

# Effect of Some Selected Leaf Extracts on *Fusarium oxysporum* from Anaswara Variety (*V. unguiculata* (subsp. *sesquipedalis* L. Verdcourt)

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## ABSTRACT

This study was aimed to evaluate the efficacy of the leaf extracts at different concentrations on the seed borne pathogenic fungus from Anaswara variety of *Vigna unguiculata* (sub sp. *sesquipedalis*) L. Verdcourt. The Ethanolic leaf extracts of the selected plants with different concentrations were tested for its antifungal efficacy on the endophytic fungus *Fusarium oxysporum* in Potato dextrose agar (PDA) medium. Among the four leaf extracts, the leaf extracts of *Murraya koenigii* showed the maximum inhibition on the endophytic fungal pathogen.

**Keywords:** *Fusarium oxysporum*, Anaswara variety, Isolation, *Murraya koenigii* L., Antimycological activity.

## I. INTRODUCTION

*Vigna unguiculata* subsp. *sesquipedalis* is a member of Fabaceae family, commonly known as yard long bean, is cultivated throughout world for its nutritional advantage of seeds and pods. It is known by several names as yard long bean or long-podded cowpea because it has long pods, under certain circumstances, it is called as asparagus bean, pea bean, Chinese long bean or snake bean. It is estimated that cow pea of 100grams contains 47 calories, 0 grams of total fat, 4 milligrams sodium, 8 grams of total carbohydrates, and 3 grams of protein. Besides, it contains Vitamin C or Ascorbic acid (19.00%), Vitamin B9 or Folate (14.00%), Magnesium (9.52%), Isoleucine (8.13%), Manganese (8.13%), Vitamin B1 (Thiamin) (8.08%), Phosphorus (7.71%), Vitamin B2 or Riboflavin (7.69%), Valine (6.96%), Histidine (6.66%). Therefore, it is valued as a nutritional supplement for both human beings and animals.

*Fusarium oxysporum* is an abundant as well as active saprophyte in soil and organic matter, with some specific forms that are plant pathogenic (Smith et al., 1988). Its saprophytic ability enables it to survive in the soil between crop cycles in infected plant debris. The pathogen itself or parts of it can stick on or it can be mixed with the seeds during any stage of growth or development. The fungus can survive either as mycelium, or as any of its three different spore types

(Agrios, 1988). The hosts of *Fusarium oxysporum* include: potato, sugarcane, garden bean, cowpea, Prickly pear, cultivated zinnia, pansy, Assam rattlebox, Baby's breath, and *Musa* sp. (Raabe et al., 1981). *Fusarium oxysporum* characterized by causing the following symptoms: vascular wilt, yellows, corm rot, root rot, and damping-off. Since having many hosts, management of this pathogen is a great concern (Gonsalves and Ferreira, 1993).

Plants or Natural herbal remedies have been highly recommended for fungal treatments, since thousands of years (Arunkumar and Muthuselvam, 2009). Plant extracts have a wide range of biochemicals and they have been widely used for various purposes (Roopa, 2012). They are more effective and have no side effects. They are enriched with various bioactive elements. *Aloe vera* L., *Bougainvillea glabra* Comm., *Murraya koenigii* L., *Coleus aromaticus* Benth. were used for the present study to evaluate its antifungal efficacy on the seed borne fungi *Fusarium oxysporum* in Ethanolic extract.

## II. METHODS AND MATERIAL

### Source of Anaswara variety:

*Vigna unguiculata* (var. *sesquipedalis*) were collected from Agriculture Research Institution Kallungal, Thiruvalla.

### Isolation of fungal pathogen:

The fungal pathogen was isolated by slant culture of water agar medium. It was incubated on  $27\pm 1^{\circ}\text{C}$  and stored at  $4^{\circ}\text{C}$ .

### Preparation of PDA medium:

The PDA culture medium was prepared by boiling 200:20:20 Potato infusion, Bacteriological agar agar, dextrose respectively, and dissolved in 1000ml of distilled water. Then the medium autoclaved at 15 lbs pressure for 15 min for avoiding contamination. The pH of the medium maintained as 4.5 using 0.1 N HCl and 0.1N NaOH.

### Preparation of leaves material:

The selected four plant leaves were collected, identified with keys and washed thoroughly under running water to clean out dirt and dust and washed with 0.01g of  $\text{HgCl}_2$  solution and finally with sterile distilled water thrice.

### Preparation of extract:

The method of extraction was carried out in accordance with Alkhail (Alkhail, 2005). The test plant materials were crushed well and the ethanolic extracts of the soluble ingredients were taken by macerating the 25%, 50%, 75% and 100% in 100ml of solvent and placed it for 24 hours. (Gabriel et al., 2014). The extracts filtered by Whatman No.1 filter paper and stored at  $4^{\circ}\text{C}$  temperature for further studies and to get free from contamination and other physio-chemical alternating changes occurring in the extracts. The different leaf concentrations were tested against the endophytic fungi, *F. oxysporum* on PDA medium of sterile Stien  $100\times 17\text{mm}$  petric plates.

The percentage of inhibition of the leaves extract was calculated by the control in treatment to that of control in growth.

$$\text{Percentage of inhibition} = \frac{\text{Control in treatment} - \text{Control in growth}}{\text{Control in treatment}} \times 100$$

## III. RESULTS AND DISCUSSION

It was found out that in the solid potato dextrose agar medium, the aerial mycelia of *F. oxysporum* have had different manifestations, as white and it changed to diverse colours, was depending up on the strain of *F. oxysporum* from violet to dark purple. If sporodochia were abundant, the culture may appear cream or orange in colour (Smith et al., 1988). The spores abundantly and frequently produced by the fungus under all conditions.

The anti mycological activities of the tested fungi, *F.oxysporum* on different concentrations of the selected leaves extract was monitored by poisoned food method after 6days of incubation at  $27\pm 1^{\circ}\text{C}$ . The leaf extracts were prepared on absolute Ethyl alcohol with different concentrations. Each 10ml of the medium in the plate was poisoned by 2ml of different concentrations of extracts.

From the results, all extracts have shown considerable level of inhibitory effects on the endophytic fungal pathogen, *F. oxysporum* from the anaswara variety of *V. unguiculata, sesquipedalis L.Verdcourt.*, as presented in the Table 1. The maximum inhibitory capacity of the extract exhibited by the leaf extracts of *Murraya koenigii L.* at 100% concentration and the minimum inhibition was found at 25% of the extracts by *Murraya koenigii L.* itself as shown in the Fig.1. The *Bougainvillea glabra* had shown a very good tendency to inhibit the growth of the spreading mycelia and sporulation of the tested fungus for a remarkable extent. *Aloe vera* had shown 91% where as *Coleus aromaticus* had shown 89.7 % of effect at 100% of concentration.

Thus, all extracts at the maximum concentration showed a very incredible level of inhibition against the fungal growth in the PDA medium. Comparison of the growth inhibition by various leaves extracts and their respective dilutions showed a strong reliant effect of extract concentrations of the phytochemicals contained in it. These results showing the intention of antifungal activity of various extracts were enhanced by increasing the concentration of the extracts. Thus, the inhibition activity of the extracts showed dependence on concentration (Gabriel and Vincent, 2014). By the increasing plant extract concentration, fungicide property of the extracts also had been increased (Sherwin, 2012).

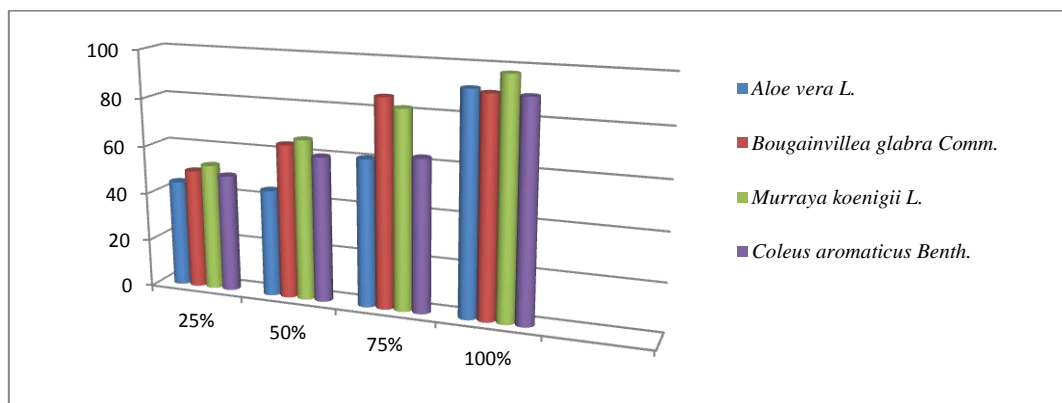
The present observation which agrees with the findings of *Banso (Banso et al., 1999)*, who had found out that with the increase in the concentrations of the substance, the growth inhibition also increased. The bioactive compounds of *Murraya koenigii* even at a lower concentration showed the antimycological effects against the growth of the endophytic fungi, thus it has showed an ideal medicine for formulating particular

medicinal substance against the fungus. Further study of extracting compounds, which is more responsible on the inhibitory action of the fungal growth, will be a remarkable advantageous discovery in the manufacturing of accurate medicine against the fungal attack.

**Table 1. Inhibition of Different leaves extract on the radial (mm) growth of *F.oxysporum***

Treatments	Ethanollic concentrations* on radius growth in treatments (mm)				Growth in Control (mm)
	25%	50%	75%	100%	
<i>Aloe vera</i> L.	25	25.1	18	3.9	45
<i>Bougainvillea glabra</i> Comm.	22.6	16	6.3	4.4	45
<i>Murraya koenigii</i> L.	21.3	15	7.8	1.0	45
<i>Coleus aromaticus</i> Benth.	23	18.1	16.6	4.6	45

\*Each treatment was replicated three times.



**Figure 2. Percentage of inhibition on different concentrations of leaves extracts**

#### IV. CONCLUSION

In this study, the antimycological results have proved that the leaf extracts of *Murraya koenigii* as an excellent source of phytochemical constituents than the other four leaves and hence it inhibited the fungal growth to a

remarkable extent. The identification and isolation of its chemical formulation will be a greater step in the manufacturing of natural fungal drugs against the treatment of seed borne fungi especially, which causes greater loss in the economy of Nations, which have the main source of income from agriculture. This kind of

treatment also helps to enhance the quality of seeds and its viability. The finding of this study also throws light to a significant level towards crop protection strategies, for the manufacturing of improved cheaper bio fungal medicines by this natural product.

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