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In vitro antifungal screening of some medicinal plants against *Macrophomina phaseolina*

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Abstract
The medicinal plants present enormous reservoir of potential microbial compounds that could be useful alternative to synthetic microbicides and are being used to develop drugs. In the present study of five selected medicinal plants the leaf powder extracts were tested against *Macrophomina phaseolina* by disc diffusion method. It was found that ethanol extract strongly effective against *Macrophomina phaseolina,* is a causal agent for charcoal rot of sunflower fungal pathogen.

Keywords: *Antifungal activity, medicinal plants, pathogen*

INTRODUCTION

Antibiotics and chemically synthesized medicines for 4th generation to cure microbial infections very fast but they may also disturb the natural immunity of body and cause variety of side effects. This aroused interest in plant products and these products are certainly an answer and which may partially support of substitute synthetic drugs. Thus, keeping this view in mind medical communities are now trying to seek the solution of above said problems from plants based on medicine in allopathic. Indian medicinal plants it is expected that screening and scientific evaluation of plant extract for their antimicrobial substance may prove beneficial for the man kind. Further, synergistic interaction among crude extract of phytoconstituents *in vitro* may be useful in the preparation of improved poly herbal or drug formulations (Parihar *et al*., 2003). Leaf of *Adhatoda vasica* is astringent, tonic, antiperiodic, used in chronic infantile dysentery and as external application for ulcer, and it is used for wounds, blood disorders and haemorrhage (Yoganarasimhan 2000).

MATERIALS AND METHODS

The selected medicinal plants used for this study were bought fresh leaf materials are collected from various parts of Annamalai nagar. A virulent pathogen of *Macrophomina phaseolina* were obtained from Department of Plant pathology, Faculty of Agriculture, Annamalai university. The isolated pathogen were maintained on nutrient agar slants (Hi – media).

The fresh leaf sample were powdered in a grinder after drying in shade and subsequently sieved. Thereafter five gram of each fine powdered sample was weighed and separately soaked in the solvent by mixing 20ml of distilled water with 20ml of 90% ethyl alcohol. They were placed on a rotary shaker for 12h this process was repeated till the extract was free from colour and odour. It was then cold centrifuged, concentrated and stored in sterile vials at10Ċ. The pH of the extracts was adjusted to 6.7.

The antifungal activity of the extract of medicinal plants was determined by using (1) microbial assay (2) paper impregnated disc method. Nutrient broth was prepared and distributed in several test tubes each with 10ml autoclaved, allowed to cool and the tubes were inoculated with 0.5ml of 24h old culture of *Macrophomina phaseolina* (Plate – I). Then each test tube was added with 1.5ml of the extract of each species of medicinal plants separately and incubated at 37Ċ for 24h. Control was maintained without adding the extract of any medicinal plant. After incubation 0.5ml of formaldehyde solution was added in each test tube. The rate of growth was determined by measuring the optical density at 545nm. The readings were compared with control.

Table 1. Antifungal effect of distilled water (DW) and ethanol (EL) extract of some medicinal plants against *Macrophomina phaseolina*

Name of the plants	Methods used	*Macrophomina phaseolina* Zone of inhibition in mm	
		DW	EL
Adhatoda vasica Nees.	a	+ + +	+ + +
	b	18	20
Lawsonia inermis Linn.	a	+ + +	+ + +
	b	20	22
Nerium indicum Mill.	a	+ + +	+ + +
	b	16	17
Pongamia pinnata (L.) Merr.	a	+	+ +
	b	10	13
Solanum nigrum Linn.	a	--	+
	b	10	10

a – microbial bioassay method, b – paper impregnated method.
+ + + = More inhibition + + = Moderate inhibition + = Less inhibition and -- = No inhibition.

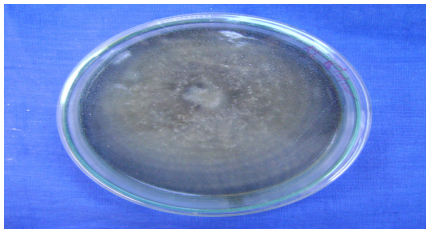

Plate 1. Culture of *Macrophomina phaseolina*

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RESULTS AND DISCUSSION

Sterilized filter paper discs (What man No. 1) of 6mm diameter were soaked in the extract of medicinal plants separately. Three pre treated filter paper discs were placed onto the solidified nutrient agar medium equidistantly and the plates were incubated at 37Ċ for 24h control was maintained by placing the filter paper discs soaked in sterile distilled water. The degree of sensitivity of the organisms was determined by measuring the visible zone of inhibition using vernier caliper.

In the present study, the antifungal activity of some selected medicinal plants with ethanol exhibition against the growth of *Macrophomina phaseolina* (Table–1).Remarkable inhibition spectrum value (+ + +) were obtained with the microbial bioassay method. All the aqueous extract of *Adhatoda vasica*, *Lawsonia inermis* and *Nerium indicum.* Some recent reports regarding the antimicrobial properties of medicinal plants support the present result (Meena and Sethi, 1994).

The antifungal activity of spices has been well documented. Antibacterial properties if *Alphinia galanga* and *Curcuma longa* were demonstrated *S.aureus* , *S.epidermis* , *S.haemolyticus , Escherchia coli* and *S.pnemoniae* (Thomas *et al* ., 1996). It has been reported that the presence of active principles such eugenol in clove and capsicin in chilli inhibited the growth of microorganisms (Choudhury & De, 1986; Karapinar & Aktug, 1987).

In the present study it was found that *Macrophomina phaseolina* was more resistant of *Adhatoda vasica*, *Lawsonia inermis* and *Nerium indicum and* less inhibitory effects of *Pongamia pinnata* and *Solanum nigrum* of both the extracts (DW & EL). Though all the selected plant species tested showed antifungal activity, it varied with the plant species and tested organisms. It is mainly due to the genetic variability of the organisms. The present finding supports the view that several ethnomedical plants might be useful as antimicrobial agents resulting in the development of novel drugs for many centuries through ethnopharmacy (Heinrich 2000, Heinrich and Simon 2001).

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