

Antifungal Activity of Essential Oils on Growth of Phytopathogenic Fungi

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Abstract: Plant essential oils are potential source of antimicrobials of natural origin. Essential oils and extracts obtained from many plants have recently gained a great popularity and scientific interest. Consumer demand for natural preservatives has increased, whereas the safety aspect of chemical additives has been questioned. The plant oil has been reported to have antibacterial, antifungal, antiviral, antiparasitic and antidermatophytic properties. It is now considered as a valuable source of natural products for development of medicines against various diseases and also for the development of industrial products. The present review is a compilation of updated information on plant essential oils with antifungal properties.

Keywords: essential oil, inhibition, plant disease, mycelial growth

1. INTRODUCTION

More than 1300 plant species are known to be potential sources of antimicrobial components but only some of them have been studied scientifically (Wilkins and Board, 1989, Paster et al., 1990.). For instance, some previous studies evaluated the inhibitory activity of essential oils on fungi. Cardwell and Dongo (1994) tested extracts from nine plant species on mycelial growth of *Apergillus flavus* L.; Manohar et al. (2001) researched origano commercial oil against *Candida albicans* (Robin) Berkhout; Marin et al. (2004) tested cinnamon, clove, oregano, palmarosa and lemongrass oils against *Fusarium graminearum* Schw., Hadizadeh et al. (2009) analyzed antifungal potential of five essential oils against *Alternaria alternata* (Fr.) Keissl. Burgiel and Smaglowski (2008) described complete growth inhibition of *Fusarium culmorum* (W.G. Smith) Sacc. and *B. cinerea* on media with a 0.5% addition of tea tree oil. In addition, some studies reported positive effect of essential oils in reducing mycotoxin accumulation in maize grain (Marin et al. 2004, Velluti et al. 2004).

The aim of this research was to test in vitro the Effect of some essential oils on mycelial growth of plant pathogenic fungi.

2. METHODS

2.1. Essential Oils

The essential oils such as neem oil, castor oil, citronella oil and camphor oil, obtained from local market and exposed to UV radiation for 10 min then these oils were used for this study. These oils were selected based on literature survey and their use in preservation of leaf articles.

2.2. Isolation of Palmyrah Leaf Decay Fungi Collection Of Sample

Palmyrah handicrafts are usually affected by fungus during rainy season. Affected tender leaf articles of Palmyrah were collected from two design centers of Palmyrah Development Board during rainy season and used for the isolation of Palmyrah leaf article decay fungus.

3. PREPARATION OF POTATO DEXTROSE AGAR MEDIA

3.1. PDA Plates

Potato Dextrose Agar medium was prepared according to the manufacturer's direction. After sterilization, the medium was allowed to cool to 50°C and poured in to sterile petridishes (20 mL/Petridish) under aseptic condition.

3.2. PDA Slants

The PDA was prepared according to the manufacturer's direction and 7 mL of the medium was poured into boiling tubes. The tubes were plugged with cotton wool and sterilized at 121°C and 15 lb/in² for 15 min. The tubes were then cooled in an inclined position and used for storage of the fungus.

3.3. Isolation of Fungal Strains

Fungus affected leaf handicrafts were brought to laboratory and using inoculation needle different colour colonies were streaked on sterile PDA plates in Zig-Zag manner and incubated at room temperature for 3- 4 days. Selected colonies were purified by repeated streaking and transferred to PDA slants and kept at 4°C.

3.4. Characterization of Isolated Fungal Strains

Selected fungal colonies were identified to species level based on macroscopic morphology and microscopic features.

3.5. Antifungal Activity Assay

PDA medium with 1, 15, 20 and 25 (ml/dl) concentrations of the essential oils such as neem, castor & citronella and 1, 5, 10 & 15 (ml/dl) of concentrations camphor oil were prepared. About 15 mL of the medium was poured into each petridish, Tween-20 (Sigma) was incorporated into the agar medium to enhance oil solubility and allowed to solidify. Nine mm disc of 5 days old culture of the test fungi from the margin of the plates were placed at the center of the petridishes and incubated at room temperature for 4 days. After incubation the colony diameter was measured in millimeter. For each treatment three replicates were maintained. PDA medium without the essential oil served as control. Growth zones were measured at 4th and 6 th days of incubation. The fungi toxicity of the oils in terms of percentage of growth inhibition of mycelia was calculated by using the formula:

$$\text{Growth inhibition } (\%) = \frac{dc - dt}{dc} \times 100$$

Where dc = Average increase in mycelial growth in control,

dt = Average increase in mycelial growth in treatment.

The antifungal agent nystatin added to the agar plates (final concentration of 1.0 mg/l) served as a positive control for *Aspergillus niger*, *A. flavus* and *penicillium*.

Table. Growth inhibition % and MIC of essential oils.

Essential oil (V/V) (ml/dl)	Fungus		
	<i>Aspergillus Niger</i>	<i>Aspergillus flavus</i>	<i>Penicillium</i>
Brasica Nigra			
10	0.00	11.52	2.74
15	2.41	22.00	2.99
20	1.77	34.02	3.24
25	5.58	36.31	2.74
Eucalyptus			
10	85.51	100	72.14
15	100	100	81.76
20	100	-	07
25	100	100	100
Cocos nucifera			
10	35.24	58.68	62.01
15	-	52.62	63.53
20	34.78	63.09	65.05
25	100	NA	100

GI: Growth inhibition (%), MIC: Minimum Inhibitory Concentra.

4. RESULT AND DISCUSSION

The results showed that two plant essential oil such as Eucalyptus and cocos nucifera oil caused 100% of growth inhibition on all species of fungi at 25 ml/dl concentration while Brassica Nigra oil not caused 100% of GI at same concentration. Therefore lower concentrations (10, 15 and 20 ml/dl) of each essential oils were used to determine the MIC on these fungi (Table). Camphor oil was the most

effective essential oil on the *A. niger*, *A. flavus* and *Penicillium* with growth inhibition average of 100, 96.38 and 84.99% respectively. Whereas the citronella oil showed minor effect on *A. niger*, *A. flavus* and *Penicillium* with growth inhibition average of 58.13, 51.32 and 72.76% respectively. GI of *A. niger* was showed higher significant difference ($p < 0.05$) for Eucalyptus oil at 25 ml/dl and all the concentrations of camphor oil when compared with other oils similarly *A. flavus* showed higher significant difference ($p < 0.05$) for Eucalyptus oil at 25 ml/dl and *Cocos nigra* at 5, 10 and 15 ml/dl when compared with other oils while *Penicillium* showed higher significant difference ($p < 0.05$) only for 25 ml/dl of Eucalyptus and 15 ml/dl of *Cocos nigra*. *Penicillium* sp. that isolated from palmyrah leaf was the most sensitive and most resistant to the studied essential oils. Essential oils have two prominent features; low toxicity for people and environment due to their natural properties and low risk for resistance development by pathogenic microorganisms. For these reasons and considering the results, we recommend the use of camphor oils for development of new and safe antifungal agent for the preservation of leafy handicrafts.

5. CONCLUSION

The results showed that eucalyptus and *Cocos nucifera* oils were very effective on *Aspergillus niger*, *Aspergillus flavus* and *Penicillium* sp. (palmyrah leaf article decay fungi) with growth inhibition average of 100% at 25 ml/dl concentration. Nevertheless, MIC of the essential oils was variable depending to species of fungi. *Penicillium* sp. was the most sensitive and most resistant to the camphor oil with 100% growth inhibition at 15 ml/dl concentration. Since growth inhibition of studied essential oils were evident in this study, they have potential to control of these palmyrah leaf article decay fungi and could be considered for developing new antifungal agent. Further field study need to be done to find out whether the use of this essential oil will prevent these fungal growth on leaf handicrafts after using this essential oil at cottage level handicraft industries in Sri Lanka.

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