



## ANTIFUNGAL ACTIVITY OF LEAF EXTRACT OF THREE CITRUS PLANTS AGAINST *COLLETOTRICHUM CAPSICI*

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### ABSTRACT

The present study was conducted with an aim of determining antifungal potential of leaf extract of three citrus plants viz., *C. limon*, *C. reticulata* and *C. aurantium* against *Colletotrichum capsici* (causal agent of anthracnose of chilli). Antifungal activity of leaf extracts was determined by Poisoned food technique. Leaf extract of *C. reticulata* and *C. aurantium* caused highest and least inhibition of fungus respectively. An inhibition of >50 % of fungus was displayed by extract of *C. limon* and *C. reticulata*. Citrus leaf extracts can be used to control chilli anthracnose.

**Keywords:** Citrus, Antifungal activity, Poisoned food technique, *Colletotrichum capsici*

### INTRODUCTION

Plants are vulnerable to diseases caused by a number of microorganisms. Among these, fungi cause more number of diseases in plants resulting in lower yield. One of the main strategies being widely used for control of fungal diseases is the use of synthetic fungicides. However, these chemical fungicides suffer from several drawbacks such as high cost, residue problems, deleterious effect on non-target micro biota and resistance development. Besides, carcinogenic, teratogenic, oncogenic and genotoxic properties of synthetic fungicides are known. Hence, the development of new strategies for management of fungal diseases is of interest. Plants are considered as best alternatives and many studies revealed the potential of plants to inhibit a variety of phytopathogenic fungi<sup>1-6</sup>. The genus Citrus belongs to the family Rutaceae and is native to tropical and subtropical areas in Southeast Asia. The citrus plants are grown worldwide and ranks top in world production and trade among the fruit trees. The fruits of citrus plants such as *C. limon*, *C. reticulata*, *C. aurantium* and *C. sinensis* are consumed fresh or in the form of juices and also used to prepare pickles and other food stuffs. The leaf, peel and essential oil from citrus plants are shown to exhibit a range of biological activities<sup>3,7-12</sup>. The objective of the present study was to determine antifungal activity of leaf extract of three citrus plants viz., *C. limon* (lemon), *C. reticulata* (orange) and *C. aurantium* (bitter orange) against *Colletotrichum capsici* (causative agent of anthracnose of chilli).

### MATERIALS AND METHODS

#### Collection and extraction of plant materials

The leaves of *C. limon*, *C. reticulata* and *C. aurantium* were collected at a place called Dantaga, Hosanagara Taluk of Shivamogga District, Karnataka, India during February 2014. The leaves were washed well using clean water, dried under shade and powdered. A known quantity of leaf powder (25 g) was added to 100 ml of methanol (HiMedia, Mumbai, India) taken in a conical flask and stirred well. The flask was allowed stand for 48 hours and mixed occasionally. Later, the content of flask was filtered through sterile Whatman No. 1 filter paper and the filtrate was evaporated in oven to dryness<sup>6</sup>.

#### Antifungal activity of leaf extracts

Poisoned food technique was performed to investigate antifungal efficacy of leaf extract of selected citrus plants. Potato dextrose agar (HiMedia, Mumbai, India) was poisoned with the leaf extract (1 mg/ml). The control (without extract) and poisoned Potato dextrose agar plates were inoculated with the spore suspension of *Colletotrichum capsici* and incubated for 5 days at 28°C. The size of the colony test fungus (diameter) on control and poisoned plates was measured. The extent of growth inhibition of *C. capsici* was calculated using the formula:

$$\text{Inhibition of mycelial growth (\%)} = (A - B / A) \times 100,$$

Where 'A' and 'B' is colony diameter of fungus on control and poisoned plates respectively

The experiment was repeated two times and the result is mentioned as Mean  $\pm$  Standard Deviation (S.D)<sup>6</sup>.

### RESULTS

The leaf extracts of selected citrus plants were shown to exhibit inhibition of mycelial growth of *C. capsici*. The colony diameter of *C. capsici* on plates poisoned with citrus leaf extracts was comparatively lesser when compared to colony diameter of fungus on control plates (Table 1). Over 50 % inhibition of fungal growth was caused by leaf extract of *C. reticulata* and *C. limon*. Among leaf extracts, marked inhibitory activity was shown by *C. reticulata* followed by *C. medica* and *C. aurantium* (Figure 1).

**Table 1: Colony diameter (cm) of *C. capsici* in control and poisoned plates**

| S. No. | Treatment            | C.D (Mean $\pm$ S.D) |
|--------|----------------------|----------------------|
| 1      | Control              | 3.2 $\pm$ 0.0        |
| 2      | <i>C. aurantium</i>  | 1.9 $\pm$ 0.1        |
| 3      | <i>C. reticulata</i> | 1.4 $\pm$ 0.1        |
| 4      | <i>C. limon</i>      | 1.5 $\pm$ 0.0        |

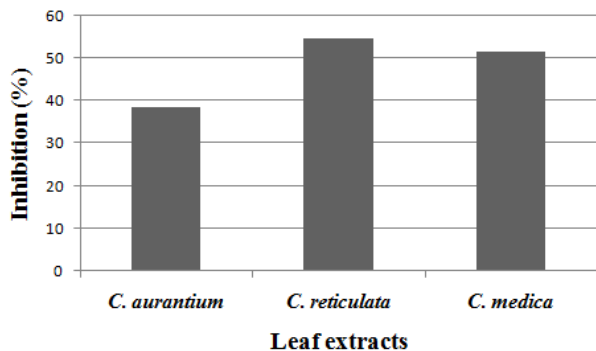


Figure 1: Inhibition (%) of *C. capsici* by leaf extracts

## DISCUSSION

Chilli (*Capsicum annum* L.) is an important economic crop grown for domestic use and export. It is used both as vegetable and spice. Anthracnose is one of the important diseases of chilli in tropics and subtropics. Various species of *Colletotrichum* are known to cause anthracnose among which *C. capsici* is an important pathogen. The disease causes drastic reduction in the yield and deterioration of the fruit quality and hence low returns to farmers. In severe cases, the crop loss may exceed 50 %. The management of anthracnose includes the use of fungicides such as mancozeb, captan, bavistin, thiram, copper oxychloride, cosan, benlate and ziram. However, resistance against most of these fungicides has been noticed in *C. capsici*. Natural products, in particular plants are considered as safe alternate for disease management<sup>6,13-16</sup>. In the present study, we determined antifungal effect of leaf extract of three citrus plants by poisoned food technique. Leaf extract of *C. limon* and *C. reticulata* inhibited mycelial growth of *C. capsici* to more or less similar extent (>50 % inhibition) while leaf extract of *C. aurantium* inhibited *C. capsici* to least extent. Marked inhibitory activity was demonstrated by leaf extract of *C. reticulata*. It has been shown that leaf, peel and essential oil of citrus plants exhibit inhibitory activity against fungi<sup>3,5,17,18</sup>.

## CONCLUSION

The leaf extracts of selected citrus plants caused marked inhibitory activity against *C. capsici* in terms of inhibition of mycelial growth. These plants can be used as natural fungicides for the control of chilli anthracnose. Further studies in fields are to be done in order to ascertain the possible application of these botanicals for disease management.


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