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Research Article

In vitro antifungal potential of plant extracts against *Fusarium oxysporum, Rhizoctonia solani* and *Macrophomina phaseolina*.

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Abstract: The antifungal activity of aqueous extracts of nine plants viz, *Azadirachta indica, Parthenium hysterophorus, Momordica charantia, Allium sativum, Eucalyptus globules, Calotropis procera*, *Aloe vera*, *Beta vulgaris* and *Datura stramonium* were assessed *in vitro* against *Fusarium oxysporum* f. sp. *melongenae, Rhizoctonia solani* and *Macrophomina phaseolina*, the soil borne phytopathogens. The assessment of fungitoxic effect was carried out by using three different concentrations i.e., 5, 10 and 20% against the test fungi, in terms of percentage of mycelial growth inhibition. The extract of *A. sativum* completely inhibited the mycelial growth of *M. phaseolina* at all the concentrations. The extracts of *D. stramonium* and *E. globulus* inhibited the mycelial growth of *R. solani* of 72%, and 70.7% respectively at 20% concentration, that of *A. sativum, E. globulus* and *D. stramonium* exhibited inhibition percentage of 63.3%, 61.8% and 61.1% respectively at 20% concentration on *Fusarium oxysporum* f. sp. *melongenae*. The application of plant extracts for disease management could be less expensive, easily available, non-polluting and eco-friendly.

Keywords: *Fusarium oxysporum, Rhizoctonia solani, Macrophomina phaseolina*, phytopathogens, antifungal activity

Introduction

Brinjal (*Solanum melongina* L.) is grown as a vegetable crop in India and the plant is affected by various fungal diseases which in turn produces low crop yield. Among the fungal diseases caused by *Fusarium oxysporum, Macrophomina phaseolina* and *Rhizoctonia solani* are the major constraints in fruit field. *Fusarium oxysporum* f. sp. *melongenae* (Fom) is the most destructive pathogen causing Fusarium wilt of brinjal. This soil-borne fungus invades the vascular bundles, causes severe wilting and death of the above ground parts of plants by blocking the xylem transport system (Altınok, 2005). The pathogen *Rhizoctonia solani* causing germination failure, damping off and seedling rot is the major constraint to brinjal cultivation (Seema and Devaki, 2010). *Macrophomina phaseolina* causing charcoal rot is cosmopolitan in distribution and is potential threat to crop production in arid regions (Hoes, 1985).

Various disease management methods have been implemented to combat and eradicate pathogenic fungi. These include cultural, regulatory, physical, chemical and biological methods (Kata, 2000). Pathogens being soil borne, causes a huge problem in controlling the diseases. Synthetic chemicals used to control plant diseases not only pollute the environment, but are also harmful to human health. Intensive use of fungicides for the control of plant diseases has resulted in the accumulation of toxins to human beings as well as to the environment. Restrictions on the use of chemical pesticides have been increasing. Knowing the ill effects of these chemical residues found in eatables, plant growers are being challenged to maintain plant health with reduced input from agricultural chemicals.

Use of natural products for the management of fungal diseases in the plants is considered as a good alternate to synthetic fungicides, due to their less negative impact on the environment. Many higher plants and their constituents have been successful in plant disease control and proved to be safe and non-phytotoxic. Plant serves as renewable natural resources for a variety of biologically active chemicals. These chemicals bear a variety of properties viz, antibacterial, antifungal, antiviral, antihelminthic, anticancer, sedative, laxative, cardio tonic, diuretic and others (Parajuli *et al.,* 1998).

Because of environmental and economic considerations, plant scientists are involved to find the cheaper and more environmental friendly bio-compounds for the control of plant diseases using extracts from different plants (Gerresten and Haagsma, 1951; Kumar *et al.,* 1979; Naidu and John, 1982). Thus, control strategies are now directed towards the use of natural products. Botanical pesticides are cheaper than their synthetic counter parts and their crude extracts are easy to prepare even by farmers. These are also less likely to the development of resistance or resurgence in pests. The benefits of natural pesticides have aroused

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interest in protection of crop plants. Keeping in view, the plant extracts were evaluated *in vitro* for their antifungal activity against, *Fusarium oxysporum, Rhizoctonia solani* and *Macrophomina phaseolina.*

Materials and Methods

Test fungus

The three pathogens viz., *Fusarium oxysporum* f. sp. *melongenae* (Fom), *Rhizoctonia solani* (Rs), and *Macrophomina phaseolina* (Mp) were selected for the experimental work. Parts of plants with symptoms of fusarium wilt, damping off, root rot, dry root rot, charcoal rot, stem canker were surface sterilized with 70% ethanol and immersion in 0.3% sodium hypochlorite for 10 min, rinsed thoroughly in sterile distilled water and transferred to potato dextrose agar (PDA) medium. Plates were incubated at 26 ± 2^{0}C and observed daily for emergence of colonies. Sub-culturing was done from single spore to obtain pure culture. The pure culture of the test fungus was maintained on PDA slants and stored at 4^{0}C in the refrigerator.

Collection of Plant Material

Fresh disease-free plant parts were collected locally. Selected plants were available in sufficient quantities. Nine native plants were selected in the present study, to evaluate their antifungal activity. The plants were selected from local flora on the basis of criteria such as the presence of antimicrobial properties according to literature, easy availability in bulk with very little commercial value. The selected plants were well adapted to the climatic conditions and were well known, among local natives, for their medicinal properties. The plants were identified by using standard flora (Pullaiah, 2015) and by comparison with herbaria of Department of Botany, Osmania University, Hyderabad, India. The plants and their parts used in the study are shown in Table 1.

Table 1. The aqueous plant extracts tested against mycelial radial growth of fungal phytopathogens.

Common name	Local name	Botanical name	Family	Plant part used
Neem	Vepa	*Azadirachta indica* A.Juss.	Meliaceae	leaves
Congress weed	Vayyari bama	*Parthenium hysterophorus*.L	Asterceae	twigs with leaves and flowers
Bitter gourd	kakara	*Momordica charantia* L.	Cucurbitaceae	leaves
Garlic	Ellipayalu	*Allium sativum* L	Allium sativum L	cloves
Blue gum	Eucaliptus	*Eucalyptus globules* Labill	Myrtaceae	leaves
Milk weed	Jilledu	*Calotropis procera* (Aiton) Dryand	Apocynaceae	leaves
Indian aloe	Kalabanda	*Aloe vera* (L.) Burm.f.	Asphodelaceae	leaves
Beet root	Beet root	*Beta vulgaris* L.	Amaranthaceae	Storage root
Devil's snare	Ummetta	*Datura stramonium* L.	Solanaceae	leaves

Preparation of aqueous extract

The leaves of *Azadirachta indica, Parthenium hysterophorus, Momordica charantia, Eucalyptus globules, Calotropis procera, Aloe vera* and *Datura stramonium,* root of *Beta vulgaris,* bulb (clove) of *Allium sativum* were washed with tap water followed by sterilised distilled water. 100 gm. of fresh leaves/bulb of each plant were taken, washed thoroughly and crushed in 100 ml of sterilized distilled water (1: 1 v/v) and were ground separately in an electric grinder. In order to remove plant debris, the extracts were passed through a muslin cloth and the filtrate was centrifuged for 10 minutes at 5000 rpm and the clear supernatant was collected. Then this material was taken in a beaker and boiled at 80^{0}C for twenty minutes in a hot water bath. The stock solution, thus obtained was used for evaluating their antifungal activity and it was designated as 100%. From this standard / stock solution(s), required concentrations (5%, 10% and 20%) were prepared by adding sterile distilled water to PDA before use.

Antifungal assay

The plant extracts were evaluated at three concentrations (5%, 10% and 20%) for their antifungal activity. To study the antifungal mechanism of plant extracts by using poisoned food technique (Nene and Thapliyal, 1993). Extracts were added in potato dextrose agar (PDA) medium at 5%, 10% and 20% concentration into petri dishes. PDA medium added only with sterile distilled water served as control. Each Petri dish was inoculated with 6mm plug of isolate taken from margins of actively growing culture of pathogen. Then Petri plates were incubated at 25° ± 2°C. Mycelial growth was recorded when the growth of pathogens were completed (90mm) in the control plates. Each treatment was repeated three times.

Statistical analysis

Per cent inhibition in growth was determined with the help of mean colony diameter and calculated by using the following formula suggested by Vincent (1947).

$$\text{Mycelial growth inhibition (\%)} = \frac{C - T}{C} \times 100$$

Where, C = Radial growth of the pathogen (mm) in control; T = Radial growth of the pathogen (mm) in treatment

Results

Inhibition in radial mycelial growth of Fom, Rs and Mp induced by plant extracts showed considerable difference in performance.

Effect of different plant extracts on radial mycelial growth of *Fusarium oxysporum* f. sp. *melongenae*

The highest percentage inhibition in radial mycelial growth was induced by Fom exhibited by *A. sativum* (63.3%), followed by *E. globulus* (61.8%), *D.*

stramonium (61.1%), *M. charantia* (24.4%), *A. vera* (16.6%), *P. histerophorus* (30.0 %), *B. vulgaris* (9.2 %), *A. indica* and *Calotropis* (0.0 %), (Table 2). *D. stramonium* (61.1), *A. vera* (16.6), and *A. indica* exhibited no inhibition of radial growth at all the experimental concentrations. *P. hysterophorus, M. charantia, E. globules, B. vulgaris, A. sativum* exhibited increasing percentage inhibition with increase in concentration of the extract (Table 2).

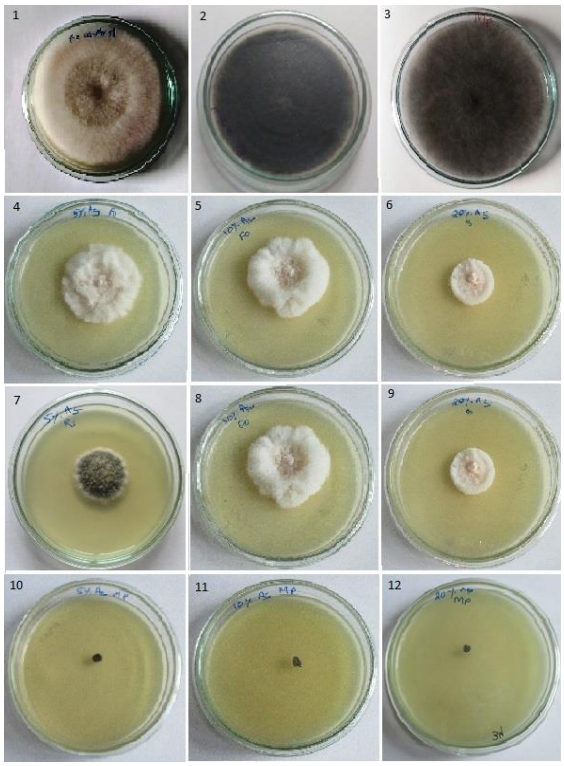

Plate 1. Fig. 1-3: Control petriplates of *Fusarium oxysporum*, *Rhizoctonia solani*, *Macrophomina phaseolina*, petriplates showing effect of *Allium sativum* at 5%, 10%, 20% concentrations- Figs. 4-6: on Fom, 7-9: on *R. solani*, 10-12: on *M. phaseolina*.

Effect of different plant extracts on radial mycelial growth of *Rhizoctonia solani*

The highest inhibition percentage (72.2%) was recorded at 20% concentration by *D. stramonium* in radial mycelial growth of pathogen, followed by *E. Globules* (70.7%), A. sativum, *M. charantia* (63.3% and 61.1%) inhibition respectively. Whereas, *A. sativum* has shown the same inhibition percentage of 63.3% at 10% and 20% extract concentration. *Calotropis* exhibited least percentage inhibition (11.1%) of mycelial growth (11.1%) at 5%, 10% and 20% concentrations.

Effect of different plant extracts on radial mycelial growth of *Macrophomina phaseolina:*

Maximum inhibition in radial mycelial growth percentage induced by *M. phasiolina* was exhibited by *A. sativum* (100.00%) at 5%, 10% and 20% concentrations. *D. stramonium* showed 0.0%, 27.7% and 57.7% inhibition at 5%, 10% and 20% concentration respectively. No inhibition in radial growth was recorded by *A. indica, P. hysterophorus, M. charantia, E. globulus, Calotropis, A. vera and B. vulgaris.*

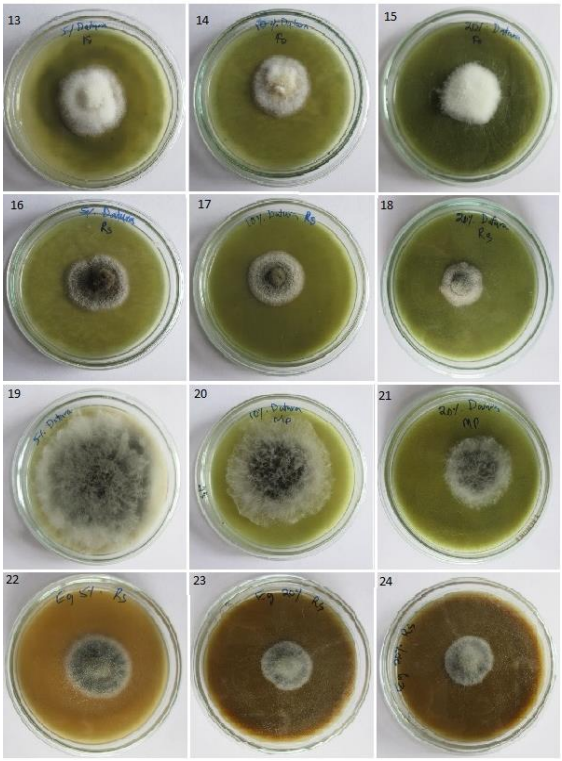

Plate 2. Petriplates showing effect of *D. stromonium* at 5%, 10%, 20% concentrations- Figs. 13-15: on Fom, 16-18: on *R. solani*, 19-21: on *M. phaseolina*; Fig. 22-24: Petriplates showing effect of *E. globulus* at 5%, 10%, 20% concentrations- Fig. 22-24: on *R. solani.*

Table 2. Effect of plant extracts on radial growth of *Fusarium oxysporum*

Plant extract	Control (mm)	Inh (%)	5% (Mean±SD)	Inh (%)	10% (Mean±SD)	Inh (%)	20% (Mean±SD)	Inh (%)
A. indica	90	0	90.00±0.00	0.0	90.00±0.00	0.0	90.00±0.00	0.0
P.histerophorus	90	0	87.66±1.45	2.6	85.00±1.52	5.5	75.66.33±	15.9
M.charantia	90	0	77.00±1.00	14.4	75.00±0.00	16.6	68.00±0.00	24.4
A.sativum	90	0	42.66±1.45	52.6	42.33±0.33	52.9	33.00±0.00	63.3
E.globulus	90	0	47.00±1.00	34.3	48.33±1.45	46.0	34.33±0.66	61.8
Calotropis	90	0	90.00±0.00	0.0	90.00±0.00	0.0	90.00±0.00	0.0
A.vera	90	0	75.00±0.00	16.6	75.00±0.00	16.6	75.00±0.00	16.6
B.vulgaris	90	0	84.33±0.66	6.3	81.66±1.66	9.2	80.66±0.66	10.3
D.stromonium	90	0	35.00±0.00	61.1	35.00±0.00	61.1	35.00±0.00	61.1

Inh (%)= Inhibition (%)

Table 3. Effect of plant extracts on radial growth of *Rhizoctonia solani*

Plant extract	Control (mm)	Inh (%)	5% (Mean±SD)	Inh (%)	10% (Mean±SD)	Inh (%)	20% (Mean±SD)	Inh (%)
A.indica	90.00±0.0	0	70.00±0.00	22.2	70.00±0.00	22.2	70.00±0.00	22.2
P.histerophorus	90.00±0.0	0	72.33±1.45	19.6	70.00±0.00	22.2	61.00±3.05	32.2
M.charantia	90.00±0.0	0	56.00±3.05	37.7	44.00±3.51	51.1	35.00±5.00	61.1
A.sativum	90.00±0.0	0	34.00±1.00	62.2	33.00±0.00	63.3	33.00±0.00	63.3
E.globulus	90.00±0.0	0	36.33±0.88	59.6	35.00±1.00	61.1	26.33±0.33	70.7
Calotropis	90.00±0.0	0	80.00±0.00	11.1	80.00±0.00	11.1	80.00±0.00	11.1
A.vera	90.00±0.0	0	55.00±0.00	38.8	58.00±0.00	38.8	60.00±0.00	33.3
B.vulgaris	90.00±0.0	0	57.33±0.66	36.3	53.66±0.88	40.3	55.66±0.66	38.1
D.stromonium	90.00±0.0	0	40.00±0.00	55.5	33.00±0.00	63.3	25.00±0.00	72.2

Inh (%)= Inhibition (%)

Table 4. Effect of plant extracts on radial growth of *Macrophomina phaseolina*

Plant extract	Control (mm)	Inh (%)	5% (Mean±SD)	Inh (%)	10% (Mean±SD)	Inh (%)	20% (Mean±SD)	Inh (%)
A.indica	90.00±0.0	0	90.00±0.0	0	90.00±0.0	0	90.00±0.0	0
P.histerophorus	90.00±0.0	0	90.00±0.0	0	90.00±0.0	0	90.00±0.0	0
M.charantia	90.00±0.0	0	90.00±0.0	0	90.00±0.0	0	90.00±0.0	0
A.sativum	90.00±0.0	0	00.00±0.0	100	00.00±0.0	100	00.00±0.0	100
E.globulus	90.00±0.0	0	90.00±0.0	0	90.00±0.0	0	90.00±0.0	0
Calotropis	90.00±0.0	0	90.00±0.0	0	90.00±0.0	0	90.00±0.0	0
A.vera	90.00±0.0	0	90.00±0.0	0	90.00±0.0	0	90.00±0.0	0
B.vulgaris	90.00±0.0	0	90.00±0.0	0	90.00±0.0	0	90.00±0.0	0
D.stromonium	90.00±0.0	0	90.00±0.0	0	65.00±0.0	27.7	38.00±0.0	57.7

Inh (%)= Inhibition (%)

Discussion

The inhibitory effect of the plant extracts might be attributed to the presence of some antifungal toxicants. Several authors have also reported the fungicidal activity in wide variety of taxa. The presence of antifungal compounds in higher plants is well recognised and considered valuable for plant disease control (Singh and Dwivedi, 1987).

In the present study, it was noticed that the extract of *A. sativum* revealed considerable antifungal activity against the tested pathogens. Our results are in correlation with earlier work of Avasthi *et al.,* (2005), Sehajpal et al. (2009) on *A. sativum*, which showed 100% inhibition of the mycelial growth of *A. niger* at 20% concentration. This fungicidal activity of *A. sativum* possibly related to organo sulphur compound including allicin (Hovana *et al.,* 2011). These compounds showed better antifungal activity than both antibiotics streptomycin and ampicillin (Illic *et al.,* 2012).

Antifungal activity of *Azadirachta indica* has been reported to have inhibitory effects on *Rhizoctonia solani* (Sivakadadacham, 1988; Sharma and Jnandaik, 1994). The bioactivity of neem extracts has been attributed by various compounds such as nimbin, nibbidin, and salannin and most important antifungal compound is azadirachtin. Our results demonstrate that *D. stramonium, E. globulus, A. sativum, M. charantia* extracts effectively suppressed the radial mycelial growth of *R. solani*. *M. charantia* contains an array of biologically active plant chemicals including triterpenes, proteins and steroids. The antifungal activity *E. globulus* extract may be attributed to the presence of some compounds. The major component was 1, 8-cineole (85.8%), B-pinine (7.2%) and B-myrcene (1.5%).

Conclusion

Results of the present study indicate that the tested extracts showed fungicidal activity against the tested pathogens. Plant extracts can be exploited as natural fungal toxicants to manage the growth of pathogenic fungi and thus reduce the dependence on fungicides. All over the world, attention has been drawn towards the exploitation of higher plant products as novel chemo-therapeutants in plant protection. Because of non-phytotoxicity, systemicity, easy bio-degradability and the stimulatory nature of host metabolism, plant products possess the potential to be of value in pest management (Mishra and Dubey, 1994). Some plant extracts such as *A. sativum, D. stramonium, E. globules* could be a good antifungal efficacy, which may be used for formulating new, safer and eco-friendly fungicides. It is therefore, encouraging to identify and characterize the active principle. Moreover, because of the water-soluble nature of the toxic principle, it is ideal for developing into herbal pesticides.

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