

SCREENING OF ESSENTIAL OILS OF ANGIOSPERMIC PLANTS FOR THEIR FUNGITOXICITY AGAINST *Alternaria alternata* FR. KEISSLE

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ABSTRACT

Fresh parts of angiospermic plants were collected from different places of the local area and were subjected to hydrodistillation through Clevengers apparatus so as to isolate the volatile plant products (essential oils). The isolated essential oils were tested for their antifungal activity at 1000 ppm, against *Alternaria alternata*, the causal agent of postharvest *Alternaria* rot (black rot) of Tomato by the poisoned food technique using potato dextrose agar medium. Out of 28 essential oils tested, most of the oils showed either poor or moderate activity. However, the essential oils of seeds of *Anethum graveolens* and *Cuminum cyminum* exhibited absolute fungitoxicity inhibiting the growth of *A. alternata* test fungus completely at 1000 ppm. Therefore the oils of seeds of *Anethum graveolens* and *Cuminum cyminum* were selected for detailed investigation, because of their absolute fungitoxicity

KEYWORDS: Angiospermic plants, *Alternaria alternata*, *Anethum graveolens* and *Cuminum cyminum* etc.

Although various essential oils have been screened for their fungicidal activity against various fungi but proper detailed study has not been done with most of the oils. There is an urgent need to reassess the fungicidal property of different essential oils and their *in vitro* and *in vivo* investigations are required for their recommendation as fungicides. It may be mentioned that simply recording fungicidal property in an oil on the basis of *in vitro* studies may not indicate its successfulness during *in vivo* trials. Their pharmacological investigations are required to know their animal toxic nature.

The essential (volatile) oils produced by different plant genera are in many cases biologically active, endowed with allelopathic antioxidant and bioregulatory properties (Elakovich, 1988; Deans *et al.* 1990, Caccioni and Guizzardi, 1994). The volatility, ephemeral nature and biodegradability of flavour compounds of angiosperms will be specially advantageous if they are developed as pesticides (French, 1985). The efficacy of essential oils of *Ocimum canum* and *Citrus medica* volatile fungitoxicants in protection of some spices against their post harvest fungal deterioration was demonstrated by Dubey *et al.* (1983a). The essential oil of *Cymbopogon citratus* has shown its *in vivo* fumigant activity in the management of storage fungi and insects of some cereals without exhibiting mammalian toxicity on albino rats (Mishra *et al.*,

1992). Fungi adversely affect stored seeds in a variety of ways. Several methods such as use of fungicides (Szejriberg *et al.* 1975) and physical treatments have been suggested to protect the seeds. However there are many limitations on the physical methods of control of post harvest deterioration. Fries (1973); Charya & Redey (1980) and Malick & Nandi (1982) have recommended the use of volatile compounds in control of mould infestation during storage.

Therefore in the present study, it has been thought desirable to test some essential oils isolated from some plants of the locality against *Alternaria alternata*, a dominant fungus causing post-harvest rot of tomato during storage and transportation

MATERIALS AND METHODS

Higher plants of different angiospermic taxa of different families were randomly selected from different areas of Northern India and identified with the help of different floras (Bailey, 1958; Duthie, 1960; Maheshwari, 1963; Santapau, 1967 and Srivastava, 1976; Dubey, 2004). Confirmations of identity of the plants were done with the help of authentic herbarium specimens lodged in the Herbarium of National Botanical Research Institute, Lucknow, India.

500gm. of fresh parts of each plant were cut separately into small pieces and then thoroughly

washed with sterilized water. The volatile fractions (essential oils) were isolated by hydrodistillation by Clevenger's apparatus. In case of essential oil bearing plants, the collecting funnel of the Clevenger apparatus showed two distinct layers an- upper oily layer and the lower aqueous layer. Both the layers were separated and the essential oils were stored in clean glass vials after removing water traces with the help of capillary tubes and anhydrous sodium sulphate.

The fungitoxicity of essential oils was tested by the poisoned food technique of Grover and Moore (1962). Potato dextrose agar medium (42 gm. of Hi-PDA medium dissolved in 1000ml of distilled water) was prepared, autoclaved and cooled down to 40⁰C. Ten mg. of streptomycin was added to it and mixed thoroughly to prevent bacterial contamination as suggested by Gupta and Banerjee (1970). Requisite amounts of the oil were dissolved separately in 0.5 ml of acetone in pre-sterilized Petriplates (9.5mm diam). 9.5ml of PDA medium was pipetted to each Petriplate and mixed thoroughly so as to obtain 1000 ppm concentration. The plates were swiveled thoroughly in order to obtain homogenous medium. For control sets, requisite amount of sterilized water in place of the oil was

added to the medium. Fungal discs (4 mm diameter) cut from the periphery of a seven day old culture of *Alternaria alternata* was placed aseptically into the centre of each Petri plate of treatment and control sets with the help of sterilized cork borer separately. The Petri plates were incubated at 25 ± 2⁰C for six days in incubation chamber. Diameters of fungal colony of treatment and control sets were measured in mutually perpendicular directions on the seventh day. The percentage mycelial inhibition was calculated by the mean value of colony diameters by the following formula:

$$dc - dt$$

$$\text{Percentage of mycelial inhibition} = \frac{dc - dt}{Dc} \times 100$$

Where, dc = Average diameter of fungal colony in control sets dt = Average diameter of fungal colony in treatment sets.

RESULTS

The experiment was run in triplicate and the mean values ± SD are presented in Table-1. Where the plants screened are arranged alphabetically with their families.

Table-1: Screening of Some Essential Oils of Angiospermic Plants for their Fungitoxicity against *Alternaria alternata* at 1000 ppm

Name of the Plants	Family	Essential Oil isolated from plant part	Percent mycelial inhibition of <i>Alternata</i> ± SD
<i>Aegle marmelos</i> (L) correa	Rutaceae	Leaf	80 ± 4.08
<i>Ageratum haustonianum</i> Mill	Asteraceae	Leaf	65 ± 18.71
<i>A. conyzoides</i> L.	Asteraceae	Leaf	96.67 ± 4.7
<i>Alpinia galangal</i> (L.) Sw.	Zingiberaceae	Leaf	63.33 ± 16.99
<i>Anethu mgraveolens</i> Linn.	Apiaceae	Leaf	59.52 ± 8.91
<i>A. graveolens</i> Linn.	Apiaceae	Seed	100
<i>Boswellia serrata</i> Roxb.	Burseriaceae	Bark	55.67 ± 4.92
<i>Callistemon lanceolatus</i> DC.	Myrtaceae	Leaf	73.33 ± 6.23
<i>Chrysanthemum indicum</i> Dc.	Asteraceae	Leaf	71.67 ± 18.40
<i>Cinnamomum camphora</i> (L.)	Lauraceae	Leaf	81 ± 19
<i>Citrus reticulata</i> Blanco	Rutaceae	Peel	53.34 ± 12.47
<i>C. sinensis</i> (L.) osbeck	Rutaceae	Peel	76.67 ± 12.48
<i>Curcuma longa</i> (L.) Koenig	Zingiberaceae	Leaf	58.67 ± 9.85
<i>C. longa</i> (L.) Koenig	Zingiberaceae	Rhizome	90.67 ± 13.19
<i>Cuminum cyminum</i> Linn.	Apiaceae	Leaf	65.71 ± 6.17

<i>C. cyminum</i> Linn.	Apiaceae	Seed	100
<i>Elettaria cardamomum</i> Maton.	Zingiberaceae	Leaf	45 ± 10.80
<i>Eupatorium cannabinum</i> L.	Asteraceae	Leaf	64 ± 17.57
<i>Hyptis suaveolens</i> L. (Poit)	Lamiaceae	Leaf	74.67 ± 10.5
<i>Leucas aspera</i> (willd) spreng	Lamiaceae	Leaf	93.34 ± 9.43
<i>Leucas aspera</i> (willd) spreng	Lamiaceae	Leaf	93.34 ± 9.43
<i>Murraya koeninghi</i> (L.) spreng	Rutaceae	Leaf	73.33 ± 24.94
<i>Nepeta hindostana</i> Roth.Haines	Lamiaceae	Leaf	50.33 ± 14.26
<i>Salvia plebeian</i> R. Br	Lamiaceae	Leaf	41.33 ± 9.84
<i>Seseli indicum</i> Wight & Arn	Asteraceae	Leaf	39.33 ± 15.52
<i>Tagetes erecta</i> Linn	Asteraceae	Leaf	63.33 ± 24.94
<i>Vetiveria zizaniodes</i> (L.) Nash	Poaceae	Root	68.67 ± 12.12
<i>Vitexne gundo</i> Linn	Verbenaceae	Leaf	43 ± 13.49

It is evident from Table-1 that among 28 essential oils of angiospermic plant parts belonging to 10 families, screened against the test fungi, most of the oils showed either poor (below 50%) or moderate (above 50% and below 100 %) activity. The essential oils of seeds of *Anethum graveolens* and *Cuminum cyminum* were found to exhibit absolute toxicity inhibiting the growth of the test fungi completely. None of the essential oils accelerated the growth of the test fungi. therefore, amongst these, the essential oils of seeds of *Anethum graveolens* and *Cuminum cyminum* were selected for further detailed studies because of their strong fungitoxicity against test fungus.

DISCUSSION

Recently, some higher plant products have proved their fruitfulness as promising fungitoxicants

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