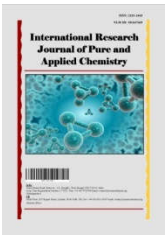

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Eco Friendly Management of Fusarium Wilt of Chickpea with Botanicals and Bio Agents in *In-vitro*

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Authors' contributions

This work was carried out in collaboration among all authors. Author KLN carried out laboratory experiments and performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SNH designed the study and guided me. Author GDG provided biocontrol agents and corrected the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Chickpea (*Cicer arietinum* L.) is one of the most important pulse crop grown all over India. Chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri* is one of the major disease on chickpea in Northern Karnataka, which is soil and seed borne. Heavy inoculum in the soil and favorable environment condition results in the death of infected plant and therefore total yield loss.In this study, three antagonists, and seven botanicals were studied against *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt. *In vitro* studies found that among the botanicals, turmeric rhizome extract gave maximum per cent inhibition of mycelial growth (26.73%) and least per cent inhibition of mycelial growth (9.96%) was observed in cassia tora at 15 per cent concentration. Among the antagonists, *Trichoderma harzianum* was effective in per cent inhibition of *Fusarium oxysporum* f. sp. *ciceri* with (76.47%) and *Pseudomonas fluorescens* found least effective in per cent mycelial inhibition with (34.41%).

Keywords: Chickpea wilt; Fusarium oxysporum f. sp. Ciceri; botanicals; bioagents.

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1. INTRODUCTION

Chickpea is (*Cicer arietinum L.*) is one of the most important and oldest pulse crop after beans and peas. Chickpea seeds contain an average of 23 per cent protein, 38-59 per cent carbohydrate, 4.8-5.5 per cent oil, 47 per cent starch, 5 per cent fat, 6 per cent crude fibre, 6 per cent soluble sugar and 3 per cent ash, minerals such as calcium (202 mg), phosphorous (312 mg), iron (10.2 mg), vitamin C (3.0 mg), calorific value (360 cal), small amounts of B complex, fibre (3.9 g) and moisture (9.8 g). There are two main commercial types of chickpea. The Desi type with smaller and darker coloured seeds which may vary from yellow to black and the Kabuli type with large, smooth and light coloured seeds [1].

Chickpea crop is attacked by 172 pathogens (67 fungi, 22 viruses, 3 bacteria, 80 nematodes and phytoplasma) across the world [2]. Among all, only a few of them have the potential to devastate the crops. Some of the serious diseases in order of their importance are wilt, dry root rot, collar rot, colletotrichum blight, alternaria blight, rust and ascochyta blight caused by *Fusarium oxysporum* f. sp. *ciceri, Macrophomina phaseolina*, *Sclerotium rolfsii*, *Colletotrichum dematium, Alternaria alternata*, *Uromyces ciceris-arietini* and *Ascochyta rabiei* respectively [3].

Losses of chickpea from Fusarium wilt have been reported to vary from 10 to 15 per cent [4,5] but losses of up to 70 per cent have been reported in some years in Northern India and Pakistan[6]. As a facultative saprophyte, *Fusarium oxysporum* f. sp. *ciceri* can survive in soil and on crop residues as chlamydospores for upto six years. The pathogen is also seed-borne and may therefore be spread by means of infected seed [7]. *Fusarium oxysporum* f. sp. *ciceri* is considered to be a major threat to chickpea production in India, Iran, Pakistan, Nepal, Burma, Spain and Tunisia [4].

In the light of present day, constraints in plant disease management practices especially those on the use of botanicals and bioagents is increasingly occupying the minds of scientists all over the world as they are eco-friendly and cost effective. These antagonistic organisms act on the pathogen by different mechanisms *viz.,* competition, lysis, antibiosis, siderophore production and hyperparasitism [8]. Formulations of antagonistic organisms are available at cheaper rate and these organisms once introduced into the soil survive for a longer period.

Therefore, the present study was carried out to evaluate the bio-agents and botanicals against the growth of *Fusarium oxysporum* f.sp. *ciceri* causing chickpea wilt in *in-vitro.*

2. MATERIALS AND METHODS

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

Fusarium oxysporum f. sp. *ciceri* was isolated from infected chickpea plants from different districts of Northern Karnataka, India. The *Fusarium oxysporum* f. sp. *ciceri* was identified, purified and preserved in PDA medium and confirmation of *F. oxysporum* f. sp. *ciceri* by Koch`s postulation and based on the morphological characters described by Booth [9].

2.2 *In-vitro* Evaluation of Botanicals against *F. oxysporum* f. sp. *ciceri*

Seven phytoextract were tested *in-vitro* for their antifungal efficacy against growth of *Fusarium oxysporum* f.sp. *ciceri* through poisoned food technique [10]. Details of the botanicas used in this experiment is given in Table 1.

2.3 Preparation of Cold Aqueous Extract

Fresh plant materials were collected and washed first in tap water and then in distilled water. Hundred grams of fresh sample was chopped and then crushed in a surface sterilized pestle and mortar by adding 100 ml sterile water (1:1 w/v). The extract was filtered through two layers of muslin cloth. Finally thus obtained extract was used as stock solution. To study the antifungal mechanism of plant extracts, the poison food technique was used [10]. Five ten and fifteen ml of stock solution was mixed with 95, 90 and 85 ml of PDA medium respectively and sterilized, so as to get 5,10 and 15 per cent concentration. The medium was thoroughly shaken for uniform mixing of extract. Twenty ml of medium was poured into sterile petriplates, mycelial discs of five mm size from periphery of actively growing culture were cut out by sterile cork borer and one such disc was placed at the centre of each plate. Control was also maintained by growing the pathogen on PDA plates. Three replications were maintained for each treatment and then such plates were incubated at 27°C ± 1°C temperature

and radial growth was taken when maximum growth was observed in control plate. The efficacy of plant products or botanicals was expressed as per cent inhibition of radial growth over the control which was calculated by using the Vincent [11] formula.

2.4 *In-vitro* Evaluation of Bioagents against *F. oxysporum* f. sp. *ciceri*

2.4.1 Dual culture method

Bioagents were evaluated for their efficacy through dual culture technique. Both biocontrol agents and test pathogen were cultured on potato dextrose agar in order to get fresh and active growth of fungus. Twenty ml of sterilised and cooled potato dextrose agar was poured into sterile petriplate and allowed to solidify. For evaluation of fungal bio control agents, mycelial disc of test fungus was inoculated at one end of the petriplate and antagonistic fungus was placed opposite to it on the other end. In case of evaluation of bacterial antagonist the bacterium was streaked at the middle of the pertiplates and mycelial disc of the test fungus was placed on either side at the centre of each half of the plate. Seven replications were maintained for each treatment and the plates were incubated at 27 ± 1°C and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. The colony diameter of the pathogen in control plate was also recorded. The per cent inhibition of the growth of the pathogen was calculated by using the formula $I=C-T/C$ x 100 given by Vincent [11].

Where:

I = Per cent inhibition
C = Radial growth in control
T = Radial growth in treatment

3. RESULTS AND DISCUSSION

Out of the seven botanicals tested against *F. oxysporum* f. sp. *ciceri Curcuma longa* (Turmeric) rhizome extract recorded the highest inhibition (17.24%), this was followed by *Allium sativum* extract (16.86%). Maximum inhibition of mycelial growth was recorded in *Curcuma longa* (Turmeric) rhizome extract (26.73%) at 15 per cent concentration. Turmeric rhizome extract was statistically on par with *Allium sativum* extract (16.86%), Least mycelial growth inhibition was recorded in *Cassia tora* (8.16%), followed by *Azadirachta indica* (12.32%) at fifteen per cent concentration. Among the different concentrations tested 15 per cent concentration was found effective in inhibiting the mycelial growth than at ten per cent concentration (Table 2). Among the three bioagents evaluated against *F. oxysporum* f. sp. *ciceri,* highest per cent inhibition (76.47%) was observed in *T. harzianum and* the least per cent inhibition was recorded in *Pseudomonas fluorescence* with (34.41%) (Table 3).

Among the botanicals evaluated against *Fusarium oxysporum* f. sp. *ciceri* the average highest per cent inhibition was observed in turmeric rhizome extract (17.24%) which was followed by *Allium sativum* extract (16.86%), which were significantly differed with each other and the average lowest per cent inhibition was observed in *Cassia tora* (8.16%). Among the different concentrations tested, significantly highest mean inhibition was recorded in turmeric rhizome extract at 15 per cent (26.73%) followed by 10 per cent (14.91%) and 5 per cent (10.08%) concentrations of the botanicals. These results are well supported by the observations made by Shukla and Dwivedi [12].

Out of three bioagents, it was observed that the mycelial inhibition of *Fusarium oxysporum* f. sp. *ciceri* was 76.47 per cent by *Trichoderma harzianum* and the least effective mycelial inhibition of *Fusarium oxysporum* f. sp. *ciceri* was noticed in *Pseudomonas fluorescence* (34.41 per cent). Similar findings were observed by Mandhare and Suryawanshi [13] and Thaware et al. [14].

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

Sl. no.	Botanical name	Common name	Plant part used
1	*Azadirachta indica* J.	Neem	Leaf
2	*Pongamia pinnata* L.	Honge	Leaf
3	*Curcuma longa* rhizome extract	Turmeric	Rhizome
4	*Alium sativum*	Garlic	Cloves
5	*Alium cepa*	Onion	Bulbs
6	*Lantana camara*	Lantana	Leaf
7	Cassia tora	Cassia	Leaf

Table 2. *In-vitro* evaluation of botanicals against *Fusarium oxysporum* f. sp. *ciceri*

Sl. no.	Botanicals	Plant part used	Inhibition of mycelial growth concentrations (%)			Mean
			5	10	15	
1	*Curcuma longa* rhizome extract (Turmeric)	Rhizome	10.08 (18.52)*	14.91 (22.73)*	26.73 (31.15)	17.24 (24.55)
2	*Allium sativum* (garlic)	Bulb	13.30 (21.40)	16.04 (23.62)	21.25 (27.46)	16.86 (24.26)
3	*Azadirachta indica* (neem)	Leaf	11.36 (19.71)	12.21 (20.46)	13.40 (21.48)	12.32 (20.56)
4	*Allium cepa* (onion)	Bulb	8.30 (16.75)	16.05 (23.63)	13.10 (21.23)	12.48 (20.70)
5	*Lantana camara*	Leaf	9.54 (18.00)	10.95 (19.33)	16.52 (23.99)	12.33 (20.57)
6	*Cassia tora*	Leaf	5.92 (14.09)	8.61 (17.07)	9.96 (18.41)	8.16 (16.61)
7	*Pongamia* spp.	Leaf	10.33 (18.76)	18.81 (25.72)	20.96 (27.26)	16.70 (24.13)
Mean			9.83 (18.28)	13.94 (21.93)	17.41 (24.67)	13.72 (21.75)
			S.Em. ±			**C.D. at 1%**
Botanicals (B)			0.96			3.63
Concentrations (C)			0.63			2.38
B × C			0.55			2.10

**Arcsine transformed values*

Table 3. *In-vitro* evaluation of biocontrol agents against *Fusarium oxysporum* f. sp. *ciceri*

Sl. no.	Bioagents	Inhibition (%)
1	*Pseudomonas fluorescens*	34.41 (35.9)*
2	*Bacillus sp.*	49.52 (44.72)
3	*Trichoderma harzianum*	76.47 (60.98)
	S. Em. ±	0.07
	C.D. at 1 %	0.27
	C.V. (%)	0.42

**Arcsine transformed values*

Among the seven botanicals tested against *Fusarium oxysporum* f. sp. *ciceri,* turmeric rhizome extract recorded the higher inhibition with 17.24 per cent at 15 per cent concentration. Among the biocontrol agents tested against *Fusarium oxysporum* f. sp. *ciceri* the highest per cent inhibition (76.47%) was observed in *T. harzianum*.

4. CONCLUSION

Among the seven botanicals tested against *Fusarium oxysporum* f. sp. *ciceri,* turmeric rhizome extract recorded the higher inhibition with 17.24 per cent at 15 per cent concentration.

Among the biocontrol agents tested against *Fusarium oxysporum* f. sp. *ciceri* the highest per cent inhibition (76.47%) was observed in *T. harzianum*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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