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A NEW PHYTOREMEDIATION STRATEGY FOR CHICKPEA WILT DISEASE MANAGEMENT

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ABSTRACT

Extract of 58 plant spp. belonging to angiospermic families were sreened against *Fusarium oxysporum* f.sp. *ciceri* causing chickpea wilt by Poisoned Food Technique under laboratory condition. *Tylophora asthmatica* exhibited 100 percent inhibition of mycelial growth. The MIC was 400×10^3µl/l. The extract was fungistatic at its MIC but was fungicidal at higher concentration (900×10^3µl/l). The efficacy of extract was neither affected by increased inoculum density nor by temperature. *In vivo* trials it was observed to be non-phytotoxic and also managed the wilting in chickpea.

KEYWORDS: *Tylophora asthmatica* exhibited 100 percent inhibition of mycelial growth. The MIC was 400×10^3µl/l.

INTRODUCTION

Wilt of chickpea (*Cicer arietinum* L.) caused by *Fusarium oxysporum* Schl. Emend. Snyd. And Hans. f.sp. *ciceri* (Padw.) Snyder and Hans. was first described by Padwick[18] and has been reported from several countries.[16] The disease causes 20-100 percent losses in crop yield.[1,10,19] In India, it is estimated to cause 10 percent annual loss in yield.[21] Among the major pulse crops produced in India chickpea contributes nearly 42-47 percent of total pulse production.[4] Various control methods have been reported so for. Most of them are use of chemicals[11,17] micro organisms[3,9,22] or solar treatment,[12] From the literature available it was noted that not much work has been done on the influence of plant extract against *Fusarium oxysporum* f.sp. *ciceri*.[15,20]

MATERIALS AND METHODS

Cultures of *Fusarium oxysporum* f.sp. *ciceri* (ITCC No. 2789) causing wilt of *Cicer arietinum* L. was procured from IARI New Delhi. This was preserved in the Department of Botany DDU Gorakhpur in sterilized condition. Pathogenicity test was conducted to confirm the pathogenic nature of the *Fusarium oxysporum* f.sp. *ciceri* on its host *Cicer arietinum* L. Plants from families belonging to angiosperm were collected from Gorakhpur district. Freshly collected leaves of each plant was surface sterilized by dipping in 0.1 percent solution of mercuric chloride and were then washed with sterile water. The plant parts (100g) were chopped in to pieces and macerated with 100 ml of sterile water (1:1 w/v) in mortar with a pestle. To obtain the extract, the pulp was squeezed through a double layer of muslin cloth. As suggested by Gupta and Banerjee, 1970.[8]

2.0 mg of streptopenicillin was added to the extract to prevent bacterial contamination during the course of experimentation. The fungitoxicity of aquous extract against *Fusarium oxysporum* f.sp. *ciceri* was tested by the Poisoned Food Technique of Grover and Moore 1962[7] using Czapek Dox Agar medium. For treatment sets five ml of prepared extract of each plant was mixed with five ml of Czapek Dox Agar medium in pre sterilized Petriplate and agitated in sound fashion in order to mix the extract homogenously. In control sets requisite amount of sterile water was added in place of the extract of plant. Assay disc of 0.5 cm of diameter, cut from the periphery of seven days old culture of *Fusarium oxysporum* f.sp. *ciceri* was aseptically inoculated in treatment as well as control sets. The Petriplates were kept for six days. The percent inhibition of mycelial was calculated by using following formula Vincent 1947.[23]

$$\text{Percent inhibition of mycelial growth} = \frac{d_C - d_t \times 100}{d_C}$$

d_C = average diameter of fungal colony in control sets. d_t = average diameter of fungal colony in treatment sets.

The experiment was repeated twice and for each test triplicate sets were maintained.

To find out the minimum concentration of the extract for obtaining absolute inhibition of mycelia growth of *Fusarium oxysporum* f.sp. *ciceri*, experiments were conducted following the Poisoned Food Technique of Grover and Moore,1962(7) at different concentrations viz., 1000×10^3µl/l, 900×10^3µl/l, 800×10^3µl/l, 700×10^3µl/l, 600×10^3µl/l, 500×10^3µl/l, 400×10^3µl/l,

300×10^3µl/l, 200×10^3µl/l and 100×10^3µl/l.

To ascertain the fungistatic/fungicidal nature of the extract, tests were performed according to the method of Garbour and Houston, 1952.[6]

Effect of increased inoculums density of the *Fusarium oxysporum* f.sp. *ciceri* on the fungitoxicity of the extract was studied by the Poisoned Food Technique, using Czapek Dox liquid medium as recommended by Mishra, 1975.[14]

To find out the effect of temperature the extract was subjected to different temperature (15°c, 20 °c, 25 °c, 30 °c, 35 °c, 40 °c, 45 °c, 50 °c and 55 °c) treatments for one hour in an incubator. Fungitoxicity of treated extract of each set was tested separately, against the *Fusarium oxysporum* f.sp. *ciceri* at its MIC, by usual Poisoned Food Technique.

The fungitoxic spectrum of extract was determine at its MIC against some fungi causing damping off, fruit/vegetable rot, common storage and cellulolytic fungi as well as two spp. causing wilt of cotton and potato by the Poisoned Food Technique of Grover and Moore, 1962.[7]

In vivo efficacy of active plant against *Fusarium oxysporum* f.sp. *ciceri* was evaluated by the method of Bhat and Shukla, 2001.[2] Natural garden soil was collected and dried under sun, passed through 2.0 mm sieve and autoclaved for two hours at 30 lbs/inch^2 pressure for two consecutive days. The earthen pots (15 cm diameter) were sterilized with five percent NaOcl (Sodium hypochlorite) solution and thoroughly rinsed with sterile water before use. The pots were filled up to 10 cm height with a mixture of sterilized soil, five percent w/w inoculum of *Fusarium oxysporum* f.sp. *ciceri* multiplied on soil maize meal medium separately and allowed for multiplication for two days at ambient temperature i.e. 20-28°c for soil infestation. The inoculated pots were then drenched with 0.1 percent shade dried leaves of active plant in the form of powder. This was treatment set (II). Unammended soil sample without fungus inoculum served as control set (I). The third set comprised of unammended soil sample treated with fungus inoculum i.e. sick pot set (III). After two weeks of sowing seedling of control and treatment sets were observed for seed germination and symptoms of disease.

OBSERVATIONS AND RESULTS

Pathogenicity test was conducted to confirm the pathogenic nature of *Fusarium oxysporum* f.sp. *ciceri* procured from ITCC, IARI New Delhi on their host i.e. *Cicer arietinum*. Result is given in plate no. 01. The pathogenicity test was positive because it confirmed Koch's postulates.

Plate No. 1: Showing pathogenicity of *Fusarium oxysporum* f.sp. *ciceri* on *Cicer.* Arietinum.

Table 01: Screening of extracts of some angiospermic plants for their fungitoxicity against mycelial growth of *Fusarium oxysporum* f.sp. *cicero.*

Plants	Percent inhibition of mycelial growth of *Fusarium oxysporum* f.sp. *ciceri*
1	2
Acanthaceae	
Astercantha longifolia Nees.	61
Hemiadelphis polyspermus Nees.	66
Amaranthaceae	
Alternenthra sessilis (L.) DC.	50
Amaranthus viridis L.	46
Digera arvensis Forsk	43
Gomphrena globosa L.	61
Apocyanaceae	
arrisa carandas L.	79
1	2
Asclepiadaceae	
***Tylophora asthmatica* (L.) Wight and Arn**	100
Asteraceae	
Ageratum conyzoides L.	53
Blumea lanciniata Roxb.	42
Eclipta prostrata L.	50
Gnephalium leuteo-album L.	80
Sonchus asper (L.) Hill.	40

Spilanthes acmella (L.) Murr.	40
Launaea nudicaulis Les.	37
Tridex procumbance L.	35
Vernonia cineria Less.	45
Bignoniaceae	
Adenocallyma alliaceum Miers.	59
Brassicaceae	
Rorippa indica (L.) Hien.	25
Caesalpiniaceae	
Cassia occidentalis L.	89
Cassia siamea Lamk.	36
Convolvulaceae	
Ipoemea angulata Lamk.	64
Ipoemea pestigridis L.	67
Euphorbiaceae	
Acalypha indica L.	51
Jatropha gossipifolia L.	64
Phyllanthus urinaria L.	42
Ricinus communis L.	57
Fabaceae	
Aeschynomene indica L.	65
1	2
Crotolaria juncea L.	62
Desmodium gangeticum (L) D.C.	54
Erythrina vatiegata L.	78
Melilotus alba Desr.	36
Lamiaceae	
Anisomalis indica (L.) O Kuntze.	81
Hyptis suaveolens L.	73
Leonotis nepataefolia R.Br.	61
Leonurus sibricus L.	58
Ocimum bacilicum L.	66
Ocimum sanctum L.	64
Malvaceae	
Sida cordifolia L.	71
Urena lobata L.	36
Menispermaceae	
Coculus hirsutus (Diels) L.	53
Moraceae	
Ficus hispida L.	50
Myrtaceae	
Calestemon lanceolatus D.C.	59
Piperaceae	
Piper longum L.	85
Plumbaginaceae	
Plumbago zeylanica L.	88
Polygonaceae	
Antigonon leptopus Hook and Arn	48

Polygonum limbatum Meissn.	36
Rubiaceae	
Oldenlandia corymbosa L.	42
1	2
Rutaceae	65
Murraya paniculata (L.) Jack.	
Scrophulariaceae	56
Mazus pumilus (Burm.f.) Steenis	40
Micardonia dienthera (SW) Pannell	
Solanaceae	57
Solanum nigrum L.	80
Solanum tarvum Sw.	
Telliaceae	50
Triumfetta rhomboidea Jacq.	
Urticaceae	58
Pouzolzia zeylonica (L.) Benn.	
Verbenaceae	71
Clerodendrum indicum L.	50
Phyla nodiflora L.	
Zygophyllaceae	40
Tribulus terrestris L.	

Screening of extracts of leaves of the plant was done for their fungitoxicity against *Fusarium oxysporum* f.sp. *ciceri*. The results of screening of each plant have been presented in table-01. The plants in table- 01 are grouped alphabaticaly under their respective family. It is evident from the data in table- 01 that aqueous extract of 58 species of plants belonging to families of angiosperm, screened against the *Fusarium oxysporum* f.sp. *ciceri*, most of the species showed either poor (below 50 percent) or moderate (above 50 percent and below 100 percent) activity, *Tylophora asthmatica* was found to be exhibit absolute toxicity against the *Fusarium oxysporum* f.sp. *ciceri*. There were six plant species which exhibited fungitoxicity in between 80 to 90 percent. The plant species are *Gnephalium leuteo-album* (80 percent), *Cassia occidentalis* (89 percent), *Anisomalis indica* (81 percent), *Piper longum* (85 percent), *Plumbago zeylanica* (88 percent) and *Solanum torvum* (80 percent).

The minimum concentration of the extract of *Tylophora asthmatica* at which it checked the mycelial growth of *Fusarium oxysporum* f.sp. *ciceri* has given in fig. 01. From the fig. 01 it is evident that the minimum concentration of plant extract at which it checked the mycelial growth of the pathogen (*Fusarium oxysporum* f.sp. *ciceri*) was 400×10^3 µl/l.

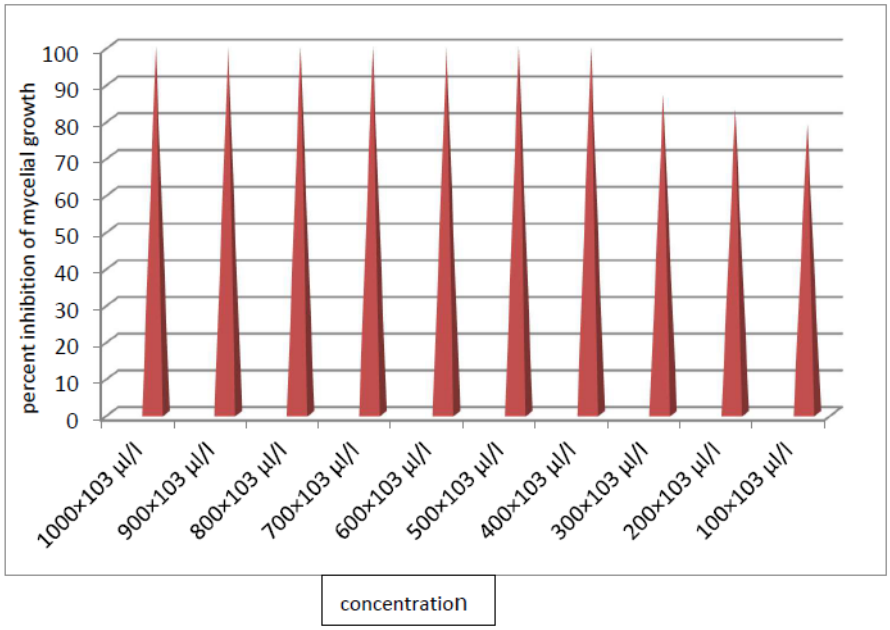

Fig. 01: MIC of plant extract of *Tylophora asthmatica* against mycelial growth of *Fusarium oxysporum* f.sp. *cicero.*

It may be noticed from the fig. 02 that the extract of *Tylophora asthmatica* was found to be fungicidal at higher concentration (900×10^3 µl/l) and fungistatic at its MIC (400×10^3 µl/l).

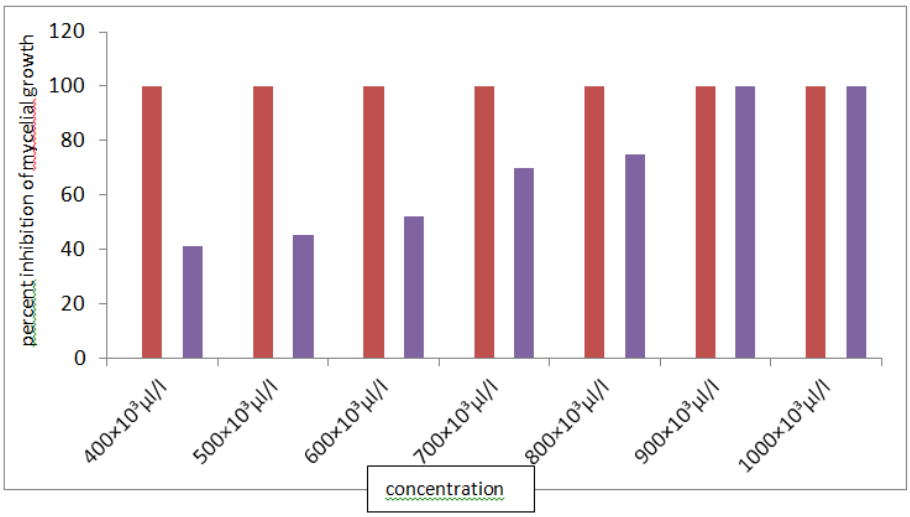

Fig. 02: Nature of toxicity of plant extract of *Tylophora asthmatica* against *Fusarium oxysporum* f.sp. *cicero.*

Increased inoculums density up to 10 disc and range of temperature i.e. 15°c, 20°c, 25°c, 30°c, 35°c, 40°c, 45°c, 50°c and 55°c were found not be affect the fungitoxic nature of the extract.

Table 02: Fungitoxic spectrum of extract of *Tylophora asthmatica* at their MIC against some fungi.

Fungi	Percent inhibition of mycelial Growth *Tylophora asthamatica* L.
1	2
Alternaria alternata	69
Aspergillus candidus	12
Aspergillus flavus	19
1	2
Aspergillus niger	20
Aspergillus sydowi	0
Colletotrichum capsici ITCC NO. 6023	65
Colletotrichum chlorophyti ITCC NO. 3535	62
Colletotrichum dematium ITCC NO. 4970	32
Colletotrichum gloeosporioides ITCC NO. 6038	31
Coleeotrichum truncatum ITCC NO. 1047	46
Fusarium oxysporum f.sp. v*asinfectum*	60
Fusarium solani	67
Lasiodeplodia theobroma	47
Penicillium chrysogenum	44
Pestalotia bicolor	35
Pestalotia foetidans	45
Pythium aphanidermatum ITCC NO. 123	62
Pythium aphanidermatum ITCC NO. 502	38
Pythium aphanidermatum ITCC NO. 4747	43
Pythium debaryanum ITCC NO. 95	61
Pythium dissotoccum ITCC NO. 5168	64
Pythium vexans ITCC NO. 5170	48
Pythium vexans ITCC NO. 5169	70
Spegazzinia intermedia	62
Trichothecium roseum	67
1	2
Ulocladium botrytis	35
Verticillium albo-atrum	48

The results given in table- 02 show the fungitoxic spectrum of plant extract of *Tylophora asthmatica*. It is evident from the data that the plant extract did not exhibited broad range of antifungal activity.

Table 03: Showing germination of seeds of *Cicer arietinum* in soil infested by *Fusarium oxysporum* f.sp. *ciceri* treated by dried leaves of *Tylophora asthmatica* in the form of powder.

Germination in days	I	II	III
1	-	-	-
2	-	-	-
3	-	-	-
4	-	-	-
5	-	-	-
6	+	+	-
7	+	+	-
8	+	+	+
9	+	+	+
10	+	+	+
11	+	+	+
12	+	+	+

I = Unamended soil sample without fungus inoculums

II = Soil sample treated with dried leaves of *Tylophora asthmatica* in the form of powder III= Soil sample treated with fungus inoculums.

+ = Presence of germination - = Absence of germination

Data of table 03 exhibits that 100 percent germination of seeds of *Cicer arietinum* was on sixth day from the date of sowing in set I and set II. However, time taken for the 100 percent germination of seeds was on 8th day in set III.

Plate 02: showing *in-vivo* efficacy of plant *Tylophora asthmatica* in the form of powder on *Cicer arietinum* inoculated by *Fusarium oxysporum* f.sp. *ciceri*

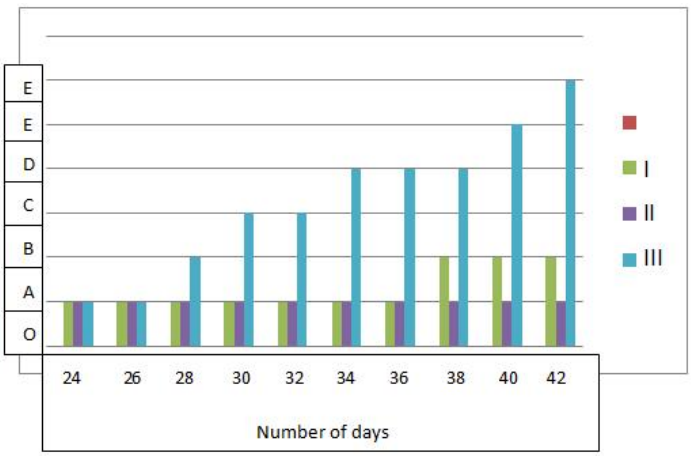

Fig. 03: Symptoms of wilting in three weeks old seedlings of *Cicer arietinum* treated by dried leaves of *Tylophora asthmatica* in the form of powder

A= Absence of any symptom. B= Chlorosis and drooping of lower leaves. C= Wilting of lower leaves. D= wilting of all leaves except terminal one. E= Complete wilting without desiccation of leaves. F= Complete wilting wilting of all the leaves and terminal portion of stem and desiccation of leaves.

From the results of fig. 03 and plate 02 it is evident that seedlings of *Cicer arietinum* did not show any symptom of wilting, there was no chlorosis and drooping of leaves when treated with dried leaves up to 42 days. There was chlorosis and drooping of lower leaves in set III after 28 days. There was wilting of lower leaves after 30 days and wilting of all leaves except terminal one after 34 days. There was complete wilting of all the leaves, terminal portion of stem and desiccation of leaves after 42 days.

DISCUSSION

Plants are primary producers of food for humans, as well as animals beneficial for human wellfare. With rapid increase in global population the demand on food sources has increased trmendously. The shortage of food can be attributed to several causes eg. less production due to poor technology, losses caused by enemies of plants and rising world population. Protection of the crop and its produces against pest, diseases is considered an approach to increase the availability of food. *Fusarium* wilts are very important among plant disease because they cause severe losses on most vegetables, field crops, plantation crop and shade trees which are used by common people in their daily life. Most of the wilt causing *Fusarium* belongs to the species, *oxysporum* and the different host plants are attacked by special forms or races of the species.

This paper described an attempt to explore the possibility of using higher plant extract as herbal fungitoxicant in order to manage wilt disease of *Cicer arietinum*.

The use of resistant varities is a chief resort of plant pathologists in controlling plant diseases but it is difficult to find out suitable parental material possessing resistant in high yielding variety. The use of cultural practices reduces wilt incidence to certain extent. But considering the various climatic zones in our country this practices has not gained popularity amongst the farmers and growers of pulses, fruit and spices. In recent past biological control has attracted much attention of scientists as a substitute of chemicals and other methods. But during evolution the saprophytes have been found to become parasite.

Tropical forests and many other tropical ecosystems are rich sources of a diversity of plant derived chemical compounds, not only because of the high species diversity but also because of the "eternal summer" which forces the plant species to the constant production of chemical defense compounds.

Usually there is no definit criteria for the selection of test organism but it is necessary to have definit objective for the selection of test organism as said by Mahadevan (1982) (13). In the present study *Fusarium oxysporum* f.sp. *ciceri* which causes wilt of *Cicer arietinum* was selected to find out the possibility of utilizing extract of higher plants.

In the present work because the cultures were obtained from IARI New Delhi it was thought desirable to confirm their pathogenic nature of the *Fusarium oxysporum* f.sp. *ciceri* on its host.

The fungitoxic substance of higher plants range in their chemical nature from simple phenols to highly complex steroids and no single organic solvent is capcble of extracting such a heterogenous group of substances. (5, 13) An ideal screening is that which extracts largrest number of sample in shortage duration with least possible cost and sensitive to detect the active principle present even in low concentration. Therefore in the present study water was used for the extraction. Some antifungal chemical present in form of inactive precursor in higher plant are liberated by enzymatic action on rupture of cells and thus there is complete release of all the active principles. In the present study therefore the leaves were macirated before making their extract.

For any fungitoxicant it is very important to know the exact concentration at which it just kills or stops the growth of the fungus. This helps in describing efficacy as well as in prescribing it appropriate dose. High dose will increase its rational value, increase its westage and may cause considerable harm to the quality of commodity treated. Keeping this fact in mind the MIC of *Tylophora asthmatica* was tested.

As said by Skinner 1995 the potency of an antibiotic is markedly dependent on the number of organisms it has to combat. In the present study effect of increased inoculum density on *Fusarium oxysporum* f.sp. *ciceri* was observed. There was no adverse effect of the increased inoculum density.

A fungitoxicant may exhibit broad range of spectrum inhibiting many fungi or it may be effctive against some specific once only. In the present study the extract was found to possess narrow range of fungitoxicity. Therefore its market value good make it uneconomical to producers on the other hand some fungitoxicant are more important or more usefull because they will cause less harm to the local soil fungal flora in case of wilt diseases.

Wellman 1977 has emphasised a compound possessing fungitoxicity must to evaluated for its phytotoxicity. The result of *in vivo* trials of the present study gave excellent result because there was no phytotoxicity.

The present invastigation, therefore indicates that the dried leaves of *Tylophora asthmatica* in the form of powder on accounts of their fungitoxicity at low MIC and phytotoxicity as well as efficacy even after increasing the dose, prolonged storage period may be recommended for large scale field trials in order to explore the possibility of its use as ecofriendly, nonpollutive herbal fungitoxicant.

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