

Original Research Article

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Eco-Friendly Management of *Fusarium oxysporum* f. sp. *ciceri* the Causal Agent of Chickpea Wilt Disease under *In-vitro* Condition

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ABSTRACT

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Chickpea (*Cicer arietinum* L.) is one of the major *rabi* pulse crop and is a cheap source of protein. It has also advantages in the management of soil fertility particularly in dry lands and the semiarid tropics. Despite of low productivity of chickpea is attributed to *Fusarium* wilt disease which caused by obligate biotroph *Fusarium oxysporum* f.sp. *ciceri* is consider one of the major limiting factor. Experiment was conducted for find out the in-vitro efficacy of bioagents and phytoextracts against *Fusarium oxysporum* f.sp. *ciceri*. Out of different bioagents tested, *T. harzianum* gave maximum inhibition (79.63 %) of mycelia growth of test fungus followed by *T. koningii* with 77.78 % inhibition and least effective is *G. virens* with 55.93 % inhibited fungus growth. In different phyoextracts tested, *A. indica* showed highest inhibition (16.30 %, 34.56 % and 52.59 %) of test fungus in spite of 2 %, 5% and 10 % respectively compare to others. This was followed by *L. camera* with 12.59%, 29.83% and 44.23 % and lowest inhibition found *J. gossypifolia* with 4.44 %, 19.26 % and 35.64 % in terms of 2 %, 5% and 10 % respectively. The above findings are very useful for the farmers for making decision over the use of bio based fungicides for management of wilt disease which is safe management practice for environment.

Introduction

Chickpea (*Cicer arietinum* L.) is one of the major pulse crops, belongs of the family Leguminosae. It is also known Bengal gram. Chickpea is a cheap source of protein compared to animal protein. Chickpea also has advantages in the management of soil fertility, particularly in dry lands and the semiarid tropics (Singh and Saxena, 1996). Low yield of chickpea is attributed to several diseases and insect. Despite of different diseases, *Fusarium* wilt disease is most important disease of chickpea causes severe damage of crop. Vascular wilt caused by an important obligate biotroph *Fusarium*

oxysporum f.sp. *ciceri* (Padwick) Matuo & K. Sato, is consider one of the limiting factor for its low productivity. Although the disease is wide spread in the chickpea growing areas of the world, it is most prevalent in the Mediterranean Basin and the Indian subcontinent (Jalil and Chand, 1992).

The fungus is a primarily soil borne pathogen, however, few reports indicated that it can be transmitted through seeds (Haware *et al.*, 1978). The pathogen can infect at all stages of plant growth with more incidences in flowering and pod filling stage. The wilt

appeared in field within three to four week after sowing, if the variety is susceptible (Haware, 1990). Early wilting causes more loss than late wilting, but seeds from late-wilted plants are lighter, rough and dull than those from healthy plants (Haware and Nene, 1980). Relatively high temperature with drought may cause up to eighty percent plant mortality (Govil and Rana, 1994).

The pathogen is facultative saprophytic and it can survive as mycelium and chlamydospores in seed, soil and also on infected crops residues, buried in the soil for up to five to six years (Haware *et al.*, 1986). If the disease inoculum establishes in the soil, it is difficult to check the disease or eliminate the pathogen except by following crop rotation for more than six years (Haware and Nene, 1982 and Gupta, 1991). Under favorable condition, the wilt infection can damage the crop completely and cause 100% yield loss (Navas-Cortes *et al.*, 2000; Halila and Strange, 1996). In India annual yield loss due to *Fusarium* wilt were estimated at 10% (Singh and Dahiya, 1973; Trapero-Casas and Jiménez-Díaz, 1985). The better way to manage the pathogen in eco-friendly approach is consider the economic way for management of the disease instead of costly and hazardous chemicals.

Biological management is considered to be antagonistic to many soil borne and plant pathogenic fungi (Prasad *et al.*, 2002). Chary *et al.*, 1984 reported that some of the toxic substances obtained from various plant species have been reported to manage a number of fungal diseases of crop plants. A number of plant species have been reported to possess some natural substances which are toxic to many fungi causing plant diseases (Mishra and Dixit, 1977). Therefore the present study was carried out to evaluate the bio-agents and phyto-extract against the growth of *Fusarium oxysporum* f.sp. *ciceri* inciting agent of chickpea wilt, under in-vitro condition.

Materials and Methods

Isolation of *Fusarium oxysporum* f.sp. *ciceri*

Wilted plants of chickpea were collected from different farmers' field of red & lateritic zone of West Bengal and surface sterilized was done by 70 % ethyl alcohol. The samples are cut into pieces of disease part along with healthy tissue. These pieces are placed aseptically on sterilized Potato Dextrose Agar (PDA) medium in Petri plates. Pure culture was done by transfer of a pinch of mycelium on sterilized Potato Dextrose Agar medium in Petri plates and incubated in BOD. The fungus was identified by colony growth, pigmentation and microscopic characteristics of *Fusarium oxysporum*.

Evaluation of bio-agents

The efficacy of biocontrol agents was evaluated *in vitro* against *F. oxysporum* f.sp. *ciceri* through dual culture technique (Denis and Webster, 1971; Dhingra and Sinclair, 1985). The bioagents i.e. *Trichoderma viride*, *T. harzianum*, *T. koningii*, *T. hamatum*, *Gliocladium virens* and *Pseudomonas fluorescense* were collected from Vivekananda Institute of Biotechnology, Nimpith and used in this experiment. The autoclaved and cooled PDA medium was poured in sterilized 90 mm Petri plate. After solidified Petri plates, 5 mm mycelium disc of bio-agent and test fungus was cut with cork borer from 7 days old culture plates, then placed both opposite end of Petri plates. In case of *Pseudomonas* sticking one end of the Petri plates and opposite end placed test fungus by sterile cork borer. Plate inoculated only with test fungus without bio-agent served as control. All plates were replicated with three times and were incubated at $26 \pm 1^{\circ}$ C for 7 days. After incubation radial growth measured and % inhibition of growth was calculated using the formula (Vincent, 1947);

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

Where,

I= Percent inhibition.

C= Radial growth of test fungus in control plate

T= Radial growth of test fungus in treated plate

Evaluation of phyto-extract

Seven phytoextract were tested *in vitro* for their antifungal efficacy against growth of *Fusarium oxysporum* f.sp. *ciceri* through poisoned food technique (Carpenter, 1942; Nene and Thapliyal, 1993). Fresh leaves, cloves of respective plants use this experiment as details shown in Table 1. Plant parts were first washed with tap water and then with sterilized distilled water and air dried. Weighted plant materials were grind in pestle and mortar using the ratio 1:1 w/v. The materials were homogenized for 5 minutes then filtered through double layered muslin cloth followed by Whatman No. 1 filter paper and filtrates were considered as standard extract (100%) (Kamlesh and Gurjar, 2002; Prasad and Barnwal, 1994). The standard leaf extracts solution were individually incorporated into Potato Dextrose Agar (PDA) medium in 250 ml conical flasks at required quantities to get 2, 5, and 10 % concentration and PDA was autoclaved. These melted PDA were poured in 90 mm sterilized Petri plate and PDA without extracts was maintained as control. All plates were replicated three times and analysis CRD.

Plates were inoculated with 5 mm mycelium disc of seven days old culture test fungus and incubated at $26 \pm 1^{\circ}$ C for seven days. The radial growth of the mycelium was measured after seven days of incubation and % inhibition of growth was calculated using the above cited formula (Vincent, 1947).

Results and Discussion

Effect of bioagents

Six biocontrol agents' viz., *Trichoderma viride*, *T. harzianum*, *T. koningii*, *T. hamatum*, *Gliocladium virens* and *Pseudomonas fluorescense* were evaluated against *F. oxysporum* f.sp. *ciceri* and the results are presented in Table 2.

The results revealed that the antagonists significantly reduced the growth of *F. oxysporum* f.sp. *ciceri* either by exhibiting inhibition zones. Among of them *T. harzianum* was found most effective than all other treatments with 79.63 % inhibition. The next best treatment was *T. koningii* with 77.78 % inhibition of mycelia growth. This was followed by *T. viride*, *P. fluorescens* and *T. hamatum* with 75.93 %, 67.78 %, and 58.15 % inhibition respectively. *G. virens* was least effective among the six antagonists treated against *F. oxysporum* f. sp. *ciceri* and exhibited 55.93 % mycelium growth inhibition. Biological control is an effective, eco-friendly and alternative approach for disease management practice. The result of dual culture technique revealed that all the bioagents significantly reduced the growth of *F. oxysporum* f.sp. *ciceri*.

The present study indicated that *T. harzianum* gave maximum inhibition of mycelial growth, than other bio-agents similar observation were reported by Dar *et al.*, 2013; Rani and Mane (2014) were observed highest growth inhibition by *T. harzianum*; Rehman *et al.*, 2013 reported that *T. harzianum* and *T. viride* alone or combination significantly inhibited the mycelia growth of the *F. oxysporum* f.sp. *ciceri*.

Effect of phytoextract

Seven phytoextracts viz. *Azadirachta indica*, *Ocimum sanctum*, *Lantana camera*,

Eucalyptus globules, *Calotropis gigantean*, *Jatropha gossypifolia*, and *Allium sativum* were evaluated against *F. oxysporum* f.sp. *ciceri* followed poisoned food technique. Phyto-extract was tested at 2, 5 and 10 % concentration and the results are presented in Table 3. The results revealed that the phytoextract significantly inhibited the growth of *F. oxysporum* f.sp. *ciceri* at all the tested concentrations. Among of them *A. indica* showed maximum inhibition (16.30 %) of mycelia growth at 2% concentration

followed by *L. camera*, *O. sanctum*, *A. sativum*, *E. globules*, and *C. gigantea*, with 12.59 %, 11.48%, 9.26%, 8.15% and 6.67 % respectively. The least effective was *J. gossypifolia* with 4.44 % inhibition.

In 5 % concentration, *A. indica* showed highest inhibition (34.56 %) of mycelium growth of fungus followed by *L. camera* 29.83 % and least inhibition by *J. gossypifolia* (19.26 %).

Table.1 List of different plant species and their parts used in experiment

SI No.	Common Name	Botanical Name	Used parts
1	Neem	<i>Azadirachta indica</i>	Leaf
2	Tulsi	<i>Ocimum sanctum</i>	Leaf
3	Lantana	<i>Lantana camera</i>	Leaf
4	Eucalyptus	<i>Eucalyptus globulus</i>	Leaf
5	Akanda	<i>Calotropis gigantea</i>	Leaf
6	Jatropha	<i>Jatropha gossypifolia</i>	Leaf
7	Garlic	<i>Allium sativum</i>	Cloves

Table.2 In vitro evaluation of bioagents against *F. oxysporum* f. sp. *ciceri*

SI No	Treatments	Diameter of the mycelium growth after 7 days of inoculation (mm)		% inhibition in mycelium growth of <i>Fusarium oxysporum ciceri</i>
		Bio-agents	<i>Fusarium oxysporum ciceri</i>	
1	<i>Trichoderma viride</i>	68.33	21.67	75.93 (60.62)*
2	<i>Trichoderma harzianum</i>	71.67	18.33	79.63 (63.17)
3	<i>Trichoderma hamatum</i>	52.33	37.67	58.15 (49.69)
4	<i>Trichoderma koningii</i>	70.00	20.00	77.78 (61.88)
5	<i>Gliocladium virens</i>	50.33	39.67	55.93 (48.40)
6	<i>Pseudomonas fluorescens</i>	61.00	29.00	67.78 (55.41)
7	Control		90.00	0.00 (0.00)
S. Em. ±			0.59	0.40
P<0.05			1.79	1.21

* Data parenthesis is Angular Transform value

Table.3 In vitro evaluation of phytoextract against *F. oxysporum* f. sp. *ciceri*

Sl. No.	Common name	Botanical name	% inhibition in mycelium growth		
			Concentration		
			2%	5%	10%
1	Neem	<i>Azadirachta indica</i>	16.30 (4.09)*	34.56 (36.00)**	52.59 (46.48)**
2	Tulsi	<i>Ocimum sanctum</i>	11.48 (3.45)	27.42 (31.57)	41.46 (40.08)
3	Lantana	<i>Lantana camera</i>	12.59 (3.61)	29.83 (33.09)	44.23 (41.68)
4	Eucalyptus	<i>Eucalyptus globulus</i>	8.15 (2.93)	24.64 (29.75)	38.57 (38.38)
5	Akanda	<i>Calotropis gigantea</i>	6.67 (2.67)	21.32 (27.48)	36.32 (37.05)
6	Jatropha	<i>Jatropha gossypifolia</i>	4.44 (2.21)	19.26 (26.01)	35.64 (36.65)
7	Garlic	<i>Allium sativum</i>	9.26 (3.11)	25.93 (30.59)	39.73 (39.07)
S. Em. \pm P<0.05			0.11 0.33	0.66 1.98	0.52 1.58

* Data parenthesis is Square Root Transform value

**Data parenthesis is Angular Transform value

The result obtained at 10 % concentration showed that among the different phytoextracts, *A. indica* inhibited maximum (52.59 %) fungus growth. The next best treatment was *L. camera* with 44.23 % inhibition of mycelia growth. This was followed by *O. sanctum*, *A. sativum*, *E. globules*, and *C. gigantean*, with 41.46 %, 39.73 %, 38.57 % and 36.32 %. The least effective phytoextract was *J. gossypifolia* with 35.64 % inhibition of fungus growth. All the treatments at 10 % concentration exhibited maximum mycelial growth inhibition as compared to 2% and 5% concentration against tested pathogen.

The present study indicated that *A. indica* leaf extract restricted the growth of *F. oxysporum* f.sp. *ciceri* at 2, 5 and 10% concentration at seven days after incubation than other treatments. Similar result was reported by

Ganie *et al.*, 2013. Singh *et al.*, (1980) reported that growth of four soil borne pathogens including *F. oxysporum* f. sp. *ciceris* was effectively inhibited by aqueous extracts of leaf, trunk bark, fruit pulp and oil of *Azadirachta indica*. Mukhtar (2007) also reported that aqueous leaf extract of *Az. indica* is highly effective in reducing the mycelial growth of *F. oxysporum* f. sp. *ciceris*.

In conclusion, the present study was *in vitro* testing of bioagents and phytoextract against *F. oxysporum* f.sp. *ciceri*. Among the different bioagents *T. harzianum* was found to best for inhibiting the growth of test fungus and least effective bioagents was *G. virens*. Among the different phytoextract tested, *A. indica* proved it supremacy in terms of growth inhibition of mycelium of pathogen. Above findings helps us to use of bioagents and

phytoextracts for management of *Fusarium* wilt disease of chickpea. Use of *T. harzianum* and *A. indica* in field against soil borne diseases can easily be practiced for minimize the menace which is also a safe management practice for environment.

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