Evaluation of antifungal activity of some medicinal plant extracts against some storage seed -borne fungi of Groundnut

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ABSTRACT
Indian medicinal plants contain antimicrobial properties. Aqueous leaf extracts of five medicinal plants viz. Ocimum sanctum (Linn), Mentha arvensis (Linn), Cymbopogon citratus (Stapf), Eucalyptus globules (Labill), Tridax procumbens (Linn) were screened for their antifungal activity at 10%, 20% and 30% concentration against eight fungal species isolated from stored seeds of groundnut. i.e. Aspergillus niger, A. flavus, A. terreus, A. fumigatus, Penecillium citrinum, Fusarium oxysporum, Alternaria alternata, Curvularia lunata. Aqueous leaf extract of Ocimum sanctum, Mentha arvensis showed highest antifungal activity. Inhibitory activity of leaf extracts on vegetative growth increased with increase in the concentration.

Key words: Aqueous leaf extract, inhibition, antifungal activity, phytopathogenic fungi.

INTRODUCTION
Nowadays, application of chemical compounds is considered as the most inexpensive and common method in plant disease control. However, their adverse effects on human health and the environment, promoted man to produce natural pesticides (Hyæs, 1991). The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Biologically active compounds found in plants appear to be more adaptable, acceptable and safer than synthetic compounds and display a wealthy source of potential pathogens control agents (Trapathi et al., 2008). Some plant species were assayed for pharmacological and biological activity such as antibacterial and antifungal activity (Al-Mughrabi, 2003, Ismail et al., 2003, Hoffman et al., 2003). Extracts of medicinal plants are effective against fungal and bacterial pathogens; meanwhile they are biodegradable compounds which have high potential for using in integrated pest management programs (Soylu, 2006). The use of biological compounds extracted from plants may be an alternative to conventionally used fungicides to control phytopathogenic fungi, due to their being bioactive chemicals such as flavonoids, phenols, tannins, alkaloids, quinones, saponins and sterols (Burt, 2004). Extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trails. Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Varma and Dubey, 1999). Biological control had attained importance in modern agriculture, due to attempts to reduce hazards of intensive use of chemicals for pests and disease control.
Mostly the aqueous extract of plants has been used to evaluate their fungitoxic properties (Thapliyal et al., 2000 and Algesaabooapathi and Balu, 2002). In view of these, the present investigation was undertaken to screen for the efficacy of antifungal potency of certain plant extracts against important phytopathogenic seed-borne fungi viz., Aspergillus niger, A. flavus, A. terreus, A. fumigatus, Penicillium citrinum, Fusarium oxysporum, Alternaria alternata Curvularia lunata known to cause significant crop loss in the fields and during storage.

MATERIALS AND METHODS
Collection of seed samples
Seeds of groundnut (Arachis hypogeal L.) were collected from local market of Nanded in presterilized cloth bags and brought to the laboratory.
Detection and Identification of mycoflora
Moist blotter plate method
The isolation of seed-borne fungi was carried out by blotter test method (ISTA, 1966). A pair of white blotter papers of 8.5 cm diameter was jointly soaked in sterile distilled water and placed in presterilized petri-plates of 10 cm diameter. 10 Seeds of groundnut were placed at equi-distance on moist blotters. Plates were incubated at room temperature (28 ± °C) for 7 days. Identification and confirmation of different fungal sp. on seeds was made. (Barnett,2000 and Mukadam,1997). The eight dominant fungi was isolated and brought to pure culture and used for further study.
Plant material
During the present study, five common and easily available plant species viz. Ocimum sanctum (Linn), Mentha arvensis (Linn), Cymbopogon citrates (Stapf), Eucalyptus globules (Labill) and Tridax procumbens (Linn) was selected and their identification was confirmed. (Naik, 1998). Fresh and healthy leaves were washed with tap water and then sterile distilled water. 10 gm, 20gm and 30 gm fresh and healthy leaves were crushed separately with mortar and pestle in distilled water. The homogenized mixture was filtered through double layered muslin cloth and then Whatman filter paper No.1. The volume of filtrate was made up to 100 ml using sterile distilled water.
Anti-fungal activity assay:
Determination of percent mycelial inhibition (mycelial dry weight method)-
Fungitoxic properties of five selected medicinal plants viz. Ocimum sanctum (Linn), Mentha arvensis (Linn), Cymbopogon citrates (Stapf), Eucalyptus globules (Labill) and Tridax procumbens (Linn) (at 10%, 20%, 30% concentration) was screened against test fungi. Glucose nitrate medium was prepared in flasks and sterilized. To this medium, the requisite quantity of the plant extract was added. The medium was autoclaved at 15 lbs pressure for 20 minutes. On cooling the medium, 1 ml fungal spore suspension was inoculated under aseptic conditions and incubated at 22 ± 1 °C temperature for seven days. Plain medium served as control. On incubation the content of the each flask was poured into a preweighed filter paper. The filter paper with the mycelial mat was dried in an oven at 60 °C until a constant weight was reached. The dry weight of the mycelia was determined by subtracting the weight of the filter paper from the total weight of the filter paper with mycelia. Three replicates were maintained for each treatment (Kumar and Prasad, 1992). The percent inhibition of mycelial growth was calculated using the formula: - Percent inhibition = C – T / C X 100 where C = Mycelial weight in control and T = Mycelial weight in treatment.
RESULTS AND DISCUSSION
Use of plant extracts against plant pathogenic fungi and plant diseases is relatively a recent approach. The antifungal activities of five medicinal plants obtained by dry mycelial weight method are shown in Table 1. All plant extracts tested exhibited different degrees of antifungal activity against Aspergillus niger, A. flavus, A. terreus, A. fumigatus, Penicillium citrinum, Fusarium oxysporum, Alternaria alternata, Curvularia lunata, isolated from seeds of Arachis hypogeal L. The maximum antifungal activity was shown by Ocimum sanctum and Mentha arvensis, followed by Eucalyptus globules. The leaf extracts of Cymbopogon citrates exhibited moderate antifungal activity and the leaf of extracts Tridax procumbens showed comparatively low antifungal activity against test fungi. It was revealed in this study, that the antifungal activity of the extracts was enhanced by increase in the concentration of the extracts. The leaf extract of O. sanctum showed maximum inhibition against F. oxysporum, Aniger A. terrus and minimum inhibition against Penicillium citrinum at 30% concentration. Similar studies have been carried out by different researcher on antifungal activity of plant
Table 1: Effect of Botanicals on dominant seed-borne fungi of Groundnut (% inhibition)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Plant species</th>
<th>% inhibition at different concentration</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Aspergillus niger</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10%  20%  30%</td>
</tr>
<tr>
<td>1</td>
<td>Ocimum sanctum</td>
<td>30.00  45.00  60.00</td>
</tr>
<tr>
<td>2</td>
<td>Mentha arvensis</td>
<td>25.00  40.00  50.00</td>
</tr>
<tr>
<td>3</td>
<td>Cymbopogon citrimum</td>
<td>20.00  25.00  30.00</td>
</tr>
<tr>
<td>4</td>
<td>Eucalyptus globules</td>
<td>10.00  35.00  40.00</td>
</tr>
<tr>
<td>5</td>
<td>Tridax procumbens</td>
<td>05.00  20.00  30.00</td>
</tr>
</tbody>
</table>

extract. *O. sanctum* is a medicinal plant having pharmacological properties like anabolic, hypotensive, cardiac depressant, and smooth muscle relaxant, antifertility and antistress activity, its extract show antifungal activity. (Singh et al., 1970, Balta et al., 1971). Singh and Prasada (1993) found that, leaf extract of *O. sanctum* inhibited the growth of *Fusarium oxysporum*. The result was an agreement with the finding of Kaur et al., 2009. The leaf extract of *Mentha arvensis* showed maximum inhibition against *A. fumigatus* and minimum inhibition against *A. niger* at 30% concentration. It also supports the earlier investigation of Varaprasad et al., (2009). *E. globulus* showed maximum inhibition against *A. fumigatus* and minimum inhibition against *P. citrinum* at 30% concentration. Similarly Luma et al. (2007) found that eucalyptus extract shows inhibitory effect on pathogenic fungi. *C. citratus* showed maximum inhibition against *F. oxysporum* and minimum inhibition against *C. lunata*. Bansod and Rai (2008) found that *C. citratus*, *E. globules* showed high antimycotic activity against *A. fumigatus* and *A. niger*. The leaf extract of *T. procumbens* showed maximum inhibition against *P. citrinum* minimum inhibition *C. lunata* at 30% concentration. Recently, Kakde et al., (2011) found that aqueous extract of *Eucalyptus angophoroides* found to be fungitoxic for the growth of *Alternaria dianthicola*, *Curvularia pellescens*, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Rhizopus stolonifer*, *Penicillium digitatum* and *Penicillium chrysogenum*. Mogle (2013) found that leaf extract of *E. globulus*, *A. mexicana*, *T. procumbens* *P. hysterophorus* were inhibitory for the growth of *Aspergillus niger*, *Penicillium digitatum*, *Botrytis cinera*, *Rhizopus arrhizus*, *Aspergillus flavus*, *Chaetomium brasiiliense* and *Rhizoctonia solani*. Recently, Jat and Agalave (2013) tested leaf extract of medicinal plants against some oil seed-borne fungi and found that aqueous extract of *Eucalyptus angophoroides* and *Withania somnifera* was found to be inhibitory for the growth of these fungi.
LITERATURE CITED


ISTA, 1996. Seed Science and Technology 21(Suppl.): 18288.


