technique at 5 and 10 percent concentration at 3 days, 5 days and 7 days of incubation.

At 5% Concentration

It may be seen from the data (Table 2) that minimum radial growth at 7 days of incubation was obtained in garlic (30.42 mm) followed by ginger (38.85 mm), neem (40.35 mm), tulsi (44.25 mm), onion (45.10 mm), turmeric (45.35mm) and ashwagandha (48.55 mm) at 5% concentration. While the maximum mycelial growth was recorded in ashwagandha (48.55 mm). The radial growth in control was (87.50 mm). The maximum percent growth inhibition was recorded in garlic (65.23) followed by ginger (55.60), neem (53.83), tulsi (49.43), onion (48.46), turmeric (48.17) and ashwagandha (44.51) at 5 per cent concentration.

At 10% Concentration

Among all the eight treatments minimum mycelial growth at 7 days of incubation was recorded in garlic (27.30 mm) followed by ginger (32.41 mm), neem (33.66 mm) tulsi (39.15 mm), onion (40.11 mm), turmeric (41.83 mm) and ashwagandha (42.74 mm). While the maximum mycelial growth was recorded in ashwagandha (42.74 mm). The radial growth in control was (87.50 mm). The maximum percent growth inhibition was recorded in garlic (68.80) followed by ginger (62.96), neem (61.53), tulsi (55.26), onion (54.16), turmeric (52.19) and ashwagandha (51.15). Similarly, Mailem *et al.* (2015)^[10] reported that garlic clove extract and neem leaf extract were the two botanicals that were most effective at preventing the growth of the test pathogen at doses of 5% and 10%. Jamwal *et al.* (2017)^[7] tested various phyto-extracts against *Fusarium oxysporum* f. sp. *ciceris* and found that the extract of *Azadirachta indica* leaves exhibited the highest degree of mycelial inhibition of the fungus, with *Allium sativum* coming in second.

Table 2: Efficacy of different botanicals against *Fusarium oxysporum* f. sp. *ciceris* in- vitro

S No.	Treatments	Concentrations							
		5%				10%			
		Mycelial Growth (mm)			Percent inhibition (%)	Mycelial Growth (mm)			Percent inhibition (%)
		3rd day	5th day	7th day		3rd day	5th day	7th day	
1.	Neem	13.8	27.20	40.35	53.83	11.66	22.56	33.66	61.53
2.	Tulsi	16.6	31.36	44.25	49.43	13.6	26.90	39.15	55.26
3.	Garlic	11.9	24.85	30.42	65.23	9.20	21.65	27.30	68.80
4.	Ashwagandha	21.5	36.70	48.55	44.51	18.4	31.22	42.74	51.15
5.	Onion	17.3	31.76	45.10	48.46	14.5	28.10	40.11	54.16
6.	Turmeric	17.1	31.90	45.35	48.17	15.2	28.40	41.83	52.19
7.	Ginger	12.42	27.33	38.85	55.60	10.3	22.05	32.41	62.96
8.	Control	32.6	50.20	87.50	0.00	31.6	50.20	87.50	0.00
	C.D. at 5%	1.13	2.58	1.51	-	1.54	1.90	1.66	-
	SE (m)	0.38	0.87	0.51	-	0.52	0.64	0.56	-

Conclusion

Among all chemicals Carboxin 37.5% DS + Thiram 37.5% DS was found most effective at 100, 250 and 500 ppm concentration and in eight botanicals garlic bulbs extract (*Allium sativum*) was found most effective at both 5 & 10% concentration.

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