Pal and Kumar, ***Current Discovery*** 2:1 (2013) 62-67
ISSN: 2320-4400
OPEN ACCESS JOURNAL

Research Article

Current Discovery
International journal of current discoveries and innovations
Volume 2 / Issue 1 / 2013

Antifungal activity of some common weed extracts against wilt causing fungi, *Fusarium oxysporum*

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Article Information

Received: 12 Nov 2012
Revised: 30 Dec 2012
Accepted 17 Jan 2013

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Key words:
Antifungal activity, *Cannabis sativa, Ageratum conyzoides, Argemone maxicana*, MIC

Acknowledgements:
Thanks are due to Head, Department of Microbiology, Chaudhary Charan Singh University, Meerut for providing the facilities.

Abstract

Herbal fungicides are mostly used to control plant disease because of their eco-friendly nature and cost effectiveness. The present investigation focuses on the antifungal activity of some weed extracts viz., *Achyranthes aspera, Parthenium hysterophorus, Cannabis sativa, Calotropis gigantean, Chenopodium album, Canada thistle, Phalaris minor, Cynoden dactylon, Argemone maxicana, Ageratum conyzoides*, and *Lantana camera* against seed-borne phytopathogenic fungi causing wilt disease. Out of 11 weed tested, the extracts of *Cannabis sativa, Ageratum conyzoides* and *Argemone maxicana*, were found most effective against phytopathogenic fungi, *Fusarium oxysporum*. The MICs of the extracts were found to be 6.25 X 10^{-4}, 3.125 X 10^{-5} and 3.125 X 10^{-5} µl/ml against the tested pathogen respectively. On the basis of present results, the methanol extracts of *Cannabis sativa, Argemone maxicana*, and acetone extract of *Ageratum conyzoides*, can be used for the development of novel broad spectrum herbal fungicidal formulations after *in vivo* and field trial. Which is in progress.

Introduction

Plants are the most important source of chemical compounds. Primary plant metabolism synthesizes essential compounds, which are present in all plant species. There is growing evidence that these compounds when applied on other plants, they can protect the plant from the pathogens and pests [1]. In the search of environmentally safer, selective and durable natural pesticides, structure identification of these compounds is required [1]. A major factor for the revival of weeds is their ability to resist pests and pathogens in their environment. Thus, they could be a potential source of antimicrobial compounds and their identification is necessary to develop cheaper pesticides [2]. The developments of resistance in weeds to the common pesticides and the increasing restrictions on the use of toxic material in the environment have given an impetus to search for novel plant protectants that interfere with the pathogenicity factors [2-3]. Herbal fungicides are gaining growing interest because of their eco-friendly attributes [4].

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Fungi are ubiquitous in the environment, and infection due to fungal pathogens has become more common. The genus *Fusarium* is widely distributed in nature and its species are among the most common fungi on the phyllosphere [5]. More than 800 million people in the developing countries do not have adequate food supplies and at least 10% of food is lost due to plant diseases [6].

As compared to other plant parasites, fungi cause the greatest impact with regard to diseases and crop production losses. The most important method of protecting the plants against the fungal attack is the use of fungicides. However, many fungicidal agents available in the market are toxic and have undesirable effects on other organisms present in the environment [7]. Some synthetic fungicides are non-biodegradable, and hence can accumulate in the soil, plants and water, and consequently effect the humans through the food chain [7]. The development of resistance of pathogenic fungi towards the synthetic fungicides is of great concern. Therefore, it is desirable to use some ecofriendly measures for the management of diseases.

Natural products seem to be a viable solution to the environmental problems caused by the synthetic pesticides and many researchers are trying to identify the effective natural products to replace the synthetic pesticides [8]. Similarly, the use of natural products for the control of diseases in plants is considered as an alternative source to synthetic pesticide due to their lower negative impacts on the environment. Besides being harmless and non-phytotoxic it has been proved that plant extracts exhibit inhibitory effect on pathogens. Several higher plants and their constituents have been successful in plant disease control and have proved to be harmless and non phytotoxic, unlike chemical fungicides. The plant based fungicides are cheap, locally available, non-toxic, and easily biodegradable [9-11].

Although there is a growing interest in the use of medicinal plants to control the plant diseases, only about 2,400 plant species among more than 250,000 higher plants have been screened for the phytoactivity [12-14]. There are evidences from earlier works that several plant species possess antifungal and antibacterial properties [15-22].

The present investigation is therefore, undertaken to test the efficacy of common weed extracts against wilt causing fungi.

Material and Methods

Plant Materials

Eleven plants viz., *Achyranthes aspera, Parthenium hysterophorus, Cannabis sativa, Calotropis gigantean, Chenopodium album, Canada thistle, Phalaris minor, Cynoden dactylon, Argemone maxicana, Ageratum conyzoides*, and *Lantana camera* were collected from local areas, near the Chaudhary Charan Singh University Meerut (Table 1).

Preparation of plant extracts

One gram dried part of each plants were powdered separately and then extracted in 10 ml of different organic solvents viz., Acetone, Benzene, Chloroform, Ethanol, and Methanol separately. The overnight extracts were filtered with a Whatman's no.1 filter paper, and then extracted with rotary evaporation in order to remove the solvents. After evaporation 10 ml of DMSO (di methyl sulphoxide) were used for final preparation.

Fungal strain

Wilt causing pathogen, *Fusarium oxysporum* were obtained from Collection of Bio-resource type Culture, Microbiology Department, CCS University, Meerut. The culture was maintained on potato dextrose agar (PDA) at 28±2°C.

Antifungal activity of different weed extracts

Antifungal screening of weed extracts were carried out by Poisoned food technique [23] with slight modifications [24]. 800 µl of PD broth were taken in 2 ml micro centrifuge tube (MCT), then 100 µl of the each solvent extracts and 100 µl of inoculums suspension (McFarland standard) were added separately. In control DMSO were added in place of extracts in appropriate amount. The test micro centrifuge tubes were mixed well and incubated at 28±2°C for 24 hours.

After 24 hours sterile disc of 0.5 mm diameter were dipped in the test as well as control suspension and transferred on plain PDA medium in petriplate separately. All inoculated petriplate were incubated at 28±2°C for 48 hours. After 48 hours, mycelia growth of the test fungus was measured and compared with control. The percentage of mycelia growth inhibition was estimated by using following formula.

MGI%= Gc-Gt X 100/Gc

Where,

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MGI% = Mycelial growth inhibition

Gc = growth diameter in control

Gt = growth diameter in treatment

Determination of MICs of extracts by microtiter plate assay

MIC (minimum inhibitory concentration) is expressed as the lowest concentration, which inhibited the growth. A broth microdilution assay was adopted using 96 well micro titer plates with resazurin [25]. It was carried out to assess the microbial growth and determine the Minimal Inhibitory Concentration.

The resazurin (oxydation-reduction indicator) solution was prepared by dissolving a 270 mg tablet in 40 mL of sterile distilled water.

A sterile 96 well microtiter plate was taken for the test. 50 µl of test extracts were pipetted into the first row of the microtiter plate A1. Wells from A2 to H2 till A12 to H12 were dispensed with 50 µl of nutrient broth. 50 µl of test extract was transferred from test solution (A1-H1) to next wells (A2-H2) and so on to create serial dilutions. 30µl of the test culture were mixed in serially descending concentrations to each well, from A2 to H2 till A12 to H12. In last 20 µl of resazurin solution was added in all tested as well as control set. A11, A12 and H11, H12 served as controls, 50 µl of DMSO were used in place of extracts.

The plates were incubated at 30^0C for 24 hours. The colour change was then assessed visually. Any colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value. The average of five values was calculated.

Determination of minimum fungicidal concentration (MFC) of the extracts

To determine the MFC for each set of wells at the MIC, a loopful of broth were inoculated on sterile agar plate separately. Plates were incubated at 30°C for 24h. After incubation the concentration at which no visible growth was observed as noted the MFC.

Results

In this study, we have tested the extracts of eleven weed plants for their antifungal activity against wilt causing fungi, *Fusarium oxysporum*. All the plant extracts showed antifungal activity against *Fusarium oxysporum.* Extracts of Cannabis sativa showed 84.21%, *Ageratum conyzoides* (84.21%) and *Argemone maxicana* (61.84%). On the contrary, *Fusarium oxysporum* was found to be more sensitive to Acetone extracts of *Ageratum conyzoides*, which was 84.21% inhibition (Table 2).

The minimum inhibitory concentration of the acetone extracts was found 3.125 X 10^{-5} µl/ml and Methanol extracts of Cannabis *sativa, Parthenium hysterophorus* and *Argemone maxicana* was to be 6.25 X 10^{-4} µl/ml (Table 3).

Discussion

Natural products from many plants are known to control plant pathogens. Antifungal activity testing of weeds remains an area of interest. However not many reports are available on the exploitation of antifungal property of weeds plants and even the data regarding use of weeds as an antifungal agents are scanty [26].

Table 1. List of weeds selected for antifungal activity

Common Name	Botanical Name	Part Used	Family
Chirchita	*Achyranthes aspera*	Stem , leaves	Amaranthaceae
Carrot Grass	*Parthenium hysterophorus*	Leaves	Asteraceae
Bhang	*Cannabis sativa*	Leaves	Cannabaceae
Aak	*Calotropis gigantea*	Leaves	Apocynaceae
Bathwa	*Chenopodium album*	Leaves	Amaranthaceae
Corn thistle	*Canada thistle*	Leaves	Asteraceae
Baluri	*Phalaris minor*	Stem, leaves, seed	Poaceae
Doab Grass	*Cynoden dactylon*	Whole Plants	Poaceae
Satyanashi	*Argemone maxicana*	Leaves	Papaveraceae
Chick weed	*Ageratum conyzoides*	Whole Plants	Asteraceae
Red Sage	*Lantana camera*	Leaves, flower	Verbenaceae

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Table 2. Antifungal screening of weed plant extracts against wilt causing fungi *Fusarium oxysporum*

Plants	Percentage of mycelial growth inhibition (MGI%)				
	AE	BE	CE	EAE	ME
Achyranthes aspera	1.31	5.26	26.31	15.78	11.84
Parthenium hysterophorus	35.52	28.94	22.36	2.6	43.42
Cannabis sativa	48.68	17.1	11.84	61.84	84.21
Calotropis gigantea	22.36	14.47	26.31	17.1	21.05
Chenopodium album	5.26	17.1	27.63	6.57	17.1
Canada thistle	1.31	10.52	18.42	9.2	1.31
Phalaris minor	3.94	13.15	22.36	3.94	22.36
Cynoden dactylon	6.57	7.8	15.78	7.89	5.26
Argemone maxicana	32.89	1.31	14.47	1.31	61.84
Ageratum conyzoides	84.21	3.94	26.31	39.47	13.15
Lantana camera	10.52	13.15	17.1	7.8	25

AE=Acetone Extract, BE=Benzene Extract, CE=Chloroform extract, EAE=Ethyl Acetate Extract, ME=Methanol extract

In the present the solvent extract of *Ageratum conyzoides, Cannabis sativa* which recorded 84.21% growth inhibition against *Fusarium oxysporum*, followed by *Argemone maxicana* 61.84% growth inhibition. Similar findings on antifungal and nature of *Cannabis sativa* were documented [27]. *Ageratum conyzoides, Cannabis sativa*, (84.21%) and *Argemone maxicana* (61.84%) showed a broad spectrum antifungal activity against wilt causing fungi *Fusarium oxysporum*. The solvents based extracts of *Ageratum conyzoides and Cannabis sativa* showed good activity against *Fusarium oxysporum* at 100 µl/ml concentrations. Our results also showed that the Methanol extract of *Argemone maxicana* is highly active against *Fusarium oxysporum* at 100 µl/ml concentrations.

Table 3. Minimum inhibitory concentrations of bioactive plant against *Fusarium oxysporum*.

Plant name	Plant extract	Minimum inhibitory concentrations (µl/ml)
Cannabis sativa	Methanol	6.25×10^{-4}
Ageratum conyzoides	Acetone	3.125×10^{-5}
Parthenium hysterophorus	Methanol	6.25×10^{-4}
Argemone maxicana	Methanol	6.25×10^{-4}

The antimicrobial potency of plants is believed to be due to tannis, saponins, phenolic Compounds, essential oils and flavonoids [28]. The antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies [29].

Thus, the extract of *Cannabis sativa,* and *Ageratum conyzoides* could be a possible source to obtain new and effective biofungicides to control *Fusarium oxysporum* caused wilt disease in various crops. Biofungicides are easily biodegradable, selective and locally produced, especially for the farmers who cannot afford expensive synthetics fungicides. By using weed plant species as raw materials for plant derived fungicides, can manage the disease, and at the same time might create economic uses for these unwanted species [30].

In order to maintain the productivity, more and more chemicals are being added in the natural environment, which enter the food chain through water, soil, and air resulting serious harmful effects on human health [31]. According to the survey made by the WHO, more than 50,000 people in developing countries are annually poisoned and 5,000 die as a result of the effects of toxic agents, used in agriculture. In India 35,000 – 40,000 tons of hazardous chemicals are sprayed on the crops every year, instead of helping the poor, these chemicals are causing cancer, sterility and death [32].

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To avoid the use of these horrible diseases causing synthetic chemicals, the plants and their products should be utilized to combat phytopathogens. As plants are known to possess various secondary metabolites, which showed inhibitory effect against the growth of pathogens. Keeping these problems in view, efforts are underway to search economic safe phytochemicals, which could be utilized for disease control.

The present investigation is important steps in developing plant based fungicides which are ecofriendly for the management of wilt diseases caused by *Fusarium oxysporum.* Further investigation will be carried out for the development of commercial formulation based on field trial and toxicological investigation.

Conclusion

The results of present study clearly demonstrates that solvent extracts of *Cannabis sativa, Ageratum conyzoides* and *Argemone maxicana* contains antifungal constituents which can be used for the eco-friendly management of the wilt disease caused by *Fusarium oxysporum.*

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