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RESEARCH ARTICLE

In vitro Studies of plant extracts on the growth of wilt causing fungi Fusarium oxysporum

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Abstract

In vitro screening of twenty five plant extracts belonging to various families were tested against wilt causing fungi *Fusarium oxyspoum* by Poisoined Food Technique. Extract of *Chenopodium ambrosioides* was the most effective of all the botanicals and showed excellent antimicrobial activity by complete inhibition of mycelial growth of the test fungi. Whereas, other plant extracts showed moderate to minimum antifungal activity. Therefore current study was further carried out to evaluate the Minimum Inhibitory Concentration (MIC) and phytotoxicity of *Chenopodium ambrosioides*. For MIC starting from $5x10^3$ µl/l dose retrogressively lower doses were used and it was observed that $0.2x10^3$ µl/l dose was the minimum dose for complete inhibition of test fungi. The phytotoxic effect of *Chenopodium ambrosioides* was also observed for seed germination and seedling growth of *cicer arietinum* which indicated that the extract has no phytotoxic effect. The extract of *Chenopodium ambrosioides* seems promising as it has a great potential for providing new drug of great benefit.

Introduction

India accounts for 68% of total global output of chickpea and incidentally it is one of the largest consumers. Chickpea forms an integral part of vegetarian diet as a protein substitute in the Indian sub-continent. Besides being a very rich source of protein, it also maintains soil fertility through biological nitrogen fixation. Chickpea is mostly grown in soils poor in fertility and moisture retention capacity. Chickpea (*Cicer arietinum* L.) wilt caused by *Fusarium oxysporum* f.sp. *ciceri* is an important and widely distributed disease in India and causes heavy losses in yield every year. Wilt of chickpea, caused by *Fusarium oxysporum* f.sp. *ciceris* (Padwick) Matuo and Ic. Sato, was first described by Padwick in India (Padwik, 1940) and has since been reported from several other countries. Currently the disease is prevalent in several countries. The pathogen attacks the seedling in soil under favourable conditions and may cause damage of crops upto 90%. It causes great economic as well as labour loss to farmers. The pathogen is both seed and soil borne; facultative saprophyte and can survive in soil up to six years in the absence of susceptible host (Haware *et al*, 1986 a & b). A major increase in the use of synthetic antimicrobial drugs is seen in the last few years for the management of plant diseases. Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to the synthetic pesticides (Verma and Dubey, 1999).

In view of these, intensive search for alternative crop protection methods has led to the detection of biocidal properties of many plant products. Experimental work done in this direction has revealed the wide occurrence of fungitoxic and insecticidal properties in various higher plants (Owolade B.F. *et al*, 1999). Considering the importance of this disease, there is a need to work for the control of the pathogen *Fusarium oxysporum* f.sp. *ciceri* for incorporating a reliable and stable resistance in chickpea crop. The objective of the present study is developing plant based formulations for plant disease management.

1.Materials and Method

1.1 Screening of botanicals against the test pathogen

Leaf samples (100 gm) of twenty five plants were thoroughly washed with 2% sodium hypochlorite solution, repeatedly with fresh water and finally with sterile distilled water, pulverized in a blender, strained through two layers of muslin cloth and finally filtered through sterilized whatman No. 1 filter paper and heat sterilized in an autoclave at 121 °C for 30 min. The clear supernatant thus obtained was used for screening. Extracts were stored aseptically in airtight bottles and served as confirmatory assay. The fungitoxicity of the plant extract was assayed by Poisoned food technique (Grover and Moore, 1962) with slight modification as describe by Dixit *et al*. (1976). PDA medium was prepared and autoclaved and poured into pre sterilized petriplates (17ml each) five ml of plant extracts were added while the medium was in molten state, simultaneously 0.02 ml of the prepared antibiotic solution was added to each assay plate (so as to obtain 20 g/ml dose of antibiotic in each plate) to check the bacterial contamination as suggested by Gupta and Banerjee (1970) and allowed to solidify. Control sets were also prepared similarly using sterilized distilled water in place of plant extract. After complete solidification of the medium, five mm disc of seven day old culture of the test fungi were placed aseptically in the centre of the petriplates and incubated at 28± 2°C for six days and observation were recorded on seventh day. For each assay three replicates were maintained. Toxicity of each extract against the test fungi was calculated in terms of percent inhibition of mycelial growth using the formula:

$$\text{Percent inhibition} = \frac{g_c - g_t}{g_c} \times 100$$

Where, gc= Average diametric growth of the colony in control sets, on the seventh day of incubation.
gt = Average diametric growth of the treatment sets, on the seventh day of incubation.
The plants were collected from different areas of Gorakhpur district and its surrounding areas. They were bought to the laboratory and identified with the help of floras (Hooker, 1872-1897, Duthie, 1903-1929, Bailey, 1958, Maheshwari, 1963and Santapau, 1967).

1.2 Minimum Inhibitory Concentration:

The MIC of the leaf extract of *Chenopodium ambrosioides* for absolute inhibition of mycelial growth of the test fungus, *F. oxysporum*, was determined by the usual Poisoned Food Technique (Grover & Moore 1962). Starting from 5.0×10^3 µl/l dose retrogressively lower doses were used till the minimum dose of the test fungi was achieved. Requisite amounts of the prepared crude leaf extract were added to pre-sterilize Petri plates containing 10 ml of molten PDA. The contents of the plates were agitated in a circular mode to mix the extract in the medium homogeneously. In control sets, the same amount of sterilized distilled water was used in place of the extract. Fungal discs (5 mm in diameter) cut from the periphery of seven days old culture of *F. oxysporum* were aseptically transferred in each Petri plate. The assay plates were incubated for six days at 28 ±1°C.

1.3 Determination of Phytotoxicity:

The phytotoxic effect of *Chenopodium ambrosioides* plant extract was observed for seed germination and seedling growth. The phytotoxicity was measured in terms of percent seed germination of *Cicer arietinum.* Aqueous extract of fresh plant was prepared. Surface sterilized seeds of *Cicer arietinum* was measured and soaked in five ml of extract for five hours. Control sets contained equal amount of distilled water instead of extract and soaked for the same time. The seeds of control and treatment sets were allow to germinate on three layers of pre sterilized moistened filter paper and were incubated at room temperature for five days. Each set contained three replicates. The germinated seeds allowed growing for seven days and the length of radical and plumule of each germinated seed in control and treatment sets were recorded.

2. Result and discussions

Attempt was made in this investigation to evaluate the efficacy of plant extracts in controlling the wilt disease of chickpea. *Chenopodium ambrosioides* of family *Chenopodiaceae* is an annual or short lived perennial herb that has been used for centuries as condiment, traditional purgative for intestinal worms and many other medicinal purposes (Ruffa *et al*., 2002; Flavia *et al*. 2005; Jamali *et al*. 2006).

2.1 Screening of Plant Extracts For Their Toxicity Against Test Fungi:

Aqueous extracts of fresh plant parts of twenty five species belonging to different families were screened against the test fungi. The screening of leaf extracts was done by the Poisoned Food Technique. The technique gives quick result, require simple equipment and little amount of the test material as well as test fungi. The technique gives ample opportunity to inoculums to be in contact with the medium in which plant extracts has been added. Thus if the culture medium gets poisoned due to presence of fungitoxic factor in the extract the growth of inoculums is affected. Among all the plant extracts tested *Chenopodium ambrosioides* completely inhibited the mycelial growth of the test fungus *i.e. Fusarium oxysporum.* Of all the plants tested fourteen plants *viz. Adhatoda vasica, Bixa Orellana, Cyperus rotundus, Euphorbia hirta, Ferula foetida, Gompherina globosa, Lawsonia inermis, Memosa pudica, Parthenium hysterophorus, Putranjiva roxburghii, Solanum nigrum, Thevetia peruviana, Vitex negundo, Xanthium strumarium* showed less than 50% growth of mycelium. *Aerva javanica, Alpinia carinata, Chenopodium ambrosioides, Cinnamomum zeylanicum, Curcuma amada, Momordica charantia, Ocimum gratissimum* were recorded as 70% or above. The screening revealed that *Chenopodium ambrosioides* was the most effective (Table1).

2.2 Minimum Inhibitory Concentration (MIC)

Determination of the minimum inhibitory concentration (MIC) of a fungitoxicant is necessary in order to know its appropriate dose for the complete mycelial inhibition of test pathogen. After screening it was found that the plant extract of *Chenopodium ambrosioides* was the one that completely inhibited the growth of the fungus therefore the experiment was conducted to in respect of MIC. In the present study the MIC of the selected plant extracts was evaluated as 0.2 $x10^3$ µl/l concentrations (table 2).

2.3 Determination of Phytotoxicity

Experiments were conducted to see whether the selected plant extracts of *Chenopodium ambrosioides* was phytotoxic to seed germination, seedling growth (Root–shoot length), general health and morphology of *Cicer arietinum*. Observation of the given experiment are presented in Table 3 and Table4. Wellman (1977) has emphasized that any compound possessing fungitoxicity must evaluated for its phytotoxicity before subjecting it to *in vivo* trials. It is therefore decided to test the phytotoxicity of plant extracts exhibiting fungitoxicity in present investigation. Previously, the extracts of *Allamanda cathartica, Lawsonia inermis and Eucalyptus citriodora, Ruellia tuberose, Ricinus communis were* found to be non-phytotoxicby Tripathi *et al*. (1978) and Dixit *et al*. (1982) respectively. Nehrash (1961 a & b), Staron *et al*. (1969), Fawcett (1969), Misra (1975) reported that a group of acids from Radish, Wyerone from *Vicia faba*, Anagalloside from *Anagallis arvensis* and the extracts of *Clematis gouriana, Ranunculus* scleratus and *Allium sativum* were found to be phytotoxic. Jefferson and Pennacchio in 2003 studied the allelopathic effects of foliage extracts from four *Chenopodiaceae* species on seed germination of *Lactuca sativa*, showed that allelochemicals play an important role, indirectly, in determining chenopod community structure. The extracts of *Chenopodium ambrosioides* exhibited no adverse effect on seed germination and seedling growth of *Cicer aritienum* which indicate the non- phytotoxic nature.

The development of natural antimicrobials and fungal pesticides would help to decrease the negative impact of synthetic agents, such as residues, resistance and environmental pollution. In this respect, natural fungicides may be effective, selective, biodegradable and less toxic to the environment as well as food and agriculture industries. The most simple and most inexpensive plant protection methods are based on the plant extracts that are producible domestically. Moreover, these plant extracts are cheaper to apply and are easily available and simple to prepare. Thus it can be concluded that the use of *Chenopodium ambrosioides* extracts could be considered as an antifungal, and can be used in making novel types of natural fungicides

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Table-1

Screening Of Plant Extracts Against Test Fungus By Poisoned Food Technique

S.No.	Name of Plant	Family	% Inhibition of mycelial growth
1.	Adhatoda vasica	Acanthaceae	46
2.	Aerva javanica	Amaranthaceae	84
3.	Aloe barbadensis	Liliaceae	68
4.	Alpinia carinata	Zingiberaceae	81
5.	Anagalis arvensis	Primulaceae	54
6.	Bixa Orellana	Bixaceae	38
7.	Chenopodium ambrosioides	Chenopodiaceae	100
8.	Cinnamomum zeylanicum	Lauraceae	76
9.	Curcuma amada	Zingiberaceae	74
10.	Cyperus rotundus	Cyperaceae	43
11.	Euphorbia hirta	Euphorbiaceae	21
12.	Ferula foetida	Apiaceae	16
13.	Gompherina globosa	Amaranthaceae	22
14.	Lawsonia inermis	Lytheraceae	16
15.	Memosa pudica	Mimosaceae	26
16.	Momordica charantia	Cucurbitaceae	72
17.	Ocimum gratissimum	Lamiaceae	73
18.	Parthenium hysterophorus	Asteraceae	24
19.	Putranjiva roxburghii	Euphorbiaceae	38
20.	Rauvolfia serpentine	Apocynaceae	68
21.	Solanum nigrum	Solanaceae	32
22.	Thevetia peruviana	Apocynaceae	24
23.	Tridax procumbans	Asteraceae	62
24	Vitex negundo	Verbinaceae	21
25.	Xanthium strumarium	Asteraceae	31

Table-2

Minimum Inhibitory Concentration (MIC) Of *Chenopodium ambrosioides*

Doses (µl/l)	% inhibition of mycelial growth
5.0	100
4.0	100
3.0	100
2.0	100
1.0	100
0.9	100
0.8	100
0.7	100
0.6	100
0.5	100

0.4	100
0.3	100
0.2	100

Table-3

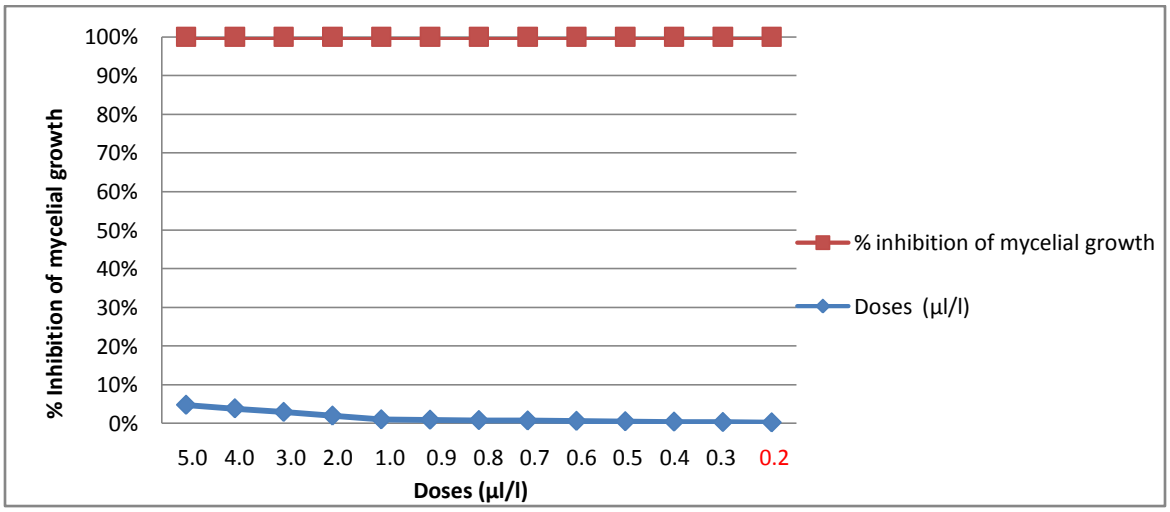
Effect of *Chenopodium ambrosioides* Plant Extract on Seed Germination of *Cice rarietinum*

S.No.	Germination period (days)	% Seed Germination	
		Control	Treated
1.	2	100	100
2.	3	100	100
3.	4	100	100
4.	5	100	100
5.	6	100	100
6.	7	100	100

Table-4

Effect of *Chenopodium ambrosioides* Extract on Root - Shoot Length of *Cicer arietinum*

Age of seedlings (days)	Radicle length of seedling (cm)		Plumule length of seedling (cm)	
	Control	Treated	Control	Treated
4	2.8	3.4	3.2	3.8
5	3.6	4.5	4.6	5.0
6	4.9	5.9	5.5	6.8
7	6.0	6.4	7.2	7.1



Minimum Inhibitory Concentration (MIC) of *Chenopodium ambrosioides*

Effect of Plant Extract on seed germination of *Cicer arietinum*

Plant of *Chenopodium ambrosioides* (Bara Bathua)

Effect of Plant Extract on Root- Shoot Length of *Cicer arietinum*