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Evaluation of Different Plant Extract and Bio-Agents against *Fusarium oxysporum* f. sp. *ciceri,* Caused Wilt Disease of Chickpea

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A B S T R A C T

Chickpea is one of the major *rabi* pulse crop grown in India. It's is a rich source of protein, minerals and vitamins for human nutrition and straw is also valued animal feed. The maximum inhibits the radial growth (16.33mm and 76.66%), (26mm and 62.85%) and (34.00mm and 51.42%) at 30, 20 and 10% concentration respectively against *Fusarium oxysporum* f. sp. *cicei* was showed in Neem extract. Onion extract was least effective which showed 48.33, 49.00 and 58.66 mm radial growth and 30.95, 30.00 and 16.20% inhibits mycelial zones at 30, 20 and 10% concentration respectively. Out of the five fungal antagonists tested *Trichoderma viride* was found to be most effective and recorded least mycelial growth (16.66mm) with highest mycelial inhibition (74.36%) of *Fusarium oxysporum* f. sp. *ciceri*. The least effective fungal bio-agent were *Trichoderma verens* (22.33mm and 65.64%) and *Trichoderma hamatum* (31.33mm and 51.80%) over control. The bacterial antagonist *Bacillus subtilis* was comparatively least effective than the fungal bio-agent and observed (38.33mm and 41.03%) against *Fusarium oxysporum* f. sp. *ciceri* as compared to untreated control. The fungal growth range varied from 16.66 to 38.33 mm and these were showed all the bio-agents suppressed the colony growth of the pathogen.

Introduction

Chickpea (*Cicer arietinum* L.) is the world's third most important pulse crop after dry beans (*Phaseolus vulgaris* L.) and dry peas (*Pisum sativum*) Vishwadhar and Gurha (1998). It's can be provides several dietary nutrients, these includes protein, essential minerals and vitamins for beneficial to the promotion of good health of humans and straw is also valued animal feed (Iqbal *et al.*, 2006). It provides the major protein requirement of millions of people of the world. It is usually grown after the rainy season on conserved soil moisture. Its productivity is constrained by sever abiotic and biotic stress. Among the abiotic stress high temperature is major factors associated

with yield reduction (Summerfield, *et al*., 1990). The crop suffers from many diseases, among the fungal disease of chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri*, is sever threat to the chickpea growers in the world. The disease appears all the growth stage of plants from seedling to pod formation stage. In the year of an average 10% and in sever epidemic crop losses has been observed up to 61% and 43% at seedling and adult stage respectively (Nema and Khare, 1973 and Dubey *et al*., 2007). Similarly early wilting reduced the number of plant and caused more yield than late wilting (Hawre and Nene, 1980). The yield losses vary between 10 to 100% depending on the agro climatic condition (Grewal and pal, 1970 and Navas-Cortes, *et al*., 2000). If therefore, need a constant watch and effort to evolve new fungicides and biopesticides are using for controlling the diseases (Jamil and Kumar, 2010). In a search for better alternatives to pesticides and insecticides and other chemical control agents, natural products are considered to be environmentally safe for control of pest and diseases. In the present study experiment has been given on the effect of different bio-agent and different plant extract check the radial growth of *Fusarium oxysporum* f. sp. *ciceri* causing wilt disease of chickpea.

Materials and Methods

Collection of the samples and isolation of causal pathogen

Fresh diseased plants were collected from the field of different location of Bundelkhand region. Samples of wilt affected root tissues were collected in paper bags for the isolation of *Fusarium oxysporum* f. sp.*ciceri*. Tissue isolation technique was followed after through surface sterilization of root pieces (2-3 mm size) with 0.1 per cent mercuric chloride solution for a minute. After this, the cut pieces thoroughly washed thrice in sterile distilled water to remove mercuric chloride from the treated pieces. These pieces were transferred in petri plates containing solidified PDA medium. The inoculated plates were then incubated at 25±2° temperature. These plates were regularly observed for the fungal growth. The fungal growth appearing on the root pieces were examined and purified by following single hyphal tip cut methods (Rangaswami, 1958). *Fusarium oxysporum* f. sp.*ciceri* was identified based on the spores and conidiophores morphology.

Effect of different plant extracts against the pathogen of *Fusarium oxysporum* f. sp.*ciceri*

To test the different plant extracts against the test pathogen. In order to find out the efficacy of various plant extracts against the Fusarium wilt of chickpea, six plant extract such as Neem kernel (*Azadirachta indica*), Garlic clove (*Allium sativum*), Mustard seed (*Brassica juncea*), Aloevera leaves (*Aloe barbadansis*), Datura leaves (*Datura stramonium*) and Onion bulb (*Allium cepa*) were used. Fresh plant material were collected and washed thoroughly in distilled water. Hundred gm of each washed plant material was grinded by adding equal amount (100ml)of sterilized distilled water (1:1w/v) and heated at 40 to 50 ° for 10 minutes in hot water both to avoid contamination. The material was filtering passing through double layered muslin cloth and centrifuged at 1000rpm for 10 minutes. The supernatant was collected and finally filtered through whatman no.1 filter paper and treated as standard plant extract (100%).

The functioning of six plant extracts is tested against the test pathogen. 10, 20 and 30 ml of stock solution of extracts was incorporated in 90, 80 and 70 ml medium to make 10, 20 and 30 per cent concentration of the extract. 15 ml melted PDA was poured in sterilized petri plates. After solidification all the plates were inoculated individually with 5 mm diameter culture disc of *Fusarium oxysporum* f. sp. ciceri.PDA plates without plant extracts but inoculated with *Fusarium oxysporum* f. sp. *ciceri* served as control. Three times were maintained for all the treatments and plates were incubated at 25±2°. Toxicity of each extracts against the test fungus was calculated in terms percent inhibition of mycelia growth using the inhibition zone was formed and expressed as per cent inhibition using

the formula given by Vincent (1927) and data was analyzed by using OPSTAT statistical program Sheoran (2006).

$$\text{Percent inhibition} = \frac{\text{Colony diameter in Control (mm)} - \text{Colony diameter in treatment (mm)}}{\text{Colony diameter in control (mm)}} \times 100 \quad \ldots \text{(Eqn.1)}$$

Effect of different bio-agents against the pathogen of *Fusarium oxysporum* f. sp. *ciceri*

Six bio-agents *Viz. Trichodema viride, Trichoderma harzianum, Trichoderma virens, Aspergillus niger, Trichoderma hamatum,* and *Bacillus subtilis* were assessed for their efficacy against *Fusarium oxysporum* f. sp. *ciceri* by using dual culture techniques (Nene and Thapliyal, 1993) the culture of test fungus and antagonists was multiplied on PDA medium. 5mm disc of test fungus and the bio-agents cut from the edge of seven days old culture plates were placed on PDA medium in petri plates. The test fungus and bio-agent were placed opposite to each other at a distance of 5mm form the circumstance of petri plates. The same disc of the test fungus was placed alone only one side on PDA medium as control. Each treatment was replicated three times and incubated at 25 ±2 °. The data were recorded after seven days of bio-agent placement, when percent inhibition formula was given in equation 1.

Results and Discussion

Effect of different plant extracts against the pathogen of *Fusarium oxysporum* f. sp. *ciceri*

Use of fungicides is one of the major components in the disease management but regular use of fungicides caused adverse effect on environment. It also encourages development of resistance among pathogen. Therefore, six pants extract contents of Neem kernel, Garlic clove, Mustard seed, Aloe vera, Datura leaves and Onion bulb tested against radial growth of *Fusarium oxysporum* f. sp. *ciceri*. The result obtained from (Table 1), reveled the maximum inhibits the radial growth (16.33mm and 76.66%), (26mm and 62.85%) and (34.00mm and 51.42%) at 30, 20 and 10% concentration respectively against *Fusarium oxysporum* f. sp. *ciceri* was showed in Neem extract. The next best of Garlic tested against radial growth of (18mm and 74.28%),(27.33mm and 60.95%) and (35.33mm and 49.05%) followed by Mustard, Aloe vera and Datura at 30, 20 and 10% concentration respectively, they were statistically at par with each other.

Onion extract was least effective which showed 48.33, 49.00 and 58.66 mm radial growth and 30.95, 30.00 and 16.20% inhibits mycelial zones at 30, 20 and 10% concentration respectively.

All the treatments were significant superior than control. In the similar *Azadirachta indica, Curcuma longa* and *Zinziber officinale* and *Allium sativum* have been reported to have fungitoxic effect against *Fusarium udum* and *Fusarium oxysporum* f. sp. *lycopersici, In vitro* (Chaudhary *et al*., 2019 and Mishra *et al*., 2014) and similar result that several higher plants are known to posses fungitoxicity against spore germination or mycelia growth (Dixit *et al.*, 1982; Dubey *et al.*, 1983). Amongst several metabolites of higher plants, the essential oil has been reported to be highly efficacious against different plant pathogens (Gaur and Raychaudhury 1970; Pandey and Dubey, 1992). In conclusion present study demonstrated that different bio-agent and different plant extract can be used for protecting of huge losses with wilt disease of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri.*

Effect of different bio-agents against the pathogen of *Fusarium oxysporum* f. sp. *ciceri*

The use of chemicals is being discouraged now a-days for the reason that the fungicides are not eco-friendly for being hazardous to mammalian group and responsible for creating the environmental pollution in air, soil and water. The five fungal and one bacterial antagonist *Viz. Trichoderma viride, Trichoderma harzianum, Trichoderma verens, Aspergillus niger, Trichoderma hamatum,* and *Bacillus subtilis* were evaluated against *Fusarium oxysporum* f. sp. *ciceri, In vitro*.

Table.1 Effect of different plant extracts against the pathogen*In vitro.*

S.No.	Treatments	Av. diameter of fungal growth (mm)			% Inhibition of mycelium growth		
		10%	20%	30%	10%	20%	30%
1	Neem kernel	34.00	26.00	16.33	51.42	62.85	76.66
2	Garlic clove	35.33	27.33	18.00	49.05	60.95	74.28
3	Mustard seed	45.33	39.00	37.66	35.24	44.28	46.20
4	Aloe vera leave	47.00	44.66	34.33	32.85	36.20	38.10
5	Datura leaves	50.00	48.66	48.00	28.57	30.48	31.42
6	Onion bulb	58.66	49.00	48.33	16.20	30.00	30.95
7	Control	70.00	70.00	70.00	-	-	-
CD at 5%		7.76	6.78	5.40			
SE(m)		2.53	2.21	1.76			

Table.2 Effect of different bio-agents against the pathogen *In vitro.*

S.No.	Treatments	Av. Diameter of radial growth(mm)	% inhibition
1	*Trichoderma viride*	16.66	74.36
2	*Trichoderma harzianum*	18.33	71.80
3	*Trichoderma verens*	22.33	65.64
4	*Aspergilus niger*	20.00	69.23
5	*Trichderma hamatum*	31.33	51.80
6	*Bacilus subtilis*	38.33	41.03
7	Control	65.00	-
CD at 5%		4.66	
SE(m)		1.52	

The result presented in (Table 2), revealed that all the bio-agent evaluated exhibited fungistatic/antagonistic activity against *Fusarium oxysporum* f. sp. *ciceri.* Out of the five fungal antagonists tested *Trichoderma viride* was found to be most effective and recorded least mycelial growth (16.66mm) with highest mycelial inhibition (74.36%) of *Fusarium oxysporum* f. sp. *ciceri*.

The next best antagonist found were *Trichoderma harzianum* (18.33mm and 71.80%) and *Aspergillus niger* (20mm and 69.23%) respectively, which were statistically at par with each other. The least effective fungal bio-agent were *Trichoderma verens* (22.33mm and 65.64%) and *Trichoderma hamatum* (31.33mm and 51.80%) over untreated control. The bacterial antagonist *Bacillus subtilis* was comparatively least effective than the fungal bio-agent and observed (38.33mm and 41.03%) against *Fusarium oxysporum* f. sp. *ciceri* as compared to untreated control. All the treatments were significantly superior than control.

In Similar observations on mycelial growth inhibition of soil borne fungal pathogens by *T. harzianum. T. viride* and *T. virens* were reported by Mukherjee and Tripathi (2000); Upadhyay and Mukhopadhyay (1986). Our finding is close agreement with the finding of the Choudhary and Mohankain (2012) that reported *T. harzianum* is the most effective and reduce radial growth of *Fusarium oxysporum* f. sp. *lentis.*

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