

Antifungal activities of selected leaves extracts on *Aspergillus niger* van Tiegh. isolated from *Vigna unguiculata* (subsp. *sesquipedalis* L. Verdcourt)

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Antifungal activities of selected leaves extracts on *Aspergillus niger* van Tiegh. isolated from *Vigna unguiculata* (subsp. *sesquipedalis* L. Verdcourt)

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The objective of the present study was to determine the inhibition efficacy of the selected leaves extracts on the mycelial growth of the endophytic fungal pathogen isolated from *Vigna unguiculata* (sub sp. *sesquipedalis*) L. Verdcourt. The Aqueous leaf extracts of the selected plants with different concentrations were tested for its antifungal capability in Potato Dextrose Agar (PDA) medium. The inhibition zone was measured with the inhibition zone of the control taken without leaves extract. Here, the leaves of five plants were used to determine the efficiency of inhibition against the endophytic fungus *Aspergillus niger*.

Key words: *Bougainvillea glabra*, Mycelial growth, Poisoned food method

INTRODUCTION

In agricultural practices, the quality of seed is very important criteria especially to increase the yield. New pathogens or new diseases can transmit through seed movements by importing activities (Harahap *et al.* 2015). Fungi form the largest group among microorganisms causing seed damage on the development which causes more destruction as post and pre-infections and deteriorates considerable seed quality. The fungus, *Aspergillus niger* is one of the most common species of the genus *Aspergillus* and a worldwide distributed member of ascomycotina. *Aspergillus niger*, a filamentous ascomycete having ability of fast growth is commonly found as a saprophyte growing on stored grains or seed, it also causes disease on certain kinds of fruits and vegetables and is a toxin for food, can produce mycotoxins which are carcinogenic and nephrogenic immunological in nature (Sharma, 2012).

The repeated and indiscriminate use of drugs favour the microbes to acquire more resistance over the drugs since very long time. So, more

adequate and eco-friendly based drugs are found to be essential. Here, five plants-*Aloe vera* L., *Bougainvillea glabra* Comm., *Murraya koenigii* L., *Coleus aromaticus* Benth., and *Strobilanthes ciliatus* Nees. were tested against the endophytic fungus *Aspergillus niger* van Tiegh. from *Vigna unguiculata* (sub sp. *sesquipedalis*) L. Plant extracts have a wide range of biochemicals and they have been widely used for various purposes (Roopa, 2012; SaiPrabha, 2014).

MATERIALS AND METHODS

Source of fungal organism

Agriculture Research Institute Kallungal, Thiruvalla provided the seed samples of cowpea (*Vigna unguiculata* (var. *sesquipedalis*)). The seeds were incubated on water agar medium at a temperature of 27±1°C. The fungal pathogen was isolated and stored at 4°C temperature.

Preparation of medium for antimycological study

The culture medium (PDA medium) was prepared by adding appropriate proportion of Potato

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infusion, Bacteriological agar and dextrose and made upto 1000ml of distilled water. Then the medium autoclaved for 15 min at 121°C for avoiding contamination. The pH of the medium maintained as 4.5 using 0.1N HCl and 0.1N NaOH for the fungal growth.

Preparation of leaf extracts

The selected plant leaves were collected, identified with keys and washed thoroughly under running water to clean out dirt and dust from the leaves and washed with 0.01g of HgCl₂ solution and finally with sterile distilled water thrice.

The leaves materials were crushed well and the aqueous extracts of the soluble ingredients were made by macerating the leaves at different concentrations (25%, 50%, 75% and 100%) (Alkhail, 2005; Hadian, 2012) in 100ml of solvent and placed it for 24 h (Olusanmi and Amadi, 2009). The extracts filtered by Whatman No.1 filter paper and stored at 4°C temperature for avoiding contamination for future studies. A small 2 mm disc was cut from an actively growing fungal culture medium, with the help of a sterile cork borer and was transferred aseptically into the centre of the sterile petriplates having the extract mixed with Potato Dextrose Agar (PDA) (Swami and Alane, 2013). The inhibition capacity of the leaves extracts at various concentrations were tested against the endophytic fungus and the percentage inhibition of the leaves extracts were calculated by the growth in control to that of the growth in treatment.

$$\text{Percentage(\%)} \text{ inhibition} = \frac{G_c - G_t}{G_c} \times 100$$

'G_c' the mycelial growth of the fungal colony in control and 'G_t' the mycelial growth of the fungal colony in treatment

RESULTS AND DISCUSSION

The antimycological activities was conducted by the poisoned food method at different concentrations of the aqueous extracts of leaves on the mycelial growth of the tested fungus, *Aspergillus niger* after 5-7days of incubation at 27±1°C.

The level of inhibition at various concentrations of leaves extracts on the mycelial growth of the endophytic fungal pathogen, *Aspergillus niger* has

Table.1: Effect of different concentrations leaf extracts on the radial growth of *Aspergillus niger*

Treatments	Concentrations of extracts on radial mycelial growth in treatments' (mm)				Growth in Control (mm) 0%
	25%	50%	75%	100%	
<i>Aloe vera</i>	31	30	24	21	45
<i>Strobilanthes ciliatus</i>	37	25	23	22	45
<i>Coleus aromaticus</i>	39	38	26	24	45
<i>Bougainvillea glabra</i>	38	37	20	18	45
<i>Murraya koenigii</i>	27	25	22	19	45

*Each treatment was replicated three times and mean value was in mm

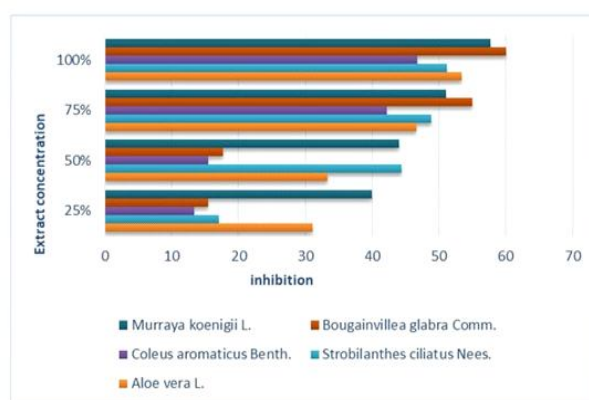


Fig.1: Percentage Inhibition (%) with different concentrations of aqueous extracts of leaves

been shown in Table 1. The leaf extracts of *Bougainvillea glabra* and *Murraya koenigii* proved highly inhibitory to the mycelial growth of the endophytic fungus *Aspergillus niger*, which showed 60% and 57.7% of inhibition respectively at the maximum concentration of the extracts taken i.e., 100%. The aqueous extracts of *Aloe vera* showed 53.3% inhibition whereas *Strobilanthes ciliatus* showed 51.1% inhibition and was followed by the extracts of *Coleus aromaticus* which proved 46.6% of inhibition. The results clarifying that the antimycological activities of the extracts were improved with the increasing extract concentrations (Senthilkumaran and Pavithra, 2014).

The minimum inhibition efficiency of the extracts found at the minimum concentration of extract, i.e., 25% of the extract concentration by the aqueous extracts of *Coleus aromaticus* (13.3%) and was followed by *Bougainvillea glabra* and *Strobilanthes ciliates* ie, 15.5 and 17%

correspondingly. The aqueous extracts of *Aloe vera* had shown 31.1% inhibition and *Murraya koenigii* shown 40% of mycelial growth inhibition at 25 % extract concentration. At 50% extract concentration, *Murraya* and *Strobilanthes* showed a good tendency to inhibit the mycelial growth than other plants and *Bougainvillea glabra* proved as highly inhibitory to the mycelial growth at 75% extract concentration. The inhibition capacity of the aqueous extracts was found to be increased with the concentration of the extract of the leaves. Evaluation of the inhibition of mycelial growth by different concentrations of leaf extracts showed a strong dependent effect of extract concentrations of the phytochemicals contained in it. The extracts concentration shows noticeable dependent inhibition on the mycelial growth of the test fungus, *Aspergillus niger*. It indicates the existence of antimycological efficacy of the aqueous extracts (Balamurugan, 2014). The present study agrees with other antimycological findings such as the report where extracts of *Bougainvillea* leaves has been shown to have potential to treat various micropathogens (Umamaheswari, 2008) and proved its effectiveness here against the mycelial growth of the pathogenic fungus, *Aspergillus niger* (Gabriel, 2014).

CONCLUSION

Aspergillus niger causes heavy loss in agricultural industries all over the world. Recent times, the herbal based remedies are highly recommendable because they cause zero health complications. This study has proved that the aqueous leaves extracts of *Murraya koenigii* and *Bougainvillea glabra* had a good potential to reduce the fungal mycelial growth and have proved as effective as well as cheaper in the treatment of pathogenic fungus and can be used in formulating new biological agents in future.

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