Antifungal activity of essential oils derived from some plants against phytopathogenic fungi

Habung Yami1 and A.K. Shukla2

1Department of Botany, Rajiv Gandhi University, Itanagar-791112, Arunachal Pradesh, India.
2Department of Botany, Indira Gandhi National Tribal University, Amarkantak-484887, Madhya Pradesh, India.

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Abstract: Essential oils were extracted from different plant species (Acorus calamus, Artemisia nilagirica, Erigeron canadensis) to evaluate their effect on the growth of four phytopathogenic fungi viz. Alternaria alternata, Botrytis cinerea, Fusarium oxysporum and Penicillium expansum following poisoned food technique method. Different concentration of oil such as 125ppm, 250ppm, 500ppm, 1000ppm and 5000ppm were taken to evaluate the effect. There was 100% inhibition in the growth of phytopathogenic fungi at 5000ppm concentration. At 500ppm concentration also 100% inhibition was found up to 7th day on F. oxysporum. Essential oil of A. nilagirica inhibits the growth of all phytopathogenic fungi at higher concentration. In case of P. expansum at 500ppm concentration 100% inhibition was recorded even after 15 days of incubation. The decrease in colony diameter or growth of fungus was corresponding to the concentration of oil. In comparison to others, essential oil of Erigeron canadensis was less effective against phytopathogenic fungi. It can be stated on the basis of results that the use of Acorus calamus and Artemisia nilagirica essential oil could be an alternative to synthetic fungicides for management of post harvest phytopathogenic fungal diseases caused by A. alternata, B. cinerea, F. oxysporum and P. expansum.

Key words: Essential oil; fungitoxic; fungicidal; Phytopathogenic fungi; Acorus; Artemisia; Erigeron

Introduction

Essential oils or volatile oils are very complex mixture of compounds whose constituents of the oils are mainly monoterpenners and sesquaepenens. Generally, the action of essential oils is the result of the combined effect of both their active and inactive compounds. These inactive compounds might influence resorption, rate of reactions and bioavailability of the active compounds. Until recently, essential oils have been studied most from the viewpoint of their flavor and fragrance only for flavoring foods, drinks and other goods. Actually, essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use (Ormancey 2001).

Biologically active plant extracts, including essential oils, represent rich potential sources of alternative and perhaps environmentally more acceptable disease management compounds. Besides, higher plants also contain a wide spectrum of secondary substances viz. phenols, flavonoids, quinines, tannins, alkaloids, saponins and sterols. Plant diversity serves the humankind as renewable natural resources for a variety of biologically active chemicals. These chemicals bear a variety of properties viz. antibacterial, antifungal, antiviral, anthelmintic, anticancer, sedative, laxative, cardiotonic, diuretic and others (Parajuli et al., 1998). Naturally occurring biologically active compounds from plants are generally assumed to be more acceptable and less hazardous than synthetic compounds and represent a rich source of potential disease control agents. The secondary metabolites performs defensive role in plant from their invaders. The factors that affect biochemical profiles and secondary metabolite production in plant include physiological, genetics, and environmental variables. Active constituents of the medicinal and aromatic plants have been found to be less phytotoxic, more systemic and easily biodegradable (Fawcett and Spencer 1970).

The general antifungal activity of essential oils is well documented (Deans and Ritchie, 1987; Reuveni et al., 1984; Tripathi and Shukla, 2007) and there have been some studies on the effects of essential oils on post harvest pathogens (Bishop and Thornton, 1997; Anthony et al, 2003; Tripathi et al., 2008). Biologically active essential oils represent a rich potential source of an alternative and perhaps environmentally more acceptable disease management compounds. There is need for an alternative approach to control phytopathonic fungi without toxicity problems that are ecotriendly and cost effective. Present study was aimed to evaluate the antifungal activity of some plant based essential oils against phytopathogenic fungi.

*Corresponding Author:
Prof. A. K. Shukla,
Department of Botany,
Indira Gandhi National Tribal University,
Amarkantak, Madhya Pradesh, India.
E-mail: ashukla21@rediffmail.com

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Materials and Methods

Isolation and identification of phytopathogenic fungi

Isolation of post harvest pathogens of kiwifruits were carried out from infected fruits on rose Bengal agar and potato dextrose agar medium (Johnson and Curi, 1972). Infected kiwifruits were randomly collected from market. Fruits were surface sterilized by 4% sodium hypochlorite and then by 75% alcohol and finally with sterilized distilled water. Small pieces of fruit were cut and placed in the petriplates containing sterilized medium and incubated at 27°C for 7-10 days. Identification of fungal pathogens was done on the basis of morphological, cultural and microscopic characteristics as detailed in available literature (Barnett and Hunter 1972, Domsch et al., 1980). In process of culture the isolated fungal pathogens were cultivated on Potato Dextrose Agar (PDA) medium and Peptone Dextrose Rose Bengal Agar medium. Potato dextrose agar medium (39 gm of Hi-PDA medium dissolved in 1000 ml of distilled water) medium was used throughout the investigation. The medium was autoclaved and cooled to 40°C ±2°C. Thirty milligram of streptomycin was added to it and mixed thoroughly so as to prevent bacterial contamination. A requisite amount of the oil was dissolved separately in 0.5 ml of 0.01 percent aqueous solution of Tween -80 in presterilized Petri plates (7cm. diam.). While using Tween-80 as solvent care was taken in designing the experiments to evaluate the true effect of essential oils on the pathogenic fungi. PDA medium (9.5 ml) was pipetted to each Petri plate and was mixed so as to obtain the requisite concentrations viz. 5000ppm, 1000ppm, 500ppm, 250ppm and 125ppm. For control sets, requisite amount of sterilized water in place of the oil was added to the medium.

Discs of test fungi (5 mm diam) were cut with the help of sterilized cork borer from the periphery of a seven-day old culture and were inoculated aseptically to the center of each petriplate of treatment and control sets. The petriplate were incubated at 27± 1°C for six days in incubation chamber. Measurement of colony diameters of the test fungus in treatment and control sets were done in mutually perpendicular directions and were recorded in terms of percent mycelial inhibition using the following formula

\[
\text{Percentage of mycelial inhibition} = \frac{dc-dt}{dc} \times 100
\]

Where \( dc \) = mean colony diameter of control sets
\( dt \) = mean colony diameter of treatment sets

Standardization of essential oils through fungitoxic properties

The standardization of essential oils was done through fungitoxic properties viz. minimum inhibitory concentration and nature of toxicity (Thompson, 1989).

Minimum inhibitory concentration (MIC)

To find out the minimum inhibitory concentration at which the oil showed absolute fungitoxicity (complete inhibition of growth of test fungi), experiments were carried out by the usual poisoned food technique. Different concentrations of the oils were prepared by dissolving separately their requisite amount in 0.5 ml of 0.01 per cent of aqueous solution of Tween-80 and then mixing with 9.5 ml potato dextrose agar medium. The
medium of control sets contained requisite amount of sterilized water dissolved in 0.5 ml of Tween-80 in place of oils. As usual the prepared plates were inoculated upside down aseptically with the assay disc of the test fungi to the center of petriplate of treatment and control sets. The petriplates were incubated at 27±1°C for six days in BOD incubator. Diameters of fungal colony of treatment and control sets were measured in mutually perpendicular directions on the seventh day and percentage inhibition calculated.

Nature of toxicity
Nature of toxicity (fungitstatic / fungicidal) of essential oils against the fungi was determined as suggested by Thompson (1989). Requisite amount of the oil was dissolved separately in 0.5 ml of 0.01 per cent of aqueous solution of Tween-80 and mixed with 9.5 ml potato dextrose agar medium to get final concentrations. Sterilized water was used in control sets in place of the oils. The plates were inoculated upside down aseptically with fungal disc (5mm diam.) taken from the periphery of a seven day old culture of the test fungi and were incubated for six days at 27± 1°C. On seventh day the inhibited discs were taken out from the plates, washed with sterilized water and reincubated aseptically to plates containing fresh potato dextrose agar medium. The revival of the growth of the fungal discs was observed and the per cent inhibition of growth of the test fungi were calculated on the seventh day with respect to control sets.

Results
Evaluation of Essential Oils against Different Fungi
Essential oils extracted from different plant species were evaluated to visualize their effect on the growth of four phytopathogenic fungi viz. Penicillium expansum, Fusarium oxysporum, Botrytis cinerea and Alternaria alternata following Poisoned food technique method. Different concentration of oil such as 125ppm, 250ppm, 500ppm, 1000ppm and 5000ppm were taken to evaluate the effect. Simultaneously, a control was also maintained by inoculating culture disc on the medium without adding any oil.

Acorus calamus
Essential oil of A. calamus was found effective against the growth of all tested fungi. In case of P. expansum and F. oxysporum 100% inhibition of growth was recorded at 5000 and 1000ppm concentration by essential oil of A. calamus. At 500ppm concentration also 100% inhibition was found up to 7th day on F. oxysporum. But growth was observed during subsequent period of incubation. The decrease in colony diameter or growth of fungus was corresponding to the concentration of oil. On A. alternata and B. cinerea also the effect of oil was significant inhibitory. At 5000, 1000 and 500ppm concentration 100% inhibition was recorded. At 250ppm also in case of A. alternata it restricts 100% up to 7th day and after that slight growth was noticed during subsequent period of incubation.

Essential oil of A. calamus inhibited the growth of all four phytopathogenic fungi, at 5000, 1000 and 500ppm concentration and at lower level i.e 125 and 250ppm concentration of oil colony growth was recorded. But it always remains lesser than the control.

Artemisia nilagirica
Essential oil of A. nilagirica inhibits the growth of all phytopathogenic fungi at higher concentration. In case of P. expansum at 5000ppm concentration 100% inhibition was recorded even after 15 days of incubation. The decrease in colony diameter or growth of fungus was corresponding to the concentration of oil. On A. alternata and B. cinerea there was 100% inhibition up to 11th day at 5000ppm concentration of oil. However, slight growth was seen on subsequent period of incubation. But in case of F. oxysporum even at 5000ppm concentration of oil slight growth was recorded. Increase in diameter of fungus colony was recorded at lower concentration of oil however it always remains lesser than control.

Erigeron canadensis
Essential oil of E. canadensis also inhibits the growth of phytopathogenic fungi. In case of P. expansum and F. oxysporum at higher concentration of oil inhibition was drastic. The decrease in colony diameter or growth of fungus was corresponding to the concentration of oil. While in case of A. alternata and B. cinerea at 5000ppm concentration of fungus colony was reduced initially but during subsequent period of incubation growth of colony increases but it always remain lesser than the control. At lower level of concentration of oil at 125ppm the growth of fungus colony was almost similar to the control. There was not much effect on growth of fungus colony by E. canadensis oil.

Minimum Inhibitory concentration (MIC)
Inhibitory evaluation of essential oils against phytopathogenic fungi showed the effective results. Essential oil of Acorus calamus was found fungitoxic at 250ppm for A. alternata and 500ppm for rest of the three fungi. EO of Erigeron canadensis was inhibitory at higher concentration for all the phytopathogenic fungi. Artemisia nilagirica EO was found fungitoxic at 5000ppm concentration against A. alternata B. cinerea, and P. expansum.
Nature of toxicity

*Artemisia calamus* oil was found fungicidal for all the phytopathogenic fungi. Oil of E. Canadensis was fungistatic for all the pathogens. *Artemisia niligirica* oil was fungicidal at 500ppm for all the tested fungi except *F. oxysporum* for which it was fungistatic.

**Table 1:** Effect of *Artemisia calamus* essential oil on the phytopathogenic fungi

<table>
<thead>
<tr>
<th>Period</th>
<th><em>Artemisia calamus</em></th>
<th><em>Alternaria alternata</em></th>
<th><em>Botrytis cinerea</em></th>
<th><em>Fusarium oxysporum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5000ppm</td>
<td>1000ppm</td>
<td>500ppm</td>
<td>250ppm</td>
</tr>
<tr>
<td>5th</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>7th</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>9th</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>11th</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>13th</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>15th</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
</tbody>
</table>

**Table 2:** Effect of *Artemisia niligirica* essential oil on the phytopathogenic fungi

<table>
<thead>
<tr>
<th>Period</th>
<th><em>Artemisia niligirica</em></th>
<th><em>Alternaria alternata</em></th>
<th><em>Botrytis cinerea</em></th>
<th><em>Fusarium oxysporum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5000ppm</td>
<td>1000ppm</td>
<td>500ppm</td>
<td>250ppm</td>
</tr>
<tr>
<td>5th</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>7th</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>9th</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>11th</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>13th</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>15th</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
</tbody>
</table>

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Table 3: Effect of *Erigersa Canadensis* essential oil on the phytopathogenic fungi.

<table>
<thead>
<tr>
<th>Period</th>
<th>Alternaria alternata</th>
<th>Botrytis cinerea</th>
<th>Fusarium oxysporum</th>
<th>Penicillium expansum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
<td>5000ppm</td>
<td>1000ppm</td>
<td>500ppm</td>
<td>250ppm</td>
</tr>
<tr>
<td>5th</td>
<td>4.95±0.05</td>
<td>4.95±0.05</td>
<td>2.95±0.05</td>
<td>1.90±0.05</td>
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<tr>
<td>7th</td>
<td>3.95±0.05</td>
<td>3.95±0.05</td>
<td>2.95±0.05</td>
<td>1.90±0.05</td>
</tr>
<tr>
<td>9th</td>
<td>2.95±0.05</td>
<td>2.95±0.05</td>
<td>2.95±0.05</td>
<td>1.90±0.05</td>
</tr>
<tr>
<td>11th</td>
<td>2.05±0.05</td>
<td>2.05±0.05</td>
<td>2.05±0.05</td>
<td>1.05±0.05</td>
</tr>
<tr>
<td>13th</td>
<td>1.15±0.05</td>
<td>1.15±0.05</td>
<td>1.15±0.05</td>
<td>0.55±0.05</td>
</tr>
<tr>
<td>15th</td>
<td>0.50±0.05</td>
<td>0.50±0.05</td>
<td>0.50±0.05</td>
<td>0.25±0.05</td>
</tr>
</tbody>
</table>

Table 4: Minimum inhibitory concentration of essential oils against pathogenic fungi

<table>
<thead>
<tr>
<th>Essential oils of plants</th>
<th>A. alternata</th>
<th>B. cinerea</th>
<th>F. oxysporum</th>
<th>P. expansum</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acorus calamus</em></td>
<td>500ppm</td>
<td>500ppm</td>
<td>500ppm</td>
<td>500ppm</td>
</tr>
<tr>
<td><em>Artemisia nilagirica</em></td>
<td>5000ppm</td>
<td>5000ppm</td>
<td>Higher Conc.</td>
<td>5000ppm</td>
</tr>
<tr>
<td><em>Erigersa Canadensis</em></td>
<td>Higher Conc.</td>
<td>Higher Conc.</td>
<td>Higher Conc.</td>
<td>5000ppm</td>
</tr>
</tbody>
</table>

Table 5: Toxicity nature of Essential oils on phytopathogenic fungi

<table>
<thead>
<tr>
<th>Essential oils</th>
<th>A. alternata</th>
<th>B. cinerea</th>
<th>F. oxysporum</th>
<th>P. expansum</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acorus calamus</em></td>
<td>Fungicidal at 500ppm</td>
<td>Fungicidal at 500ppm</td>
<td>Fungicidal at 1000ppm</td>
<td>Fungicidal at 500ppm</td>
</tr>
<tr>
<td><em>Artemisia nilagirica</em></td>
<td>Fungicidal at 500ppm</td>
<td>Fungicidal at 500ppm</td>
<td>Fungicidal at 1000ppm</td>
<td>Fungicidal at 500ppm</td>
</tr>
<tr>
<td><em>Erigersa Canadensis</em></td>
<td>Fungistatic</td>
<td>Fungistatic</td>
<td>Fungistatic</td>
<td>Fungistatic</td>
</tr>
</tbody>
</table>

Discussion

Essential oil of *A. calamus* was recorded inhibitory for the growth of all tested fungi. Colony growth of *P. expansum* and *F. oxysporum* restricted 100% at 5000 and 1000ppm concentration of *A. calamus* essential oil. Colony growth of *A. alternata* and *B. cinerea* was also inhibited by oil and at 5000, 1000 and 500ppm concentration 100% inhibition was recorded. Results indicate that *A. calamus* essential oil have fungitoxic and fungistatic property against the phytopathogenic fungi. Mazza (1985) found, that Indian calamus oil contained high amount of β-asarone (77.7%) and 6.8% α-asarone, but in European calamus oil acorenone (8.1%), isosy wholeone (6.3%), β-gurjunene (6.7%), calamenediol (5.2%) and β-asarone (5.2%) were found to be major components. The complexity in essential oils is due to terpene hydrocarbons as well as their oxygenated derivatives, such as alcohols, aldehydes, ketones, acids and esters (Wiješekara et al., 1997). Radušiene (2007) reported that essential oils of *A. calamus* were dominated by the presence of phenolic compounds: (Z)-asarone (15.7–25.5%) and (Z)-methyl isoeugenol (2.0–4.9%). Other identified major components were (E)-caryophyllene, α-humulene, germacrene, linalool, camphor and isoborneol. Satyal (2013) isolated a number of compounds from the essential oil of *A. calamus* and noted cytotoxicity and antifungal activity against *Aspergillus niger*. Sharma et al., (2007) reported antifungal activity of *Acorus calamus* oil against *Sclerotia rolfsii* and *Rhizoctonia batatula*. Devi and Ganjewala (2009) found remarkable antifungal activity of *A. calamus* oil against *Aspergillus niger, A. flavus, Microsporum canis* and *Penicillium chrysogenum*. Lee et al., (2007) attributed antifungal activity of α-asarone and aldehydes present in the *A. calamus* oil. Due to presence of a number of compounds and high quantity of phenolics in essential oil of *A. calamus*, perhaps any one of that or in combination would...
have inhibited the colony growth of the tested phytopathogenic fungi.

Essential oil of *A. nilagric* inhibits the growth of all phytopathogenic fungi at higher concentration. In case of *P. expansum* at 5000ppm concentration 100% inhibition was recorded even after 15 days of incubation. The decrease in colony diameter or growth of fungus was corresponding to the concentration of oil. On *A. alternata* and *B. cinerea* there was 100% inhibition up to 11th day at 5000ppm concentration of oil. However, slight growth was seen on subsequent period of incubation. At low concentration nature of oil was fungistatic. Sati *et al.*, (2013) reported that essential oil contained approximately 79.91% monoterpenoids and 18.25% sesquiterpenoids. - Thujone (36.35%), -thujone (9.37%), germacrene D (6.32%), 4-terpinol (6.31%), -caryophyllene (5.43%), camphene (5.47%) and borneol (4.12%) as the major constituents. The essential oil exhibited significant antifungal activity against *Rhizoctonia solani* (ED50, 85.75 mg L1), Sclerotium rolfsii (ED50, 87.63 mg L1) and Macrophomina phaseolina (ED50, 93.23 mg L1). Padalia *et al.*, (2014) found that essential oils were mainly composed of monoterpenoids (59.0%–77.3%) and sesquiterpenoids (15.7%–31.6%). The major constituents identified were artemisia ketone (38.3%–61.2%), chrysanthene (1.5%–7.7%), germacrene D (3.1%–6.8%), -caryophyllene (1.9%–6.8%), germacr-4,5, 10- trien-1- x-ol (1.9%–4.9%) and artemisia alcohol (1.4%–3.6%). Stappen *et al.*, (2014) reported that *A. nilagric* essential oil have nonselective antifungal activity against plant pathogens *Colletotrichum acutatum*, *Colletotrichum fragariae* and *Colletotrichum gloesporioides*. Presence of terpenoids in large quantity and other compounds in small quantity would have perhaps played antifungal property against the phytopathogenic fungi.

Essential oil of *Erigeron canadensis* also inhibits the growth of phytopathogenic fungi *P. expansum* and *F. oxysporum* at higher concentration. The decrease in colony diameter or growth of fungus was corresponding to the concentration of oil. While in case of *A. alternata* and *B. cinerea* at 5000ppm concentration of fungus colony was reduced initially but during subsequent period of incubation growth of colony increases but it always remain lesser than the control. Unnithan (2014) reported in the essential oil of *Erigeron canadensis* a total of 23 components and main constituents were monoterpenoids (limonene 57.2%), camphene (2.5%) x and -pinenes (1.9 % & 2.1%) and sesquiterpenoids (-caryophyllene (6.7%), germacrene D (4.9%) and x-curcumene (3.0%). A few non-terpenoid acetylenic compounds (4.8%) were also detected. Curini *et al.*, (2003) found essential oils of *E. canadensis* under in vitro condition as growth inhibitors against phytopathogenic fungi *Rhizoctonia solani* Kuhn, *Fusarium solani* and *Colletotrichum lindemuthianum* but with weak fungicidal activity.

It can be concluded on the basis of present findings that the use of *Acorus calamus* and *Artemisia nilagrica* essential oil could be an alternative to synthetic fungicides for management of post-harvest phytopathogenic fungal diseases caused by *A. alternata*, *B. cinerea*, *F. oxysporum* and *P. expansum*.

**References**


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