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Evaluation of Botanicals for the Management of *Fusarium oxysporum* f. sp. ciceri of Chickpea

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A B S T R A C T

With respect to *in-vitro* studies under laboratory conditions was conducted to check the effect of botanicals against *Fusarium oxysporum* f. sp. *ciceri* in vitro of chickpea. The experiment was analysed by using CRD with three replication and sixteenth treatments with T_0-Control, T_1-Neem leaves (*Azadirachta indica*), T_2-Arjun leaf extract, T_3-Jamun leaf extract, T_4-Marigold leaf extract, T_5-Onion (bulb), T_6-Garlic (bulb), T_7-Guava leaf extract, T_8-Lemon leaf extract, T_9-Mint leaf extract, T_{10}-Congress grass leaf extract, T_{11}-Dhatura leaf extract, T_{12}-*Lantana* leaves (*Lantana camara*), T_{13}-Eucalyptus leaf extract, T_{14}-Tulsi leaf extract and T_{15}-Ashoka leaf extract were taken up. It was observed that the treatment with Neem leaves (*Azadirachta indica*) was found to be most effective against *Fusarium oxysporum* f. sp. *Ciceri* showing minimum disease incidence followed by Guava leaf extract. It was observed that Neem leaves (*Azadirachta indica*) treatment was significantly superior as compared to other 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).

Pulses being legume crops plays vital role in improving soil fertility and conserve natural resources which are essential for sustainable agriculture. India, being a major pulse producing country, they are grown over an area of 10.84 m ha with a production of 6.43 m t and productivity of 593 Kg per ha (Singh, 2013).

Chickpea (*Cicer arietinum* L.) is the world's third most important pulse crop, after dry beans (*Phaseolus vulgaris* L.) and dry peas (*Pisum sativum* L.) is a vital source of plant derived edible protein in many countries. Chickpea also has advantages in the management of soil fertility, particularly in dry lands and the semiarid tropics. Chickpea (*Cicer arientinum* L.) is a premier Rabi season pulse crop of the Indian subcontinent.

It is grown in semi-arid and tropical climate. It originated from middle east and now grown in 45 countries across the world. Indian subcontinent accounts for 90 per cent of the total world chickpea production (Vishwadhar, 1998; Juan *et al.*, 2000).

The yield potential of present day Chickpea cultivar exceeds 4 t /ha. However, average yield is less than 0.8 t/ha, the gap between average and potential yield is mainly due to diseases and poor management practices. It is attacked by about 67 fungi, 3 bacteria, 22 viruses and mycoplasma and 80 nematodes. Major diseases in order of their global importance are *Ascochyta* blight, *Fusarium* wilt, *Botryti s*gray mould, dry root rot, stem rot, foot rot, black root rot, *Verticillium* wilt, rust etc. *Fusarium oxysporum* f. sp.*ciceri* is a wilt fungus causing severe damage wherever this crop is grown. *Fusarium* wilt is one of the major diseases of chickpea and at national level the yield losses encountered were reported to the tune of 60 per cent. At national level the yield losses encountered due to wilt may vary between five to ten per cent.

Haware *et al.*, (1978) reported that the fungus *Fusarium oxysporum* f. sp. *ciceri* is a primarily soil borne pathogen, however, few reports indicated that it can be transmitted through seeds. Attempts were made to estimate loss in yield on a per plant basis. Early wilting caused more loss than late wilting. Seeds harvested from wilted plants were lighter than those from healthy plants Haware and Nene (1980). Singh and Reddy (1991) reported that the average annual yield losses due to wilt have been estimated to be 10 to 90 per cent in wilt affected soils.

The disease occurs at seedling and flowering stage of plant growth. The symptoms which can be observed are drooping of petioles and rachis, yellowing and drying of leaves from base to upward, browning of vascular bundles, improper branching, withering of plants and finally death of plants Westerlund *et al.*, (1974); Prasad and Padwick (1939). Erwin (1958) reported that the foliage of the *Fusarium oxysporum* f. sp. C*iceri* infected plant turns yellow before wilting and the xylem tissue shows light brown discoloration. Saxena and Singh (1987) reported that internal discoloration of pith and xylem can be seen if stem and root of the wilted plants split vertically.

Though, there are scattered reports on its fruit and foliar diseases, however, heavy decline in chickpea due to *Fusarium oxysporum* have been recorded in different parts of the India (Sharma and Gaur, 2014). The use of synthetic fungicides for the management of disease is not only hazardous for human beings but also increases different types of environmental pollution and toxicological problems. Botanicals have assumed special significance for plant disease management in present day strategy being ecologically safe. The popularity of botanical pesticides is increasing and some plant products are being used globally as green pesticides (Gurjar *et al.*, 2012). Several botanicals have been demonstrated to possess excellent fungicidal properties (Bhardwaj, 2012). Inhibition of plant pathogenic fungi by many antifungal compounds of plant origin led to the present study to explore the feasibility of using extract of several plants for the management of *Fusarium oxysporum* causing wilt disease of chickpea under in vitro conditions.

Materials and Methods

The experiment was conducted in the research laboratory, Department of Plant Pathology, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj during the year 2018-2019.

Cleaning and sterilization of glass wares

Glass wares used during experiment were dipped in the tap water overnight and then washed with detergent powder in running tap water and sun dried. The cleaned glass wares were wrapped with clean paper separately and sterilized in hot air oven at temperature of 150˚C to 180˚C for two to four hours.

Sterilization of inoculating needle

Cleaned inoculating needle was dipped in spirit and heated over the flame until red hot. It was repeated two to three times. Inoculation needle was used in transferring inoculums from Petri plates to culture tubes for maintaining the pure culture of *Fusarium oxysporum* f. sp.*ciceri.*

Preparation of media

For isolation and growth of pathogen *Fusarium oxysporum* f. sp. *ciceri.* Potato dextrose agar (PDA) medium was used, the composition of which is as follows,

Agar	:	15-20 gm
Dextrose	:	20 gm
Peeled Potato	:	200 gm
Distilled water	:	1000 ml
pH	:	6 - 6.5

Plating of media

Autoclaved media in which Agar–Agar had been mixed was cooled down to around 45˚c and 200 ml poured in sterilized Petri plates.

Slanting of media

The test tube containing 5 ml liquid medium was slanted by putting them into a wooden slanter after autoclaving. After solidification they were used for culturing.

Isolation of pathogen from Plant Materials

Infected chickpea roots showing symptoms of the disease were obtained from sick blots from field. The roots were cut into small sections (0.5-1.0 cm), washed thoroughly with tap water, surface sterilized with Clorox (NaOCl) for 5 minutes, rinsed three time in changes of sterilized distilled water and dried on sterilized filter papers. The sterilized roots sections were plated at the rate of five sections/ plate onto potato dextrose agar (PDA) medium supplemented with chloramphenicol (0.05 g/L) in 9cm Petri dishes. The Petri dishes were incubated at 25°C. After incubation for 7 days, isolated fungi were subculture on PDA. When free from contamination; Isolates were maintained on PDA slants and examined visually for their growth patterns and pigmentation on the adverse side of the agar.

Further microscopic examinations were carried out for mycelia and conidia structure using pure culture of F. oxysporum f.sp.ciceri was obtained by using Hyphal Tip Technique. Pure culture of the isolated fungi was transferred to PDA slants and kept in refrigerator at 40°C for further use. Sample of the obtained colonies were sub cultured by transferring small mycelia from the colony margins. Pure cultures were obtained by sub-culturing three times and slides were prepared and examined microscopically to confirm identity (x: 40).

Microscopic characteristics of Pathogen

Mycelium is branched, septate and hyaline which inhabits the vessel of the roots

Conidia are two types, macro conidia and micro conidia.

Macroconidia are borne on cushion-like sporodochia, are long and multi-septate & slightly curved.

Microconidia are very small having one or

two septa, spherical elongated and are borne on simple or branched single hyphae.

Maintenance and preservation of culture

The PDA slants and preserved in refrigerator at 5˚C. The pathogen was sub cultured at a regular intervals of 1 month for maintain the live culture.

Re-isolation of the test fungus

The test fungus was re-isolated to confirm the Koch's postulates. For this purpose, roots were washed thoroughly with tap water and cut into small pieces. After surface sterilization with 5% bleach solution for 1–2 min, root pieces then placed on PDA plates. Five days after incubation, the recovery of inoculated fungus was recorded.

Detail of treatments

	Treatment	Botanical name	Dosage
T_0	Control		
T_1	Neem leaves	*Azadirachta indica*	**(5%,10%,15%**
T_2	Arjun leaf extract	*Termenelia arjuna*	**(5%,10%,15%**
T_3	Jamun leaf extract	*Syzygium cumini*	**(5%,10%,15%**
T_4	Marigold leaf extract	*Tagetes erecta*	**(5%,10%,15%**
T_5	Onion (bulb)	*Allium cepa*	**(5%,10%,15%**
T_6	Garlic (bulb)	*Allium sativum*	**(5%,10%,15%**
T_7	Guava leaf extract	*Psidium guazava*	**(5%,10%,15%**
T_8	Lemon leaf extract	*Citrus indica*	**(5%,10%,15%**
T_9	Mint leaf extract	*Mentha sp*	**(5%,10%,15%**
T_{10}	Congress grass leaf extract	*Parthenium hysterophorus*	**(5%,10%,15%**
T_{11}	Dhatura leaf extract	*Datura stramonium*	**(5%,10%,15%**
T_{12}	*Lantana* leaves	*Lantana camara*	**(5%,10%,15%**
T_{13}	Eucalyptus leaf extract	*Eucalyptus globulus*	**(5%,10%,15%**
T_{14}	Tulsi leaf extract	*Osimum sanctum*	**(5%,10%,15%**
T_{15}	**Ashoka leaf extract**	***Saraca asoca***	**(5%,10%,15%**

In-vitro evaluation of effect of botanicals on radial growth of *Fusarium oxysporum* f. sp.*ciceri*

Botanical extracts 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 of respective plants were used in this experiment. 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. 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 5% 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 analyzed

using CRD.

Plates were inoculated with 5 mm mycelium disc of seven days old culture test fungus and incubated at 26±10 C for fifth days. The radial growth of the mycelium was measured after seventh day of incubation and % inhibition of growth was calculated using the below cited formula Vincent (1947).were evaluated against *F. oxysporum* f. sp. *ciceri* followed poisoned food technique. Phyto-extract was tested at 5% concentration. The results revealed that the phytoextract significantly inhibited the growth of *F. oxysporum* f. sp. *ciceri* at all the tested concentrations Patra and Biswas (2017).

% inhibition of growth was calculated using the formula,

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

Where,

I= Percent inhibition.

C= Radial growth of test fungus in control plate.

T= Radial growth of test fungus in treated plate.

Results and Discussion

Inhibition of Mycelial growth (%) as affected by different treatments on *Fusarium oxysporum* f.sp. *ciceri in- vitro* at 5%, 10% and 15% concentration.

The treatments showed significant inhibition at 5% concentration in the mycelial growth as compared to control (T_0). Maximum inhibition was recorded from T_1-Neem leaves (*Azadirachta indica)* (86.61), T_6-Garlic (bulb) (84.95),T_{10}-Congress grass leaf extract (73.79), T_{12}-*Lantana* leaves (*Lantana camara*) (65.38), T_5-Onion (bulb) (63.72), T_{13}-Eucalyptus leaf extract (55.25), T_{14}-Tulsi leaf extract (53.59), T_2-Arjun leaf extract (43.53), T_9-Mint leaf extract (36.08), T_{15}-Ashoka leaf extract (30.4), T_8-Lemon leaf extract (28.75), T_{11}-Dhatura leaf extract (21.73), T_4-Marigold leaf extract (20.08), T_7-Guava leaf extract (16.26), T_3-Jamun leaf extract (14.61), Statistically, all the treatments were significant over control. However at 10% concentration, the maximum inhibition was recorded from T_1-Neem leaves (Azadirachta indica) (89.66), T_2-Arjun leaf extract (55.48), T_3-Jamun leaf extract (15.12), T_4-Marigold leaf extract (57.2), T_5-Onion (bulb) (20.78), T_6-Garlic (bulb) (76.39), T_7-Guava leaf extract (87.94), T_8-Lemon leaf extract (29.76), T_9-Mint leaf extract (37.35), T_{10}-Congress grass leaf extract (16.84), T_{11}-Dhatura leaf extract (65.96), T_{12}-*Lantana* leaves (*Lantana camara*) (22.5), T_{13}-Eucalyptus leaf extract (67.68), T_{14}-Tulsi leaf extract (45.06), T_{15}-Ashoka leaf extract (31.48),Statistically, all the treatments were significant over control. Incase of 15% concentration the treatments showed significant inhibition in the mycelial growth as compared to control (T_0). Maximum inhibition was recorded from T_1-Neem leaves (Azadirachta indica) (94.29), T_2-Arjun leaf extract (47.39), T_3-Jamun leaf extract (69.37), T_4-Marigold leaf extract (21.86), T_5-Onion (bulb) (15.9), T_6-Garlic (bulb) (80.33), T_7-Guava leaf extract (92.48), T_8-Lemon leaf extract (31.3), T_9-Mint leaf extract (39.28), T_{10}-Congress grass leaf extract (58.35), T_{11}-Dhatura leaf extract (23.66), T_{12}-*Lantana* leaves (*Lantana camara*) (71.18), T_{13}-Eucalyptus leaf extract (60.15), T_{14}-Tulsi leaf extract (17.71), T_{15}-Ashoka leaf extract (33.1), Statistically, all the treatments were significant over control.

Table.1 Radial growth (mm) and IOC (inhibition over control) of *Fusarium oxysporum* f. sp.*ciceri on different botanicals at 5%, 10% and 15%* concentration

Treatments	*at 5%*		*at 10%*		*at 15%*	
	Radial growth	% Inhibition	Radial growth	% Inhibition	Radial growth	% Inhibition
T_0-Control	34.37	0	33.2	0	31.57	0
T_1-Neem leaves (Azadirachta indica)	4.6	86.61	3.43	89.66	1.8	94.29
T_2-Arjun leaf extract	19.41	43.53	14.78	55.48	16.61	47.39
T_3-Jamun leaf extract	29.35	14.61	28.18	15.12	9.67	69.37
T_4-Marigold leaf extract	27.47	20.08	14.21	57.2	24.67	21.86
T_5-Onion (bulb)	12.47	63.72	26.3	20.78	26.55	15.9
T_6-Garlic (bulb)	5.17	84.95	7.84	76.39	6.21	80.33
T_7-Guava leaf extract	28.78	16.26	4.00	87.94	2.37	92.48
T_8-Lemon leaf extract	24.49	28.75	23.32	29.76	21.69	31.3
T_9-Mint leaf extract	21.97	36.08	20.8	37.35	19.17	39.28
T_{10}-Congress grass leaf extract	9.01	73.79	27.61	16.84	13.15	58.35
T_{11}-Dhatura leaf extract	26.9	21.73	11.3	65.96	24.1	23.66
T_{12}-*Lantana* leaves (*Lantana camara*)	11.9	65.38	25.73	22.5	9.1	71.18
T_{13}-Eucalyptus leaf extract	15.38	55.25	10.73	67.68	12.58	60.15
T_{14}-Tulsi leaf extract	15.95	53.59	18.24	45.06	25.98	17.71
T_{15}-Ashoka leaf extract	23.92	30.4	22.75	31.48	21.12	33.10
C.D. at 5%	5.743		7.080		9.459	
S. Em. (±)	2.709		3.340		4.462	

Biological control of soil borne plant pathogens is a vital area of plant pathological research all over the world. It is regarded as highly important, as use of chemicals against plant pathogens is becoming very expensive and ecologically dangerous leading to serious health problems in human beings and animals besides polluting soil and water by chemical residues.

Fusarium wilt is a serious disease threat (Khan, 1980), especially in low rainfall areas, where weather conditions are favorable for disease development. The pathogen, *Fusarium oxysporum* f. sp. *ciceri* is a soil inhabitant, enter into the xylem vessels and cause blocking leading to seedling death. The wilted plant shows marked discoloration of the vascular tissues in the stem (Haware *et al.*, 1982).

Therefore, in the present investigation, different aspects of wilt disease and the pathogen have been investigated viz. isolation and identification of pathogens, Isolation and identification of botanicals in vitro assessment, management of the disease under a lab trial.

Isolation, identification, and pathogenicity of pathogen

The causal organism, *F. oxysporum* f. sp. *ciceri*, was isolated from diseased parts of chickpea by following standard tissue isolation method. The pure culture of F. oxysporum f. sp. *ciceri* was obtained by hyphal tip method.

Fisher *et al.*, (1982) isolated *F. oxysporum* f. sp. ciceri from infected chickpea plant. Nikam

et al., (2011) also collected wilt infected chickpea plant samples from different locations and isolated *F. oxysporum* f. sp. *Ciceri* on potato dextrose agar (PDA) in the laboratory.

Identification of pathogen

The pathogen was identified as *F. oxysporum* f. sp. *ciceri* based on its morphological characters as described by Booth (1971). The pathogen produced white dense cotton like radiating mycelium on PDA.

Present results are in conformity with the results obtained by Deepak *et al.,* (2013) who identified the culture based on morphological, cultural characteristics of the pathogen and symptomatology, the pathogen was identified as *F. Oxysporum* f. sp. *ciceri.* Nikam *et al.*(2011) identified the culture based on morphological, cultural characteristicsof the pathogen and symptomatology, the fungal pathogen was identified as *F. oxysporum* f. sp. *ciceri.*

In vitro evaluation of botanicals against wilt pathogen

To minimize the use of fungicides and encourage organic farming 15 botanicals were tested in laboratory against *F. oxysporum* f. sp. *ciceri.*

Management of soil borne plant pathogens could be achieved by different fungicides, soil fumigants (Methyl bromide) and bioagents. Hence management of soil borne plant pathogens is difficult to achieve chemically, in this context plant extracts can be used as an alternative source for controlling soil-borne diseases since they comprise a rich source of bioactive substance. Plants extracts are eco-friendly possess protective, curative and antagonistic activity against many diseases.(Kandasamy *et al.,* 1974; Hale and Mathers, 1977; Rahber, 1986; Kalo and Taniguchi, 1987).

The data revealed that, most of the plant extracts showed fungistatic nature at higher concentration (15%). All the plant extracts showed more than 30 per cent inhibition of mycelial growth, maximum was found in Neem leaf extract.

At 10 per cent concentration, four plants extracts namely Eucalyptus leaf extract, Lantana leaf extract, Congress grass leaf extract, and Thulsi leaf extract showed more than 25 per cent inhibition of mycelial growth. Effectiveness of Neem leaf and lantana leaf extract against *F. oxysporum* f. sp. *ciceri* is supported by the work of Kamdi *et al.,* (2012) and Hossain *et al*.(2013).

The inhibitive action of garlic bulb crude extract on fungal growth has been attributed to the existence of allicin, as the major anti-bacterial, anti-fungal and anti-viral component (Miron *et al.,* 2000). The fungicidal spectrum of *Azadirachta indica* has been attributed to presence of azadiractin which belongs to C25 terpenoides compounds (Subramanian 1993).

Further, it can be concluded that the efficacy of botanicals differed significantly in inhibiting the radial growth of the test fungus at all the three concentrations (5, 10 and 15%). The higher concentration of all the botanicals offered significantly higher percent mycelial growth inhibition as compared to their lower levels.

Among the different botanicals, Neem leaf extract had significant antifungal properties against wilt pathogen of Chickpea followed by Guava leaves extracts. These botanicals could be further subjected to lab trials to access their effectiveness under lab conditions.

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