

Original Research Article

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To Evaluate the Bio-efficacy of Botanical Leaf Extracts against *Fusarium oxysporum* F. SP.ciceri Causing Wilt in Chickpea under *in-vitro* Condition

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ABSTRACT

Keywords

Fusarium, *In-vitro* evaluation, Plant leaf extracts and wilt

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Gram belong to the order *Fabales* and family *Fabaceae* is an herbaceous annual, having branching close to the ground with semi-erect to semi-spreading habit. This crop (chickpea) is subjected to attack by a number of fungal, viral, bacterial and nematode diseases. Chickpea wilt caused by *F. oxysporum* f. sp. *ciceri*. In vitro evaluation of four plant leaf extracts revealed that, among these extracts per cent inhibition over control was found maximum (71.75) in *Azadirachta indica* followed by *Lantana camara* (59.23) and it was found minimum in *Argemone mexicana* (41.69). *Parthenium hysterophorus* was found least (46.77) effective to inhibit the mycelial growth of *Fusarium oxysporum* f. sp. *ciceri* under *in-vitro* conditions.

Introduction

Pulse crops play an important role in Indian agriculture, besides being rich in protein. Chickpea is a cheap source of protein compared to animal protein. In India chickpea (*Cicer arietinum* L.) variously known as Gram or Bengal gram. Chickpea wilt caused by *F. oxysporum* f. sp. *ciceri*. Occurrence of chickpea wilt was first described in India by Butler (1918). In human it's also consumed as whole seed in the form of fried and boiled for

to full fill the protein deficiency. Gram flour is mixed with wheat flour to improve the protein content of wheat flour and it's used in making chapatti (Roti). One hundred-gram of chickpea seeds contain about 9.8 per cent Moisture, 21.1 g Protein, 5.3 g Fat, 61.0 g Carbohydrates and 3.9 g Fiber in addition to vitamins.

This crop (chickpea) is subjected to attack by a number of fungal, viral, bacterial and nematode diseases. According to Singh *et al.*,

(1980) they were reported that the seed treatments with Neem oil are produced disease free seedlings. To study the compatibility as well the antifungal mechanism of plant extracts the poisoned food technique (Haware and Nene, 1980) was used, they were reported that the chickpea wilt causes complete loss in grain yield if the disease occurs in the vegetative and reproductive stages of the crop and when seed harvested from wilted plants they were lighter than those collect from healthy chickpea plants. The fungus to be present into the hilum of the infected seed in the form of chlamydospores like structures Haware *et al.*, (1982). Leaf extract of *Azadirachta indica* 100 per cent controlled the spore germination of *F. oxysporum* followed by *Lantana camara* (Gupta and Bansal, 2003). Infected seeds play an important role in the long-distance dispersal of the pathogen and in its introduction in to *F. oxysporum* f. sp. *ciceri* free soils and geographic areas also Pande *et al.*, (2007). *Fusarium* wilt is one of the major diseases of chickpea and they were found that at national level the yield losses up to the tune of 60 per cent Singh *et al.*, (2010). *F. oxysporum* f. sp. *ciceri* infects chickpea at seedling as well as at flowering and pod forming stage, with more incidence at flowering and pod filling stage, if the crop is subjected to sudden temperature rise and water stress. It is more prevalent in lower latitudes (0-30°N) where growing season is relatively dryer and warmer than in the higher latitudes (30-40°N) Arunodhyan *et al.*, (2014). Test pathogen (*F. oxysporum* f. sp. *ciceri*) is the seed borne in nature and found that its mycelium was presented in the hilum region of the infected seeds which was collected from wilt affected plants of chickpea when it was isolated on Potato dextrose agar (PDA) (Trivedi and Rathi, 2016). *F. oxysporum* f. sp. *ciceri* is the most destructive and widespread fungal disease of chickpea. It has drastic effect on yield causing cent per cent loss

under favorable conditions (Suman Patra and Mohan Kumar Biswas, 2017).

Test pathogen

The pathogen (*F. oxysporum* f. sp. *ciceri*) is a facultative saprophyte or a heterotroph in nature and it can survive as mycelium and chlamydospores in seed, soil and also on infected crop residues. It can easily obtain their food from organic matter through decomposing method. Test pathogen life cycle can be divided into three stages like dormant, parasitic and saprophytic stages. Test pathogen proceeds to infect the host through its sporangial germ tube or through mycelium by invading the plants roots. If any wound are found in the roots or at the formation point of lateral roots the fungus were inserted into this. As its growth, the mycelium branched and produces microconidia, which are carried upward within the vessel by way of the plants sap stream. They are easily isolated from tap root, lateral roots and collar region, main stem, lateral branches and pod hulls also Mahendra Pal *et al.*, (1998).

Wilt

Chickpea wilt caused by *F. oxysporum* f. sp. *ciceri*. Its symptoms can be seen on the seedlings as well as at the maturity stage of the diseased plants. Yellow dry leaves are defoliated; marginal necrosis of infected leaves is also seen. Reddish to black discoloration of the xylem vessels are seen inside the infected plant near by ground stem as a line or dots in cross section. Roots of wilted plants turn black and decompose in later stage and ultimately dry up.

Materials and Methods

A study has been conducted at Department of Botany, Meerut Collage; Meerut (Uttar

Pradesh) to know the bio-efficacy of different botanicals leaf extract against *F. oxysporum* was assessed by poison food technique (Nene and Thapliyal, 1982).

The experiment was successfully conducted under *In-vitro* condition during the year 2019-20 in the laboratory of the said Department of Botany.

Botanicals

***Azadirachta indica* (Neem tree)**

It has commonly known as Neem, Nimtree or Indian lilac. Neem is a tree in the mahogany family *Meliaceae*. Neem are used as key ingredient in non-pesticidal management (NPM), providing a natural alternative to synthetic pesticides. Neem seeds kernel extract and its leaf powder are use as botanical pesticide. It acts as anti-fungal properties.

***Parthenium hysterophorus* (Weed plant)**

It has commonly known as Gajarghans and it is the most common invasive species in India. *Parthenium* is a genus of North American shrubs in the sunflower tribe within the Daisy family. All members of this genus has commonly known as feverfew.

***Lantana camara* (Ornamental plant)**

Lantana camera, commonly known as wild-sage and trickery. It's having small tubular shaped flowers. Belong to the family *Verberaceae*. Its leaf extract were found anti-fungal property in nature.

***Argemone mexicana* (Pioneer plant)**

Argemone mexicana belong to the family *Papaveraceae*. Its seeds contain 22-36% of non-edible oil which is also called as

Argimone oil or Kalkar oil. This oil contains some toxic alkaloids like; *Sanguinarine* and *Dihydrosanguinarine* act as anti-fungal property in nature.

Procedure of Isolation, purification and identification of the test pathogen

Isolation

Typical characteristic symptoms of the wilting under natural condition were taken and brought to the laboratory of the Department of Botany, Meerut College, Meerut (U.P.) India. Method of isolation was followed as according to Aneja (2007) he was suggested that isolation was making from the infected root samples and its purification of the culture was successfully done by repeated transfer of hyphal tip and with single-spore culture by dilution method. Its underground portion of roots-stem up to height of collar region was selected for the isolation of the test pathogen.

Firstly selected parts of diseased specimens were properly washed in running tap water to remove dust particle form the surface of whole parts to minimize the further contamination. The washed whole diseased root-stem parts were split open and small bits (2.5 mm) were cut from the portion of collar region along with some healthy portions with the help of sterilized blade or knife. The small bit (piece) was surface sterilized with 0.1 per cent solution of Sodium hypochlorite (NaOCl) for one minute under aseptic conditions. After that it's washed three times in sterilized water to remove the traces of solution from the surface of particular pieces. Excess moisture was removed from the surface of piece by placing them between two fold of pre-sterilized blotting paper.

Five dry pieces of sterilized root were placed in each Petri-dish four at the corner of Petri-

dish and one piece in the centre of dish. These Petri-dishes were incubated in Biological oxygen demand (B.O.D.) at 28 ± 2^0 C temperature for 2 days. After 24 hours of inoculation when mycelial growth was visible in different Petri-dishes around the incubated pieces of root, finally cut advanced growth stage (hyphal tip) of the mycelium and further it was transferred into the agar slants test tube.

Purification

Two per cent dilute spore suspension in distilled water was poured on plain agar Petri-dishes to form a very thin layer surface and spore suspension was allowed to fix down on this layer. Then fixed spores were separated singly from each other. It was marked separately by glass marker. Circled spore was lifted along with agar layer in a form of block with the help of dummy cutter from Petri-dishes. Block was transferred to Petri-dishes which were pre-contained 2 per cent sterilized PDA medium. As soon as the mycelial growth was clearly visible from single spore block, then regular sub-culturing was done, till pure culture was not obtained.

Identification

For identification of the test pathogen was firstly based on its cultural characteristics *viz.*, radial growth characteristics and sporulation of spore and secondly based on its morphological characteristics *viz.*, colony, mycelium, sporodochia, microconidia & macroconidia and chlamydo spores features. The observations on radial growth, colony growth, colony pattern, substrate colour and mycelium colour of test pathogen were recorded as suggested by (Sonkar *et al.*, 2014).

Morphological studies of the pathogen

For identification of the test pathogen, which is based on its morphological study of the wilt

disease causing fungus *F. oxysporum* f. sp. *ciceri* through the use of best suited medium (PDA) was taken for obtaining its better mycelial growth. When mycelial growth of test pathogen were clearly seen on the PDA surface, it slide was prepared with the help of mounting medium *viz.*, cotton blue and lacto phenol then observed under stereo binocular (6.4-40x).

Cultural studies of the pathogen

Identification of the test pathogen (*F. oxysporum* f. sp. *ciceri*) during the investigation which was based on its cultural characteristic which is mainly based on measurements of its radial growth of mycelium on different mediums and other on its spore-sporulation that is mainly based on average grade percentage of its spore presence on glass slide under stereo binocular microscope.

Preparation of plant leaf extracts (Botanicals)

Total four botanicals *viz.*, *Azadirachta indica* (Neem tree), *Parthenium hysterophorus* (Weed plant), *Lantana camara* (Ornamental plant), and *Argemone mexicana* (Pioneer plant) was taken for this investigation.

The plant extracts were prepared by grinding of fresh leaves in a pestle and mortar by using sterilized distilled water. All were used as a stock solution for this experiment. Total hundred g of fresh leaf material from all four botanicals, separately were harvested from natural flora and it's were washed properly with running tap water, then it were rinsed with distilled water. All rinsed leafy materials were air dried under aseptically conditions and was macerated separately with 100 ml of distilled water in a warring blender. The leaf extract was filtered separately in plastic test tubes with required dose through the help of double-layered muslin cloth and then it was

centrifuged at 5000 rpm for 5 minutes. Its supernatant was collected and filter through the use of Whatman No. 1 filter paper. Each filtrate liquid was heat sterilized and preserve as stock (100%) solution under aseptically conditions.

***In-vitro* evaluation of plant leaf extracts against test pathogen through Poisoned food technique**

Two ml of stock solution of extract was incorporated into the 100 ml medium (PDA) to make 2% concentration of the extract. 15 ml of incorporated molten PDA poured into sterilized autoclavable glass Petri-plates.

After solidification of inoculated PDA medium, all these plates were inoculate individually with the 5 mm in diameter disc of pure culture of *Fusarium oxysporum* f. sp. *ciceri* was placed on PDA surface at the centre.

Un-inoculated (with the stoke solutions-plant extracts) PDA medium was poured and after its solidification of these Petri-plates only were inoculated with the same disc size (5mm) of *Fusarium oxysporum* f. sp. *ciceri* was served as control. Then after all these plate were incubated at 25±1⁰ C under incubation room for 7 days.

The experiment was conducted in completely randomized block design with four replications and five treatments included suitable control (without adding any botanical extracts into the PDA).

Experimental details

Design : CRD
 Replication : Four
 Treatments : Five

Treatments details

Treatments	Common name	Scientific name
T ₁	Neem (Tree)	<i>Azadirachta indica</i>
T ₂	Gajarghans (Weed plant)	<i>Parthenium hysterophorus</i>
T ₃	Wild-sage (Ornamental plant)	<i>Lantana camara</i>
T ₄	Maxican poppy (Pioneer plant)	<i>Argemone mexicana</i>
T ₅	Control	-

The observation on radial mycelial growth/ colony diameter in millimetre (mm) of the test pathogen (*Fusarium oxysporum* f. sp. *ciceri*) was assessing at an interval of 24 hours and continued till untreated plates were fully covered with test pathogen mycelial growth.

The efficacy of plant leaf extracts (botanicals) was expressed as per cent of radial growth/cfu over the control which was calculated by using the formula of (Vincent, 1947).

$$\text{Per cent inhibition (I)} = \frac{C - T}{C} \times 100$$

Where,

I= Per cent inhibition

C = Growth of the test fungus in untreated control plates.

T = Growth of the test fungus in treated plates.

* Measuring scale: In millimetre (mm).

Results and Discussion

The results obtained from the present investigation as well as relevant discussion have been summarized under below head:

In-vitro evaluation of plant leaf extracts against test pathogen

Natural products which were isolated from plant appear to be one of the best alternatives in plant disease management as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides.

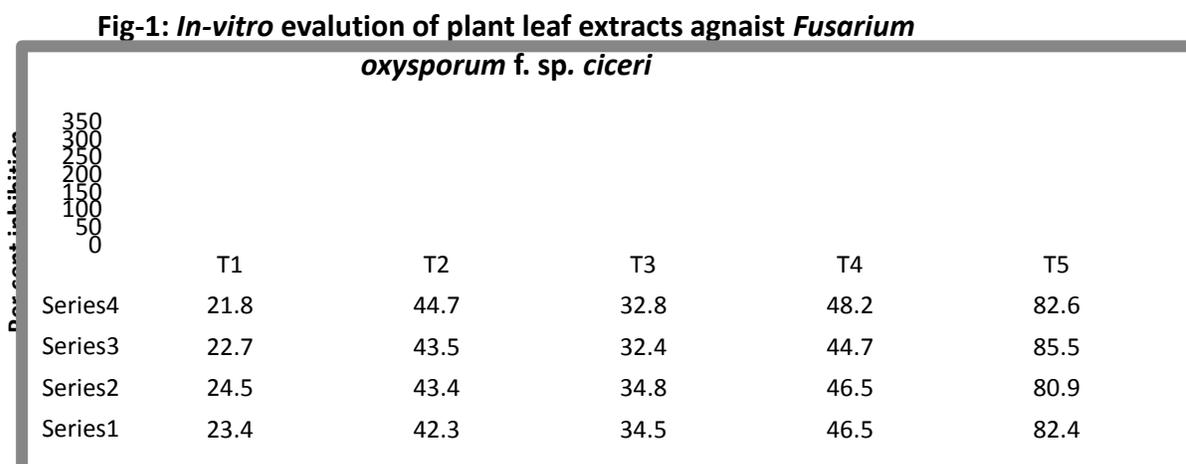
The result presented in table -1 revealed that the per cent inhibition over control was found maximum (71.75) in the leaf extracts of *Azadirachta indica* followed by *Lantana camara* (59.23) and it was found minimum into the leaf extract of *Argemone mexicana* (41.69). *Parthenium hysterophorus* was found least (46.77) effective to inhibit the mycelial growth of *Fusarium oxysporum* f. sp. *ciceri* under *in-vitro* conditions (Fig. 1).

Table.1 *In-vitro* evaluation of plant leaf extracts against *Fusarium oxysporum* f. sp. *ciceri*

Tr. No.	Treatment details	Colony growth (mm)				Mean (%)	Per cent Inhibition over control
		R ₁	R ₂	R ₃	R ₄		
T ₁	<i>Azadirachta indica</i>	22.4 (28.24)	24.6 (29.73)	21.7 (27.76)	23.8 (29.19)	23.12 (28.73)	71.75 (57.89)
T ₂	<i>Parthenium hysterophorus</i>	42.3 (40.57)	44.0 (41.55)	42.5 (40.68)	45.7 (42.53)	43.62 (41.33)	46.70 (43.10)
T ₃	<i>Lantana camara</i>	32.5 (34.75)	34.2 (35.78)	33.0 (35.06)	33.8 (35.54)	33.37 (35.28)	59.23 (50.31)
T ₄	<i>Argemone mexicana</i>	47.5 (43.56)	48.9 (44.36)	45.3 (42.30)	49.2 (44.54)	47.72 (43.69)	41.69 (40.21)
T ₅	Control	82.4 (65.19)	78.9 (62.65)	84.5 (66.81)	81.6 (64.59)	81.85 (64.78)	--
SE (m)						0.817	
CD @ 5%						2.484	

*=Mean of four replications.

*Figure in parentheses indicates transformed values



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