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In vitro antifungal activity of *Citrus aurantifolia* Linn plant extracts against phytopathogenic fungi *Macrophomina phaseolina*

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ABSTRACT

The aqueous extracts of leaf of *Citrus aurantifolia* L were assessed *in vitro* for inhibitory activity against *Macrophomina phaseolina* isolated from dry root rot specimens of Gingelly. The antifungal activity was determined by poison food technique. The extracts have shown dose dependent inhibition of mycelial growth of test fungi. The extracts were more effective in inhibiting *Macrophomina phaseolina*. The extracts of *Citrus aurantifolia* were found effective against Gingelly dry root rot pathogens. Further field experiments are to be carried out to recommend the extracts against the disease.

***Keywords*:** Phytopathogens; Gingelly; antifungal activity

1. INTRODUCTION

More than 800 million people in developing countries do not adequate food and at least 10 % of food is lost due to plant diseases. Plant diseases are caused by pathogens such as fungi, bacteria, nematodes and viruses. Compared to other plan parasites, fungi cause the impact with regard to diseases and crop production losses. This includes considerable foliage and post harvest losses of fruits and vegetables which are brought about by decay due to fungal plant pathogens (Strange *et al.,* 2005). Medicinal plants are naturally gifted with invaluable bioactive compounds which form the backbone of traditional medicines. The use of plants as medicines antedates history. Approximately 80 % of the 4000 million inhabitants of the earth rely on herbal medicines for their primary health care. There has been an increasing interest word wide on therapeutic values of natural products from plants due to disenchantment with modern synthetic drugs. Moreover, the clinical efficacy of many existing antibiotics is also being threatened by the emergence of multidrugs - resistant pathogens. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Therefore, there has been a tremendous upsurge in the demand for the drugs from natural sources (Mukherjee *et al.,* 2006).

Macrophomina phaseolina is a soil-borne fungal plant pathogen that cause charcoal rot diseases in more than 500 different monocotyledonous and dicotyledonous plant species including such important crops as sorghum, soybean, alfalfa, maize etc (Ma *et al.,* 2010). It

exists in soil as sclerotia, a compact mass of hardened mycelial structures, which can remain dormant for many years and produces hyphae under appropriate conditions which infect the roots host plants. High variation in pathogenicity of genetic diversity or both has been reported in *Macrophomina phaseolina* that confirms the ability of the pathogen to survive and adapt to the various environmental conditions (Baired *et al.,* 2010).

Antifungal agents based on natural products have always been promising in the control of fungi. The secondary metabolites produced by these plants have shown to affect the fungal agents. Moreover, these agents are not toxic and are decomposed easily. Numerous literatures have highlighted the inhibition effect of plants and their possible utilization for control of plant diseases (Farooq *et al.,* 2010; Nunez *et al.,* 2010; Gupta and Tripathi 2011).

2. MATERIALS AND METHODS

2. 1. Isolation of Fungi from diseased specimen of Gingelly

A virulent strain of *Macrophomina phaseolina* (Tassi) Goid. was isolated from dry root rot infected *Sesamum indicum* roots were collected from Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalai nagar. Axenic culture of the pathogen was obtained by single hyphal tip method and maintained in PDA slants by periodical sub-culturing and used for further studies.

2. 2. Collection and identification of plant materials

The leaves of *Citrus aurantifolia* L. were collected from the Experimental Orchards, Faculty of Agriculture, Annamalai university, Annamalainagar, during the year 2013. The plant specimens were identified and authenticated by Dr. V. Venkatesalu Professor & Head Department of Botany Wing, Annamalai universaity, Annamalainagar, Tamilnadu, India.

2. 3. Extraction

The fresh leaves were cut into small pieces, shade dried and powdered using electrical blender. For extraction, 10 g of powdered material was added to 100 ml of distilled water and boiled half an hour. The content was filtered through 4-fold muslin cloth followed by Whatman No. 1 filter paper and used for antifungal studies (Kekuda *et al.,* 2010).

2. 4. Antifungal activity of leaf extracts

The antifungal efficacy of leaf extracts was determined by poisoned food technique. PDA media amended with different concentrations of leaf extracts (5, 10, 15 and 20 %) were sterilized by autoclaving and added to labelled petridishes. The fungicide Carbendazim 50 per cent wp at 0.1 per cent concentration in the medium was used for comparison. The medium was poured into 90 mm Petri plates at 15 ml/plate. The fungal discs of 9 mm size obtained from seven days old culture were inoculated at the centre of the Petri plates and incubated at room temperature (28 ±2 °C) for 10 days. Four replications were maintained for each treatment and a suitable control was also maintained. The diameter of the mycelial growth (mm) of pathogens was measured and recorded after 7 days of incubation. Antifungal activity was recorded in terms of inhibition of mycelial growth (%) and calculated using the formula.

$$\text{Inhibition of mycelial growth (\%)} = (C - T/C) \cdot 100$$

where 'C' is average diameter of fungal colony in control plates and 'T' is average diameter of fungal colony in poisoned plates (Gupta and Tripathi, 2011).

3. RESULTS AND DISCUSSION

The poisoned food technique was employed to determine inhibitory efficacy of different concentrations of leaf extracts of *Citrus aurantifolia* against *Macrophomina phaseolina* and the result is presented in Table 1.

The average diameter of colonies of test fungi in poisoned food plates was markedly lesser than that of colony diameter in control plates which is indicative of antifungal potential of extracts. The inhibition was concentration dependent. Among fungi tested, susceptibility to extracts was revealed that an increase in the concentration of plant extracts decreased the mycelial growth of the test fungus.

Among the plant extracts used, the maximum inhibition of the mycelial growth (3 mm) was obtained with 20 per cent concentration which accounted for 96.67 per cent reduction of mycelial growth over control. This was followed by Carbendazim (0.1 %) recording 9.0 mm and 3.0 mm of the mycelial growth which was accounted for 90.0 and 96.67 per cent reduction of mycelial growth over control.

Table 1. Effect of plant extracts on the mycelial growth of *Macrophomina phaseolina* (Poison food technique).

Tr. No	Treatment	Diameter of mycelial growth (mm)				Per cent decrease over control			
		Concentration of the culture filtrate (%)				Concentration of the culture filtrate (%)			
		5%	10%	15%	20%	5%	10%	15%	20%
T1	*Citrus aurantifolia* L	22.0	18.0	11.0	3.0	75.55	80.0	87.77	96.67
T2	Carbendazim (0.1%)	--	--	--	9.0	--	--	--	90.00
T3	Control	90.0	90.0	90.0	90.0	-	-	-	-
T4	SE CD (p = 0.05)	0.05 0.19	1.25 2.89	0.67 2.03	0.24 0.53	--	--	--	--

*** The values are mean of four replications**

Crop loss due to root rot causing fungal pathogens is a significant problem. The most common method of control is the use of chemical fungicides. However, environmental concerns, cost, development of resistance in pathogens increased interest in alternatives such as plant extracts, antagonistic microbes and others to traditional synthetic chemical fungicides (Sealy *et al.,* 2007).

Plants and plant products have shown to be useful candidates for prevention and control of phytopathogenic fungi. Farooq *et al.,* (2010) showed the efficacy of plant extracts against *Sclerotium rolfsii,* causative agent of root rot of sugar beet and observed maximum inhibition of the fungus by *Azadirachta indica* followed by *Cassia fistula, Cannabis sativa* and others. Poisoned food technique has been routinely employed to screen the effect of plants and their compounds against fungi.

The antifungal activity is observed as reduction in the mycelial growth of fungus in poisoned plates when compared to the control plates. It has been employed by several researchers to evaluate antifungal activity of plants (Nunez *et al.,* 2010). In the present study, we have investigated the effect of aqueous extracts of leaf of *Citrus aurantifolia* against mycelial growth of *Macrophomina phaseolina* isolated from dry root rot of Gingelly.

The extracts have showen marked concentration dependent inhibition of mycelial growth of test fungi indicating the presence of antifungal principles in the aqueous extracts. In an earlier study, (Sagar *et al.,* 2007) showed the fungi-toxic efficacy of some plant extracts against *P. aphanidermatum* and *F. solani* isolated from rhizome rot specimen of Ginger. It was found that *Azardirachta indica* and *Ferula asafoetida* showed maximum inhibition of mycelial growth of *P. aphanidermatum* and *F. solani* respectively.

4. CONCLUSION

From the results of the present study, it is concluded that the leaf of *Citrus aurantifolia* are effective against *Macrophomina phaseolina* dry root rot pathogens. Further, field experiments are to be carried out in order to recommend the bio active extract against the disease.

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