



RESEARCH ARTICLE

ESSENTIAL OILS AS CONTROL AGENT OF POST-HARVEST FUNGAL DISEASES OF KIWI FRUITS

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ABSTRACT

Plant derived essential oils may be an alternative agent in controlling the diseases caused by phytopathogenic fungi. Antifungal activity of *Ageratum conyzoides*, *Eupatorium odoratum* and *Messua ferrae* oil was evaluated against phytopathogenic fungi namely *Alternaria alternata*, *Botrytis cinerea*, *Fusarium oxysporum* and *Penicillium expensum* those cause post-harvest diseases in Kiwi fruits. Essential oil of *A. conyzoides* inhibits 100% growth of all the four phytopathogenic fungi at 5000 and 1000ppm concentrations and at low concentrations also growth of fungi was found inhibited. Essential oil of *E. odoratum* inhibited 100% growth of tested fungi at 5000ppm concentration and at low concentration also growth was found restricted. Inhibition in the growth of fungi was also reported by *M. ferrae* but it could not restricted 100% of growth at any concentration. On the basis of results it could be concluded that essential oil of *A. conyzoides* may be an alternative for control of phytopathogenic fungi after performing pharmacological evaluations.

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INTRODUCTION

Postharvest losses of fruits and vegetables is a serious problem, because the values of fresh product significantly increase while passing from the farm to the consumers table and due to overpopulation the demand for fruits and vegetables increases in the world. Enyiukwu *et al.* (2014) stated that most important losses in agricultural production which involve the greatest costs on the farm economy occur postharvest. It is estimated that worldwide between 10 to 40% losses of agricultural produce occur are postharvest. Losses are more severe in developing than developed nations of the world. Several species of fungi and in some cases bacteria participate in postharvest deterioration and rots of tubers and agro-produce. These include species of *Aspergillus*, *Botrytis*, *Fusarium*, *Colletotrichum*, *Macrophomina*, *Penicillium* and *Rhizopus* amongst several others. Kiwifruit [*Actinidia deliciosa* (A. Chev.) C.F. Liang and A.R. Ferguson var. *deliciosa* Hayward] is a climacteric and susceptible fruit to fungal decays in postharvest stage. After harvest, fruit rot diseases cause a severe loss of kiwifruit during cold storage, transportation, marketing, and in retail stores (Koh *et al.*, 2003). Many fungi are associated with post harvest fruit rots of

kiwifruit (Pennycook 1985; Hawthorne *et al.*, 1982). Botrytis gray mold rot caused by *Botrytis cinerea* is the most important and can directly invade the fruit or enter through wounds. Kiwifruit become much more susceptible to Botrytis (and other fungi) as they soften. Other fungal pathogens *Phomopsis mali*, *Botryosphaeria dothidea* and *Diaporthe actinidiae* have also been reported to cause post harvest fruit rots of kiwifruit (Lee *et al.*, 2001; Koh *et al.*, 2003). Essential oils or volatile oils are very complex mixture of compounds whose constituents of the oils are mainly monoterpenes and sesquiterpenes. 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 re-sorption, rate of reactions and bioavailability of the active compounds (Tripathi and Shukla, 2007). 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, Tripathi and Shukla, 2009). Essential oils are complex volatile compounds produced in different plant parts, which are known to have various functions in plants including conferring pest and disease resistance (Goubran and Holmes 1993). It has long been recognized that some essential oils have antimicrobial properties (Boyle, 1955) and these have been reviewed in the

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past (Shelef, 1983; Nychas, 1995). Present study was aim to evaluate for antifungal activity of essential oils against phytopathogenic fungi of kiwifruits.

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 Curl, 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) 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.

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 *Agiratum conyzoides*, *Artemisia nilagerica*, *Erigeron canadensis*, *Eupatorium odoratum*, *Mesua ferrae*, *Mikania cordata*, *Piper mullesua* and *Pogostemon cablin* etc. Subsequently on getting results potent 3 plants were taken for detailed study.

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 except in case of *Mesua ferrae* where leaves and flowers were respectively used for the oil extraction. 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 (Guenther, 1972).

Antifungal activity assay

Fungitoxic activities of the essential oils were tested by the poisoned food technique of Grover and Moore (1962) and Perrucci *et al.* (1994). 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 0.5ml of 0.01 percent of 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

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

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 fungi toxicity (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 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 (fungistatic / 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 $27 \pm 1^\circ\text{C}$. On seventh day the inhibited discs were taken out from the plates, washed with sterilized water and reinoculated 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. *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. Simultaneously, a control was also maintained by inoculating culture disc on the medium without adding any oil.

Ageratum conyzoides

Essential oil of *A. conyzoides* was effective on the growth of all tested fungi at higher concentration 5000 and 1000ppm. In case of *P. expansum*, *F. oxysporum* and *B. cinerea* 100% or complete inhibition of growth of fungus colony was recorded at 5000 and 1000ppm 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 1000ppm concentration. By and large in comparison to the control growth of fungus at all concentration of essential oil was lesser.

Eupatorium odoratum

Essential oil of *E. odoratum* was also inhibitory against the four phytopathogenic fungi. At 5000ppm concentration growth of all the four fungi completely restricted. In case of *P. expansum*, *F. oxysporum* and *A. alternata* at 1000ppm concentration of oil during their initial days of incubation the inhibition was remarkable, but during subsequent period of incubation growth of fungus colony was recorded.

Table 1. Effect of *Ageratum conyzoides* essential oil on the phytopathogenic fungi

| Period | <i>Alternaria alternata</i> | | | | | |
|------------------|-----------------------------|-----------|-----------|-----------|-----------|-----------|
| Days | 5000ppm | 1000ppm | 500ppm | 250ppm | 125ppm | Control |
| 5 th | 0.00±0.00 | 0.00±0.00 | 1.00±0.00 | 1.75±0.05 | 2.40±0.2 | 4.50±0.10 |
| 7 th | 0.00±0.00 | 0.00±0.00 | 1.35±0.05 | 2.40±0.10 | 3.45±0.25 | 5.90±0.00 |
| 9 th | 0.00±0.00 | 0.00±0.00 | 1.75±0.05 | 3.05±0.15 | 4.65±0.25 | 6.50±0.10 |
| 11 th | 0.00±0.00 | 0.00±0.00 | 2.25±0.05 | 3.40±0.40 | 5.30±0.30 | 6.80±0.10 |
| 13 th | 0.00±0.00 | 0.00±0.00 | 2.65±0.05 | 3.85±0.65 | 5.90±0.10 | 6.80±0.10 |
| 15 th | 0.00±0.00 | 0.00±0.00 | 3.05±0.05 | 4.45±0.55 | 6.10±0.10 | 7.00±0.10 |
| | <i>Botrytis cinerea</i> | | | | | |
| Days | 5000ppm | 1000ppm | 500ppm | 250ppm | 125ppm | Control |
| 5 th | 0.00±0.00 | 0.00±0.00 | 0.40±0.40 | 1.35±0.45 | 2.25±0.05 | 4.50±0.10 |
| 7 th | 0.00±0.00 | 0.00±0.00 | 0.50±0.50 | 2.10±0.60 | 2.50±0.10 | 5.90±0.00 |
| 9 th | 0.00±0.00 | 0.00±0.00 | 0.60±0.60 | 2.25±0.45 | 2.70±0.10 | 6.50±0.10 |
| 11 th | 0.00±0.00 | 0.00±0.00 | 0.65±0.65 | 2.25±0.45 | 2.90±0.10 | 6.80±0.10 |
| 13 th | 0.00±0.00 | 0.00±0.00 | 1.35±0.65 | 2.50±0.60 | 3.00±0.00 | 6.80±0.10 |
| 15 th | 0.00±0.00 | 0.00±0.00 | 1.50±0.55 | 2.65±0.75 | 3.20±0.10 | 7.00±0.10 |
| | <i>Fusarium oxysporum</i> | | | | | |
| Days | 5000ppm | 1000ppm | 500ppm | 250ppm | 125ppm | Control |
| 5 th | 0.00±0.00 | 0.00±0.00 | 0.75±0.05 | 1.30±0.30 | 2.45±0.05 | 4.00±0.00 |
| 7 th | 0.00±0.00 | 0.00±0.00 | 0.75±0.05 | 1.30±0.30 | 3.25±0.15 | 4.50±0.10 |
| 9 th | 0.00±0.00 | 0.00±0.00 | 1.60±0.10 | 1.70±0.10 | 3.90±0.50 | 4.70±0.10 |
| 11 th | 0.00±0.00 | 0.00±0.00 | 2.05±0.05 | 2.25±0.05 | 4.20±0.80 | 5.40±0.10 |
| 13 th | 0.00±0.00 | 0.00±0.00 | 2.45±0.05 | 2.80±0.00 | 4.60±1.20 | 6.30±0.10 |
| 15 th | 0.00±0.00 | 0.00±0.00 | 2.60±0.20 | 3.25±0.05 | 4.60±1.20 | 7.00±0.10 |
| | <i>Penicillium expansum</i> | | | | | |
| Days | 5000ppm | 1000ppm | 500ppm | 250ppm | 125ppm | Control |
| 5 th | 0.00±0.00 | 0.00±0.00 | 1.35±0.15 | 2.15±0.15 | 1.95±0.15 | 4.50±0.10 |
| 7 th | 0.00±0.00 | 0.00±0.00 | 1.70±0.10 | 2.60±0.20 | 2.15±0.35 | 5.90±0.00 |
| 9 th | 0.00±0.00 | 0.00±0.00 | 1.95±0.35 | 2.80±0.40 | 2.15±0.35 | 6.50±0.10 |
| 11 th | 0.00±0.00 | 0.00±0.00 | 2.05±0.45 | 3.00±0.60 | 2.40±0.60 | 6.80±0.10 |
| 13 th | 0.00±0.00 | 0.00±0.00 | 2.15±0.55 | 3.25±0.75 | 2.40±0.60 | 6.80±0.10 |
| 15 th | 0.00±0.00 | 0.00±0.00 | 2.35±0.75 | 3.50±1.00 | 2.40±0.60 | 7.00±0.10 |

Table 2. Effect of *Eupatorium odorata* essential oil on the phytopathogenic fungi

| Period | <i>Alternaria alternata</i> | | | | | |
|------------------|-----------------------------|-----------|-----------|-----------|-----------|-----------|
| Days | 500ppm | 1000ppm | 500ppm | 250ppm | 125ppm | Control |
| 5 th | 0.00±0.00 | 1.10±0.10 | 1.65±0.15 | 2.15±0.15 | 2.60±0.10 | 4.50±0.10 |
| 7 th | 0.00±0.00 | 1.60±0.10 | 2.05±0.05 | 2.65±0.15 | 3.25±0.25 | 5.90±0.00 |
| 9 th | 0.00±0.00 | 2.10±0.10 | 2.40±0.10 | 3.10±0.10 | 4.25±0.15 | 6.50±0.10 |
| 11 th | 0.00±0.00 | 2.10±0.10 | 2.40±0.10 | 3.90±0.10 | 4.25±0.15 | 6.80±0.10 |
| 13 th | 0.00±0.00 | 2.60±0.10 | 3.25±0.25 | 4.70±0.30 | 4.50±0.10 | 6.80±0.10 |
| 15 th | 0.00±0.00 | 2.65±0.15 | 3.80±0.20 | 4.70±0.30 | 4.50±0.10 | 7.00±0.10 |
| Days | <i>Botrytis cinerea</i> | | | | | |
| Days | 500ppm | 1000ppm | 500ppm | 250ppm | 125ppm | Control |
| 5 th | 0.00±0.00 | 3.60±0.00 | 3.90±0.10 | 3.70±0.20 | 4.05±0.05 | 4.50±0.10 |
| 7 th | 0.00±0.00 | 5.40±0.10 | 4.20±0.20 | 4.75±0.35 | 4.10±0.00 | 5.90±0.00 |
| 9 th | 0.00±0.00 | 5.80±0.00 | 4.55±0.05 | 4.90±0.50 | 4.50±0.10 | 6.50±0.10 |
| 11 th | 0.00±0.00 | 6.20±0.10 | 4.70±0.20 | 4.95±0.45 | 4.50±0.10 | 6.80±0.10 |
| 13 th | 0.00±0.00 | 6.20±0.10 | 4.75±0.15 | 4.95±0.45 | 4.50±0.10 | 6.80±0.10 |
| 15 th | 0.00±0.00 | 6.20±0.10 | 4.75±0.15 | 4.95±0.45 | 4.50±0.10 | 7.00±0.10 |
| Days | <i>Fusarium oxysporum</i> | | | | | |
| Days | 500ppm | 1000ppm | 500ppm | 250ppm | 125ppm | Control |
| 5 th | 0.00±0.00 | 0.85±0.05 | 0.85±0.05 | 1.60±0.20 | 2.45±0.05 | 4.00±0.00 |
| 7 th | 0.00±0.00 | 0.85±0.05 | 1.10±0.10 | 2.05±0.15 | 3.00±0.00 | 4.50±0.10 |
| 9 th | 0.00±0.00 | 1.60±0.10 | 1.70±0.10 | 2.40±0.10 | 3.65±0.05 | 4.70±0.10 |
| 11 th | 0.00±0.00 | 1.95±0.05 | 2.05±0.05 | 2.80±0.10 | 4.00±0.10 | 5.40±0.10 |
| 13 th | 0.00±0.00 | 2.35±0.05 | 2.50±0.10 | 3.25±0.15 | 4.25±0.05 | 6.30±0.10 |
| 15 th | 0.00±0.00 | 2.35±0.05 | 2.50±0.10 | 3.65±0.15 | 4.25±0.05 | 7.00±0.10 |
| Days | <i>Penicillium expansum</i> | | | | | |
| Days | 500ppm | 1000ppm | 500ppm | 250ppm | 125ppm | Control |
| 5 th | 0.00±0.00 | 1.20±0.00 | 1.75±0.05 | 1.85±0.05 | 1.95±0.05 | 4.50±0.10 |
| 7 th | 0.00±0.00 | 1.70±0.00 | 2.05±0.05 | 2.10±0.10 | 2.45±0.05 | 5.90±0.00 |
| 9 th | 0.00±0.00 | 2.20±0.00 | 2.35±0.05 | 2.45±0.05 | 2.85±0.05 | 6.50±0.10 |
| 11 th | 0.00±0.00 | 2.45±0.05 | 2.65±0.05 | 2.75±0.05 | 3.15±0.05 | 6.80±0.10 |
| 13 th | 0.00±0.00 | 2.45±0.05 | 2.65±0.05 | 2.75±0.05 | 3.45±0.05 | 6.80±0.10 |
| 15 th | 0.00±0.00 | 2.45±0.05 | 2.65±0.05 | 3.05±0.05 | 3.45±0.05 | 7.00±0.10 |

Table 3. Effect of *Mesua ferrae* essential oil on the phytopathogenic fungi

| Period | <i>Alternaria alternata</i> | | | | | |
|------------------|-----------------------------|-----------|-----------|-----------|-----------|-----------|
| Days | 500ppm | 1000ppm | 500ppm | 250ppm | 125ppm | Control |
| 5 th | 1.35±0.15 | 1.50±0.10 | 1.30±0.10 | 1.50±0.00 | 1.55±0.05 | 4.50±0.10 |
| 7 th | 1.85±0.15 | 2.15±0.15 | 1.85±0.15 | 2.00±0.10 | 2.00±0.10 | 5.90±0.00 |
| 9 th | 2.25±0.15 | 2.60±0.10 | 2.40±0.10 | 2.65±0.05 | 2.55±0.05 | 6.50±0.10 |
| 11 th | 2.55±0.25 | 2.90±0.10 | 2.85±0.05 | 3.05±0.05 | 2.95±0.05 | 6.80±0.10 |
| 13 th | 2.85±0.25 | 3.20±0.10 | 3.30±0.00 | 3.50±0.00 | 3.40±0.10 | 6.80±0.10 |
| 15 th | 3.25±0.25 | 3.55±0.25 | 3.90±0.10 | 4.10±0.10 | 4.20±0.00 | 7.00±0.10 |
| Days | <i>Botrytis cinerea</i> | | | | | |
| Days | 500ppm | 1000ppm | 500ppm | 250ppm | 125ppm | Control |
| 5 th | 1.70±0.10 | 2.65±0.15 | 3.20±0.00 | 3.70±0.10 | 3.85±0.25 | 4.50±0.10 |
| 7 th | 2.50±0.00 | 4.25±0.15 | 4.75±0.05 | 5.10±0.10 | 5.35±0.25 | 5.90±0.00 |
| 9 th | 2.90±0.30 | 5.30±0.10 | 5.80±0.20 | 5.45±0.25 | 5.40±0.20 | 6.50±0.10 |
| 11 th | 3.05±0.45 | 5.45±0.05 | 6.10±0.10 | 5.60±0.10 | 5.40±0.20 | 6.80±0.10 |
| 13 th | 3.05±0.45 | 5.45±0.05 | 6.10±0.10 | 6.20±0.10 | 6.00±0.20 | 6.80±0.10 |
| 15 th | 3.05±0.45 | 5.45±0.05 | 6.10±0.10 | 6.20±0.10 | 6.30±0.20 | 7.00±0.10 |
| Days | <i>Fusarium oxysporum</i> | | | | | |
| Days | 500ppm | 1000ppm | 500ppm | 250ppm | 125ppm | Control |
| 5 th | 0.00±0.00 | 2.05±0.05 | 2.10±0.10 | 2.20±0.00 | 2.15±0.05 | 4.00±0.00 |
| 7 th | 0.00±0.00 | 2.35±0.05 | 2.45±0.05 | 2.45±0.05 | 2.45±0.05 | 4.50±0.10 |
| 9 th | 2.55±0.05 | 2.65±0.05 | 2.85±0.05 | 2.95±0.05 | 2.95±0.05 | 4.70±0.10 |
| 11 th | 2.55±0.05 | 2.90±0.10 | 3.15±0.05 | 3.25±0.05 | 3.15±0.05 | 5.40±0.10 |
| 13 th | 2.90±0.10 | 3.40±0.20 | 3.55±0.05 | 3.65±0.05 | 3.85±0.05 | 6.30±0.10 |
| 15 th | 2.90±0.10 | 3.40±0.20 | 3.55±0.05 | 4.05±0.05 | 4.20±0.10 | 7.00±0.10 |
| Days | <i>Penicillium expansum</i> | | | | | |
| Days | 500ppm | 1000ppm | 500ppm | 250ppm | 125ppm | Control |
| 5 th | 0.00±0.00 | 1.80±0.10 | 2.40±0.40 | 2.45±0.05 | 2.80±0.20 | 4.50±0.10 |
| 7 th | 0.00±0.00 | 2.10±0.10 | 2.75±0.25 | 2.65±0.05 | 2.95±0.05 | 5.90±0.00 |
| 9 th | 0.00±0.00 | 2.55±0.05 | 2.75±0.25 | 3.05±0.05 | 3.40±0.10 | 6.50±0.10 |
| 11 th | 1.50±0.10 | 2.90±0.10 | 3.00±0.30 | 3.50±0.00 | 4.20±0.00 | 6.80±0.10 |
| 13 th | 1.90±0.10 | 3.20±0.10 | 3.25±0.25 | 4.10±0.10 | 4.35±0.15 | 6.80±0.10 |
| 15 th | 2.25±0.15 | 3.50±0.20 | 3.45±0.05 | 4.10±0.10 | 4.45±0.25 | 7.00±0.10 |

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

| MIC of oils against fungi | | | | |
|----------------------------|-----------------------|-------------------|---------------------|--------------------|
| Essential oils of plants | Phytopathogenic fungi | | | |
| | <i>A. alternata</i> | <i>B. cinerea</i> | <i>F. oxysporum</i> | <i>P. expansum</i> |
| <i>Agiratum conyzoides</i> | 500ppm | 500ppm | 500ppm | 500ppm |
| <i>Eupatorium odorata</i> | 500ppm | 500ppm | 1000ppm. | 5000ppm |
| <i>Mesua ferrae</i> | Higher Conc. | Higher Conc. | Higher Conc. | Higher Conc. |

Table 5. Toxicity nature of Essential oils on phytopathogenic fungi

| Essential oils | <i>A. alternata</i> | <i>B. cinerea</i> | <i>F. oxysporum</i> | <i>P. expansum</i> |
|----------------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| <i>Agiratum conyzoides</i> | Fungicidal at 1000ppm | Fungicidal at 1000ppm | Fungicidal at 1000ppm | Fungicidal at 1000ppm |
| <i>Eupatorium odorata</i> | Fungicidal at 5000ppm | Fungicidal at 5000ppm | Fungicidal at 5000ppm | Fungicidal at 5000ppm |
| <i>Mesua ferrea</i> | Fungistatic | Fungistatic | Fungistatic | Fungistatic |

However, it always remains lesser than control. The decrease in colony diameter or growth of fungus was corresponding to the concentration of oil. Colony diameter of *B.cinerea* was higher than other three phytopathogenic fungi. Growth of *P. expansum* remain inhibited even at 125ppm concentration.

Mesua ferrea

Essential oil of *M. ferrea* also inhibits the growth of four phytopathogenic fungi. At 5000ppm concentration also growth of fungal colony was noticed. In all four tested fungi effect of *M. ferrea* oil was quite similar. At initial stage there was sever inhibition but during their subsequent period of incubation growth of fungus colony was recorded for all four fungi at all concentration of oil. However, the growth of fungus colony always remains lesser than the control. The decrease in colony diameter in growth of fungus colony was corresponding to their concentration of oil. Thus, effect of *M. ferrea* oil on all four phytopathogenic fungi was not so effective.

Minimum Inhibitory Concentration (MIC)

Inhibitory evaluation of essential oils against phytopathogenic fungi showed the effective results. Essential oil of *A. conyzoides* was found fungi toxic at 500ppm for all the four fungi. EO of *E. odoratum* was inhibitory at 5000 and 1000ppm concentration for all the phytopathogenic fungi. *M. ferrea* was found fungitoxic at 5000ppm concentration against all the fungi.

Nature of toxicity

A. conyzoides oil was found fungicidal for all the phytopathogenic fungi. Oil of *M. ferrea* Canadensis was fungistatic for all the pathogens. *E. odoratum* oil was fungicidal at 5000ppm for all the tested fungi but at low concentrations it was fungistatic.

DISCUSSION

Essential oil of *A. conyzoides* significantly inhibits the growth of all the four phytopathogenic fungi at 5000 and 1000ppm concentration. Increase in diameter of colony was always remained lesser than control one. Kamboj and Saluja (2008) 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 (Sharma *et al.*, 1978; Kumar *et al.*, 2010). Amadioha and Markson (2007) found that oil of *Ageratum conyzoides* and *A.melegueta* to significantly arrest the mycelial growth and biomass development of *Botrydiplochia acerina* causal agent of rot of

cassava *in vivo*. Essential oil of *Eupatorium odoratum* was inhibitory against fungi at higher concentration of oil. At lower concentrations (500, 250, 125ppm) it was fungistatic. Joshi (2013) reported a total of twenty-nine compounds from essential oil of *Eupatorium odorata* have been identified, accounting 97.6% of the total oil. The main constituents were himachalol (24.2%), 7-isopropyl-1,4-dimethyl-2-azulenol (17.6%), androencecalinol (14.1%), and 2-methoxy-6-(1-methoxy-2-propenyl) naphthalene (5.6%). The essential oil consists mainly of phenyl derivatives (41.6%), followed by oxygenated sesquiterpenes ((26.6%), long-chain hydrocarbons (18.9%), sesquiterpene hydrocarbons (6.8%), oxygenated monoterpenes (2.8%), and monoterpene hydrocarbons (0.9%). Owolabi *et al.* (2010) also determined major Components in essential oil of *E. odoratum* as -pinene (42.2%), -pinene (10.6%), germacrene D (9.7%), -copaen-4 -ol (9.4%), (E)-caryophyllene (5.4%), and geijerene/pregeijerene (7.5%). The oil was screened for antifungal activity against *Aspergillus niger* (MIC = 78 µg/mL). Presence of phenols and terpenoids found to be toxic and inhibitory for the growth of fungi. Essential oil of *Mesua ferrea* had fungistatic effect on all the fungi. At initial days there was inhibition but during their subsequent period of incubation, growth of fungus colony was recorded for all four fungi at all concentration of oil. However, the growth of fungus colony always remains lesser than the control. Chanda *et al.* (2013) reported that *M. ferrea* effect the growth of *A. niger*. The principal constituents of *M. ferrea* include mesuaferone-A & B, mesuaferrol, mesuanic acid, amyryn and sitosterol present in the stamen (Subramanyam and subba, 1977) while it is reported that seeds contain essential oils, xanthonenes and coumarins (Subramanyam and subba, 1977). Sahu *et al.*, (2014) analyzed that the different parts of the plant contain glycosides, coumarins, flavanoids, xanthonenes, triglycerides and resins. Specifically it contains -copaene and germacrene D, ve -amyryn, -sitosterol, and a new cyclohexadione compound named as mesuaferrol, mesuanic acid, triterpenoids and resins, reducing sugars, and tannins, saponins, Mesuaferone B, mesuol. On the basis of present findings it can be concluded that *A. conyzoides* oil could be an alternative to synthetic fungicide for control of phytopathogenic fungi. However, pharmacological studies are needed prior to use it as botanical pesticide.

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