Journal of Advances in Biology & Biotechnology
10(2): 1-10, 2016; Article no.JABB.29331
ISSN: 2394-1081

SCIENCEDOMAIN *international*
www.sciencedomain.org

Impact of Essential Oils on Growth of Phytopathogenic Fungi Responsible for Rotting of Fruits

Pramila Tripathi[1], Habung Yami[2] and A. K. Shukla[3*]

[1]Department of Botany, DAV-PG College, Kanpur – 208001, India.
[2]Department of Botany, Rajiv Gandhi University, Itanagar – 791112, India.
[3]Department of Botany, Indira Gandhi National Tribal University, Amarkantak – 484887, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author PT wrote the first draft of the manuscript. Author HY conducted the experiments and managed the analyses of the study. Author AKS designed the study and experimental protocol. Authors PT, HY and AKS managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2016/29331
Editor(s):
(1) Joana Chiang, Department of Medical Laboratory Science and Biotechnology, China Medical University, Taiwan.
Reviewers:
(1) Partiban Subramanian, Asia Metropolitan University, Malaysia.
(2) Mariana M. B. de Azevedo, Institute at Federal, University of Rio de Janeiro, Brazil.
(3) Yemisi Jeff-Agboola, University of Medical Sciences, Ondo City, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/16805

Original Research Article

Received 3rd September 2016
Accepted 13th October 2016
Published 5th November 2016

ABSTRACT

Aims: To evaluate the antifungal activity of essential oils against phytopathogenic fungi for control of post harvest diseases.
Place of Study: present study was carried out at Department of Botany, Rajiv Gandhi University, Itanagar, India.
Methodology: Phytopathogenic fungi were isolated from infected fruits on rose Bengal agar medium and potato dextrose agar medium. Subsequently fungi were identified and cultures were maintained on the above mentioned medium. Essential oils were extracted from a number of plants and further four plants (*Acorus calamus, Agreratum conyzoides, Artemisia nilagirica, Litsea cubeba*) essential oils were selected to evaluate their essential oils against the isolated fungi. Antifungal activity was assessed by poisoned food technique using above mentioned media. Essential oils were standardized for fungitoxic properties and nature of toxicity. Fungitoxic spectrum

**Corresponding author: E-mail: ashukla21@rediffmail.com;*

of essential oils was also determined using other storage fungi.
Results: Antifungal property of essential oils was determined at different concentration such as 125 ppm, 250 ppm, 500 ppm, 1000 ppm and 5000 ppm. There was 100% inhibition in the growth of phytopathogenic fungi at 5000 and 1000 ppm concentration by essential oil of *Acorus calamus, Agreratum conyzoides, Artemisia nilagirica and Litsea cubeba.* Essential oil of *Acorus calamus* and *Agreratum conyzoides* also inhibited growth of *Alternaria alternata* and *Botrytis cinerea* at 500 ppm concentration. The decrease in colony diameter or growth of fungus was corresponding to the concentration of oil. Minimum inhibitory concentration of oils was reported 500 ppm. Essential oils of *Agreratum conyzoides, Artemisia nilagirica* and *Litsea cubeba* were fungicidal at 1000 ppm concentration. *A. calamus* oil was found fungicidal at 500 ppm for *A. alternata* and *B. cinerea* and at 1000 ppm for fungi *Fusarium oxysporum* and P*enicillium expansum*. Fungitoxic spectrum of oils showed that there was almost 100% inhibition of all the other storage fungi.
Conclusion: On the basis of results It can be stated that *Acorus calamus, Agreratum conyzoides, Artemisia nilagirica* and *Litsea cubeba* essential oil may be used as botanical pesticides for management of post harvest phytopathogenic fungal diseases. However, further investigations are needed in light of pharmacological tests.

Keywords: Essential oils; phytopathogenic fungi; antifungal; fungitoxic.

1. INTRODUCTION

Fungi are significant destroyers of foodstuffs, grains, leaves and fruits during storage and retard the nutritive value through deterioration and by producing metabolites. On an average 25% agricultural food items become annually unsuitable for consumption due to invasion by different fungi and their toxic substances [1]. Condition is more serious in tropical and sub-tropical regions of the world due to favourable factors like temperature, relative humidity and moisture content which favours the development of fungal population. General fungi are found associated with food items include *Aspergillus* spp., *Penicillium* spp*., Fusarium* spp*., Alternaria* spp*., Curvularia* spp., members of Mucorales etc. Fungal infection in food items lead to their bio-deterioration increase in free fatty acids content, change in colour, texture and decline in nutritional value [2]. Moreover various mycotoxins secreted by food borne molds cause serious health hazards to consumers.

Acorus calamus, commonly known as sweet flag, is a seasoning and herb traditionally used in Ayurveda and Traditional Chinese Medicine for its cognitive enhancing properties. The active ingredient in *Acorus calamus* is β-asarone, which is also responsible for the plant's main mechanism. *Ageratum conyzoides* L., Asteraceae, is an annual herbaceous plant with a long history of traditional medicinal uses in several countries of the world and also has bioactivity with insecticidal and nematocidal acitivity. This tropical species appears to be a valuable agricultural resource. Essential oils of *Artemisia* spp. have been widely used for a variety of medicinal purposes for many years. *Artemisia nilagirica* (Clarke) pamp commonly called Indian worm wood, is widely found in the hilly areas of India. It is considered to produce most medicinally important secondary metabolites. The tree bears whitish-yellowish flowers that have a characteristic citrusy fragrance and fruits that resemble peppers (that's where the word 'cubeb' comes from). *Litsea Cubeba's* antibacterial and antiviral properties make it effective against common infections like cold and cough. Simply pop a few drops into a vaporizer to keep any respiratory problems at bay. *Litsea cubeba* is also very useful in treating infectious skin conditions such as Athlete's Foot because of its pronounced antifungal action.

Various strategies time to time have been developed to get rid of these noxious fungal pathogens during storage of food materials. Chemical control is applied mainly throughout the world and large numbers of synthetic fungicides are being continuously used to control fungi during storage. However, chemical biocides have negative impact due to their post side effect. Among the various strategies proposed biological control using natural products such as essential oils and plant extract show most promising and effective. Many plant essential oils and their volatile constituents have been reported to possess potent antifungal activities [3]. The advantage of plant essential oils is their bioactivity in the vapor phase that makes them attractive as possible fumigants for the post harvest control of decay fungi in fruit and grains [3,4]. Essential oils are made up of many

different volatile compounds and the composition of oil quite often varies between species [5-7]. It seems that the antifungal effect is the result of many compounds acting synergistically [7,8]. There would be negligible chance of development of resistible races of fungi after application of essential oils to fruits and vegetables. As a consequence essential oils are one of the most promising candidate groups of natural compounds for the development of safer antifungal agents [9]. There is need to search for alternative approaches to preserve stored food material without toxicity problems that are eco-friendly and cost effective.

2. MATERIALS AND METHODS

2.1 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 [10]. 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 [11,12]. In process of culture the isolated fungal pathogens were cultivated on Potato Dextrose Agar (PDA) medium and Peptone Dextrose Rose Bengal Agar medium were used. Potato dextrose agar medium (39 gm of Hi–PDA medium dissolved in 1000 ml of distilled water) 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. Similarly Peptone Dextrose Rose Bengal Agar (31.55 gm of Hi-RBA medium dissolved in 1000 ml of distilled water) medium was prepared to maintain the fungal culture.

2.2 Plant Material Collection and Essential Oils Extraction

Plants were collected from different parts of Arunachal Pradesh during the study period. Identification of plants was done by the plant taxonomist in the Department of Botany, Rajiv Gandhi University, Itanagar as well as by the Scientist from Regional Centre of Botanical Survey of India at Itanagar. Herbarium was preserved and voucher specimens were deposited in the department. Extraction of essential oils was carried out from some locally available larger number of angiospermic taxa namely *Acorus calamus*, *Ageratum conyzoides*, *Artemisia nilagirica, Erigeron canadensis, Eupatorium odoratum, Litsea cubeba, Mesua ferrae, Mikania cordata, Piper mullesua and Pogostemon cablin* etc. Subsequently on getting preliminary screening results potent 5 plants were taken for detailed study. Each time an amount of 250 gm of fresh leaves of each plant were cut separately into small pieces and were thoroughly washed with sterilized water. The volatile fractions were isolated by hydro distillation through Clevenger's apparatus. Leaves of the plants were used for extraction of essential oils. The isolated fractions of plant parts exhibited 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 [13].

2.3 Antifungal Activity Assay

Fungitoxic activities of the essential oils were tested by the poisoned food technique method [14,15]. Potato dextrose agar medium (39 gm of Hi–PDA medium dissolved in 1000 ml of distilled water) 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 of aqueous solution of Tween –80 in pre-sterilized Petri plates (7 cm. 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. 5000 ppm, 1000 ppm, 500 ppm, 250 ppm and 125 ppm. 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 petriplates 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

2.4 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 [16].

2.5 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 was added in 0.5 ml 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 petriplates 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.

2.6 Nature of Toxicity

Nature of toxicity (funtgistatic/ fungicidal) of essential oils against the fungi was determined as suggested by [16]. 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 (5 mm 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 re-inoculated 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.

2.7 Fungitoxic Spectrum

Fungitoxicity range of the essential oils was determined for *Acorus calamus*, *Ageratum conyzoides*, *Artimesia nilagirica* and *Litsea cubeba* agaisnt four available fungi namely *Aspergillus niger, Cladosporium herbarum, Periconia microspinosa* and *Rhizopus oryzae* (mostly storage fungi) by the usual poisoned food technique using potato dextrose agar medium.

3. RESULTS

3.1 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 125 ppm, 250 ppm, 500 ppm, 1000 ppm and 5000 ppm were taken to evaluate the effect. Simultaneously, a control was also maintained by inoculating culture disc on the medium without adding any oil.

3.1.1 *Acorus calamus*

Essential oil of *A. calamus* (EOAC) 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 1000 ppm concentration by essential oil of *A. calamus*. 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 500 ppm concentration 100% inhibition was recorded. At 250 ppm 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.

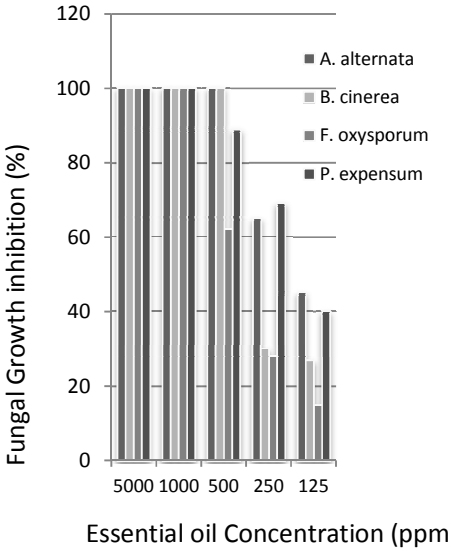

Fig. 1. Effect of *A. Calamus* oil on growth of fungi

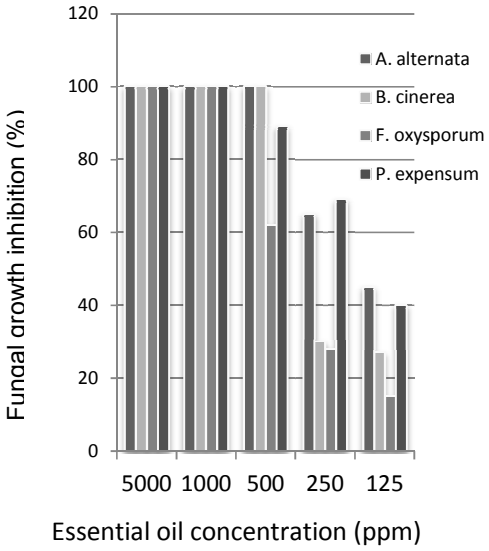

Fig. 2. Effect of *A. conyzoides* oil on growth of fungi

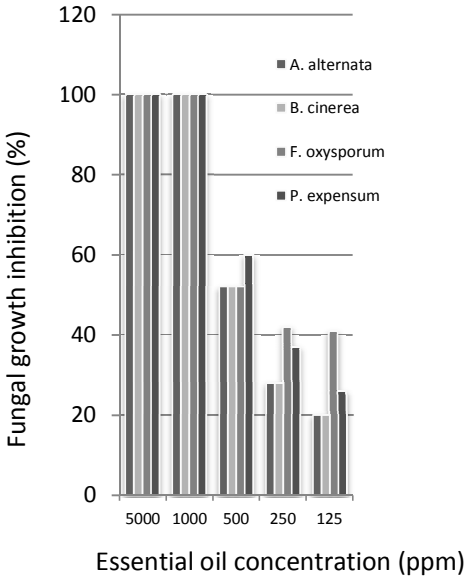

Fig. 3. Effect of *A. nilagirica* oil on growth of fungi

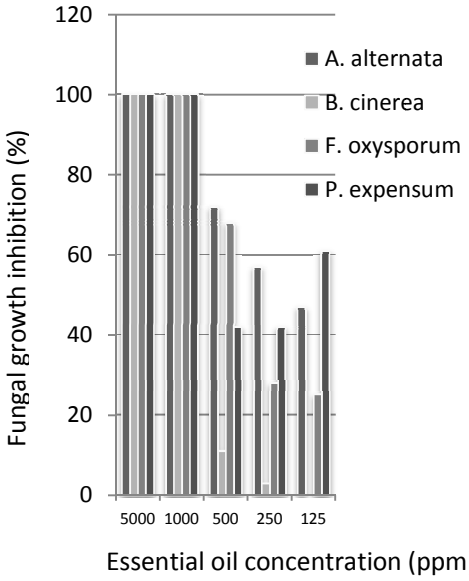

Fig. 4. Effect of *L. cubeba* oil on growth of fungi

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

3.1.2 *Ageratum conyzoides*

Essential oil of *A. conyzoides* (EOAC) was effective on the growth of all tested fungi at higher concentration 5000 and 1000 ppm. In case of *P. expansum, F. oxysporum and B. cinerea* 100% or complete inhibition of growth of fungus colony was recorded at 5000 and 1000 ppm concentration even after 15 days of inoculation. The decrease in colony diameter or growth of fungus was corresponding to the concentration of oil. Essential oil of *A. conyzoides* significantly inhibits the growth of all the four phytopathogenic fungi at 5000 and

1000 ppm concentration. By and large in comparison to the control growth of fungus at all concentration of essential oil was lesser.

3.1.3 *Artemisia nilagirica*

Essential oil of *A. nilagirica* (EOAN) inhibits the growth of all phytopathogenic fungi at higher concentration. At 5000 ppm and 1000ppm concentrations 100% inhibition was recorded even after 15 days of incubation. The decrease in fungal growth was almost 50% even at 500 ppm concentration of oil in case of all the tested fungus. Even at 125 and 250 ppm concentration of oil growth was found inhibited. Slight expansion in diameter of fungus colony was recorded at lower concentration of oil however it always remains lesser than control. At higher concentration oil was found fungicidal in nature.

3.1.4 *Litsea cubeba*

Essential oil of *Litsea cubeba* (EOLC) inhibited the growth of all phytopathogenic fungi at higher concentrations. The decrease in colony diameter or growth of fungus was corresponding to the concentration of oil. In case of *A. alternata* effect of *EOAL* was quite remarkable, at 5000 and 1000 ppm concentration 100% inhibition was recorded even after 15 days of incubation. EOAL shows drastic effect on the growth of fungus colony at higher concentration 5000 and 1000 ppm. At lower level of concentration *i,e* 125, 250, and 500 ppm of oil colony growth was recorded for all the four phytopathogenic fungi but it always remain lesser than the control. The effect of essential oil was corresponding to their concentration.

3.2 Minimum Inhibitory Concentration (MIC)

Inhibitory evaluation of essential oils against phytopathogenic fungi showed the effective results. *A. calamus, A. conyzoides, A. nilagirica* and *L. cubeba* oils inhibited the growth of fungi at 500 ppm.

3.3 Nature of Toxicity and Fungitoxic Spectrum

In general essential oils were found fungicidal at higher concentrations. *A. calamus* was fungicidal for *A. alternata* and *B. cinerea* at 500 ppm and for *F. oxysporum* and *P. expensum* at 1000 ppm. *A. conyzoides* reported fungicidal at 1000 ppm for all fungi. Similarly *A. nilagirica* and *L. cubeba* oils were reported fungicidal at 1000ppm concentrations.

Fungitoxic spectrum of the essential oils was studied applying against anumber of other fungi. Results showed that essential oil of *A. calamus, A. conyzoides, A. nilagirica* and *L. cubeba* was found fungicidal in nature and cent-percent inhibitory for fungi such as *Aspergillus niger, Cladosporium herbarum, Periconia microspinosa* and *Rhizopus oryzae*. However, inhibition of *A. niger* by *A. conyzoides* was found only 95%. In this case essential oil was found to stop the growth of fungi at initial period of incubation up to 11^{th} day but after that slight growth was occurred in petriplates.

Table 1. Minimum inhibitory concentration of essential oils against pathogenic fungi

Essential oils	MIC of oils against fungi			
	Phytopathogenic fungi			
	A. alternata	***B. cinerea***	***F. oxysporum***	***P. expansum***
Acorus calamus	500 ppm	500 ppm	500 ppm	500 ppm
Ageratum conyzoides	500 ppm	500 ppm	500 ppm	500 ppm
Artemisia nilagirica	500 ppm	500 ppm	500 ppm	500 ppm
Litsea cubeba	500 ppm	500 ppm	500 ppm	500 ppm

Table 2. Toxicity nature of essential oils on phytopathogenic fungi

Essential oils	*A. alternata*	*B. cinerea*	*F. oxysporum*	*P. expansum*
Acorus calamus	Fungicidal at 500 ppm	Fungicidal at 500 ppm	Fungicidal at 1000 ppm	Fungicidal at 1000 ppm
Ageratum conyzoides	Fungicidal at 1000 ppm	Fungicidal at 1000 ppm	Fungicidal at 1000 ppm	Fungicidal at 1000 ppm
Artemisia nilagirica	Fungicidal at 1000 ppm	Fungicidal at 1000 ppm	Fungicidal at 1000 ppm	Fungicidal at 1000 ppm
Litsea cubeba	Fungicidal at 1000 ppm	Fungicidal at 1000 ppm	Fungicidal at 1000 ppm	Fungicidal at 1000 ppm

Table 3. Fungitoxic spectrum of essential oil at 1000 ppm concentration

Fungal species	Percent inhibition of growth			
	A. calamus	***A. conyzoides***	***A. nilagirica***	***L. cubeba***
Aspergillus niger	100	95	100	100
Cladosporium herbarum	100	100	100	100
Periconia microspinosa	100	100	100	100
Rhizopus oryzae	100	100	100	100

4. 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 1000 ppm concentration of *A. calamus* essential oil. Colony growth of *A. alternata* and *B. cinerea* was also inhibited by oil and at 5000, 1000 and 500 ppm concentration 100% inhibition was recorded. Results indicate that *A. calamus* essential oil have fungitoxic property against the phytopathogenic fungi. Mazza [17] 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%), isoshyobunone (6.3%), β-gurjunene (6.7%), calamendiol (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 [18]. Radušienė et al. [19] 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 et al. [20] isolated a number of compounds from the essential oil of *A. calamus* and noted cytotoxicity and antifungal activity against *Aspergillus niger*. Sharma et al. [21] reported antifungal activity of *Acorus calamus* oil against *Sclerotium rolfsii* and *Rhizoctonia bataticola*. Devi and Ganjewala [22] found remarkable antifungal activity of *A. calamus* oil against *Aspergillus niger, A. flavus, Microsporum canis* and *Penicillium chrysogenum*. Lee et al. [23] 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. conyzoides* inhibits the growth of all the four phytopathogenic fungi at 5000 and 1000 ppm concentration. Increase in diameter of colony was always remained lesser than control one. Kamboj and Saluja [24] found a wide range of chemical compounds including alkaloids, cumarins, flavonoids, chromenes, benzofurans, sterols and terpenoids from *A. conyzoides* essential oil. Inhibitory impact attributed to the presence of flavonoids, chromenes, benzofurans, sterols and terpenoids in the oil. Fungitoxic activity of the oil is very well documented [25, 26]. Amadioha and Markson [27] found that oil of *Ageratum conyzoides* and *A.melegueta* to significantly arrest the mycelial growth and biomass development of *Botrydiplodia acerina* causal agent of rot of cassava *in vivo.*

Essential oil of *A. nilagirica* inhibits the growth of all phytopathogenic fungi at higher concentration. At 5000 ppm and 1000 ppm concentrations 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. At low concentration nature of oil was fungistatic. Sati et al. [28] reported that essential oil contained approximately 79.91% monoterpenoids and 18.25% sesquiterpenoids. Among the terpenoids thujone (9.37%), germacrene D (6.32%), 4-terpineol (6.31%), -caryophyllene (5.43%), camphene (5.47%) and borneol (4.12%) were found 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. *,* [29] 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%), chrysanthenone (1.5%-7.7%), germacrene D (3.1%-6.8%), β-caryophyllene (1.9%-6.8%), germacra-4,5, 10-trien-1-α-ol (1.9%-4.9%) and artemisia alcohol (1.4%-3.6%). Stappen et al. [30] reported that *A. nilagirica* essential oil have nonselective antifungal activity against plant pathogens *Colletotrichum acutatum, Colletotrichum fragariae* and *Colletotrichum gloeosporioides.* Presence of terpenoides in large quantity and other

compounds in small quantity would have perhaps played antifungal property against the phytopathogenic fungi.

The EOLC was found to inhibit the growth of all the phytopathogenig fungi at 5000 and 1000 ppm concentrations. Si et al. [31] reported 59 compounds from *L. cubeba* oil out of which dominant components were monoterpenes (94.4–98.4%), represented mainly by neral and geranial (78.7–87.4%), and D-Limonene was in lesser constituent (0.7–5.3%). Several components were only detected in certain regions and compounds such as o-cymene and eremophilene have never before been reported in EOLC. Su et al. [32] identified main components in oil as β-caryophyllene (13.0%), τ-cadinol (11.1%), α-cadinol (8.6%), α-humulene (7.5%), α-pinene (7.0%), globulol (6.6%), and β-eudesmol (6.1%). The anti-wood-decay fungal activity of the oil showed that the oil was inhibitory for wood-decay-fungi species and compounds were determined to be τ-cadinol, α-cadinol, and β-eudesmol. Yang et al. [33] through preliminary bioassay study showed *L. cubeba* oil has good fungicidal activities against *Sclerotinia sclerotiorum, Thanatephorus cucumeris, Pseudocer-cospora musae* and *Colletotrichum gloeosporioides* at the concentration of 588 and 272 μM, and the essential oil has good fungicidal activities against *T. cucumeris* and *S. sclerotiorum*, with IC_{50} values of 115.58 and 151.25 μg/mL, repectively.

Essential oils are mainly conjugated to phenolic compounds which accumulate in some plant cells, and have positive effects on pathogen control [34]. Reports indicated that essential oils containing carvacrol, eugenol and thymol (phenolic compounds) had the highest antibacterial performances [35]. Thus, our findings revealed that exogenous essential oils may have a positive role in preservation of plant materials and reduce decay.

5. CONCLUSION

Acorus calamus and *Agreratum conyzoides* oils inhibited growth of *A. alternata* and *B. cinerea* even at 500 ppm and other two plants oil at 1000 ppm concentrations. On the basis of results It can be stated that *Acorus calamus, Agreratum conyzoides, Artemisia nilagirica* and *Litsea cubeba* essential oil may be used as botanical pesticides for management of post harvest phytopathogenic fungal diseases. However, further investigations are needed in light of pharmacological tests.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pittet A. Natural occurrence of mycotoxins in foods and feeds –an update review. Revue de Medecine Veterinire. 1998;49: 449-92.
2. Dhingra OD, Mizubuti ESG, Napoleao IT, Jham G. Free fatty acid accumulation and quality loss of stored soybeen seeds invaded by *Aspergillus ruber.* Seed Sci. Technol. 2001;29:193-203.
3. Tripathi P, Shukla AK. Emerging non conventional technologies for control of post harvest diseases of perishables. Fresh Produce. 2007;1:111-120.
4. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. J. Appl Microbiol. 1999;86:985-990.
5. Mishra AK, Dubey NK. Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. Appl. Env. Microbiol. 1994; 60:1101-05.
6. Tripathi P, Dubey NK. Exploitation of natural products as alternative strategy to control post harvest fungal rotting of fruits and vegetables. Post harvest boil. Technol. 2004;32:235-245.
7. Tripathi P, Shukla AK. Exploitation of botanicals in the management of phytopathogenic and storage fungi. In: A. Arya and A.E. Perello editors, Management of fungal pathogens. CAB International, USA. 2010;36-50.
8. Bagamubola CF, Uyttendaele M, Devbevere J. Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalooland p-cymene towards *Shigella sonnei* and *S. flexneri.* Food Microbiol. 2004;21:33-42.
9. Hadizadeh I, Peivastegan B, Hamzehzarghani H. Antifungal activity of essential oils from some medicinal plants of Iran against *Alternaria alternate.* American J Appl. Sci. 2009;6:744-748.
10. Balali GR, Neate SM, Scott ES, Whisson, DL, Wicks TJ. Anastomosis group and pathogenicity of isolates of *Rhizoctonia solani* from potato crops in South Australia. Plant Path. 1995;44:1050-1057.

11. Barnett BB, Hunter HL. Illustrated genera of imperfect fungi. Burgess Publishing Co. Minneapolis, USA; 1972.
12. Domsch KH, Games W, Anderson T. Compendium of soil fungi. Academic Press, London; 1980.
13. Fauziah A, Subramanian P, Ibrahim H, Malek SNA, Serm LG, Lai HS. Chemical composition and antifeedant activity of the rhizomes of galangal, *Alpinia galanga* against Asian subterranean termites, *Coptotermes gestroi* and *Coptotermes curvignathus*. J. Insect Science. 2015;15: 1-7.
14. Grover RK, Moore JD. Toximetric study of fungicides against brown rot organism, *Sclerotinia fructicola* and *S. laxa*. Phytopathol. 1962;52:876-880.
15. Perrucci S, Mancianti F, Ciont PL, Flamini G, Morelli I, Macchioni G. *In vitro* antifungal activity of essential oils against some isolates of *Microsporum canis* and *M. gypseum*. Planta. Medi. 1994;60:184-187.
16. Thompson DP. Fungitoxic activity of essential oil components on food storage fungi. Mycologia. 1989;81:151-153.
17. Mazza G. Gas chromatographic and mass spectrometric studies of the constituents of the rhizome of calamus (*Acorus calamus*): 1.Volatile constituents of the essential oil. J. Chromatogr. 1985;328:179-194.
18. Wijesekara ROB, Ratnatunga CM, Durbeck K. The distillation of essential oils. Manufacturing and plant constructions handbook. Escgborn, Federal Republic of Germany: Protrade, Department of Food Stuffs & Agricultural Products; 1997.
19. Radušienė J, Judžentienė A, Pečiulytė D, Janulis V. Essential oil composition and antimicrobial assay of *Acorus calamus* leaves from different wild populations. Plant Genetic Resources. 2007;5:37-44.
20. Satyal P, Paudel P, Poudel A, Dosoky NS, Moriarity DM, Vogler B, Setzer WN. Chemical compositions, phytotoxicity and biological activities of *Acorus calamus* essential oils from Nepal. Nat Prod Commun. 2013;8:79-81.
21. Sharma PK, Raina AP, Dureja P. Evaluation of the antifungal and phytotoxic effects of various essential oils against *Sclerotium rolfsii* (Sacc) and *Rhizoctonia bataticola* (Taub). Phytopathol. Plant Protection. 2009;42:65-72.
22. Devi SA, Ganjewala D. Antimicrobial activity of *Acorus calamus* (L.) rhizome and leaf extract. Acta Biologica Szegediensis. 2009;53:45- 49.
23. Lee SO, Choi GJ, Jang KS, Lim HK, Cho KY, Kim JC. Antifungal activities of five plant essential oils as fumigant against postharvest and soilborne plant pathogenic fungi. Plant Pathol. J. 2007;23:97-102.
24. Kamboj A, Saluja AK. *Ageratum conyzoides* L.: A review on its phytochemical and pharmacological profile. Int J Green Pharm. 2008;2:59-68.
25. Sharma GP, Jain NK, Garg BD. Antifungal activity of some essential oils. Indian Drugs. 1978;15:21-23.
26. Kumar A, Shukla R, Singh P, Dubey NK. Chemical composition, antifungal and antiaflatoxigenic activities of *Ocimum sanctum* L. essential oil and its safety assessment as plant based antimicrobial. Food Chemical Toxicology. 2010;48:539-543.
27. Amadioha AC, Markson AA. Postharvest control of cassava tuber rot caused by *Botrydiplodia acerina* using extracts of plant origin. Arch Phytopathol. 2007; 40:359-366.
28. Sati SC, Sati N, Ahluwalia V, Walia S, Sati, OP. Chemical composition and antifungal activity of *Artemisia nilagirica* essential oil growing in northern hilly areas of India. Natural Product Res. 2012;27:45-48.
29. Padalia RC, Verma RS, Chauhan A, Chanotiya CS. Seasonal variation in essential oil composition of *Artemisia nilagirica* var. septentrionalis from Foot Hills of Western Himalaya. Rec. Nat. Prod. 2014;8:281-285.
30. Stappen I, Wanner J, Tabanca N, Wedge DE, Ali A, Khan IA, Kaul VK, Lal B, Jaitak V, Gochev V, Girova T, Stoyanova A, Schmidt E, Jirovetz L. Chemical composition and biological effects of *Artemisia maritima* and *Artemisia nilagirica* essential oils from wild plants of Western Himalaya. Planta Med. 2014; 80:1079-87.
31. Si L, Chen Y, Han X, Zhan Z, Tian S, Cui Q, Wang Y. Chemical composition of essential oils of *Litsea cubeba* harvested from its distribution areas in China. Molecules. 2012;17:7057-7066.
32. Su YC, HO CL. Composition and two activities of the leaf essential oil of *Litsea acuminata* (blume) kurata from Taiwan. Rec. nat. Prod. 2012;7:27-34.

33. Yang Y, Jiang J, Qunei L, Yan X, Zhao J, Yuan H, Wang M. The fungicidal terpenoids and essntial oil from *Litsea cubeba* in Tibet. Molecules. 2010;15: 7075-7082.
34. Plotto A, Roberts RG, Roberts DD. Evaluation of plant essential oils as natural postharvest disease control of tomato (*Lycopersicon esculentum*). Acta Horticulture. 2003;628:737-745.
35. Kim J, Marshall MR, Wei C. Antibacterial activity of some essential oils components against five food borne pathogens. J Agric Food Chemistry. 1995;43:2839–2845.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/16805