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

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Abstract: Essential oils were extracted from different plant species (*Acorus calamus, Artemisia nilagerica, 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 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*. 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. In comparison to others two, 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; Artemesia; Erigeron*

Introduction

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 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 ecofriendly and cost effective. Present study was aimed to evaluate the antifungal activity of some plant based essential oils against phytopathogenic fungi.

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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 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) 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. 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 *Acorus calamus*, *Artimesia nilogerica, 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 *Acorus calamus* where fruits, rhizome 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

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

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 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 (funtgistatic / 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 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. *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 fungi toxic 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

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

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

Period			***Alternaria alternata***			
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.10±0.00	2.60±0.00
7th	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.65±0.05	3.20±0.10
9th	0.00±0.00	0.00±0.00	0.00±0.00	0.50±0.00	2.05±0.05	4.90±0.10
11th	0.00±0.00	0.00±0.00	0.00±0.00	0.90±0.40	2.60±0.00	5.60±0.10
13th	0.00±0.00	0.00±0.00	0.00±0.00	2.05±0.25	3.15±0.05	6.00±0.10
15th	0.00±0.00	0.00±0.00	0.00±0.00	2.45±0.25	3.80±0.00	6.20±0.10
			Botrytis cinerea			
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.00±0.00	0.00±0.00	0.00±0.00	1.65±1.50	3.05±2.80	4.50±0.10
7th	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.30	4.25±0.65	5.90±0.00
9th	0.00±0.00	0.00±0.00	0.00±0.00	2.85±0.35	4.70±0.80	6.50±0.10
11th	0.00±0.00	0.00±0.00	0.00±0.00	4.25±0.45	4.85±0.85	6.80±0.10
13th	0.00±0.00	0.00±0.00	0.00±0.00	4.60±0.30	5.10±0.60	6.80±0.10
15th	0.00±0.00	0.00±0.00	0.00±0.00	4.90±0.00	5.10±0.60	7.00±0.10
			Fusarium oxysporum			
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.00±0.00	0.00±0.00	0.00±0.00	0.90±0.40	1.65±0.15	4.00±0.00
7th	0.00±0.00	0.00±0.00	0.00±0.00	1.75±0.25	2.50±0.00	4.50±0.10
9th	0.00±0.00	0.00±0.00	0.75±0.25	2.35±0.25	3.30±0.20	4.70±0.10
11th	0.00±0.00	0.00±0.00	1.45±0.05	3.2±0.30	4.15±0.35	5.40±0.10
13th	0.00±0.00	0.00±0.00	2.05±0.05	4.15±0.15	5.20±0.40	6.30±0.10
15th	0.00±0.00	0.00±0.00	2.65±0.05	5.05±0.25	5.95±0.25	7.00±0.10
			Penicillium expensum			
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.00±0.00	0.00±0.00	0.00±0.00	0.50±0.00	1.20±0.10	3.60±0.10
7th	0.00±0.00	0.00±0.00	0.00±0.00	1.00±0.00	1.95±0.05	4.50±0.10
9th	0.00±0.00	0.00±0.00	0.00±0.00	1.45±0.05	2.65±0.05	5.00±0.10
11th	0.00±0.00	0.00±0.00	0.50±0.00	1.95±0.05	3.15±0.15	5.50±0.10
13th	0.00±0.00	0.00±0.00	0.75±0.05	1.95±0.05	3.85±0.05	5.80±0.10
15th	0.00±0.00	0.00±0.00	0.75±0.05	2.15±0.15	4.15±0.15	6.00±0.10

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

Period			***Alternaria alternata***			
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.00±0.00	0.00±0.00	2.60±0.20	3.35±0.05	3.90±0.10	2.60±0.00
7th	0.00±0.00	0.00±0.00	3.25±0.55	3.05±0.05	4.50±0.10	3.20±0.10
9th	0.00±0.00	0.00±0.00	3.25±0.55	4.15±0.05	5.15±0.15	4.90±0.10
11th	0.00±0.00	0.00±0.00	3.25±0.55	5.00±0.10	5.25±0.25	5.60±0.10
13th	0.00±0.00	0.00±0.00	3.30±0.50	5.00±0.10	5.60±0.20	6.00±0.10
15th	0.00±0.00	0.00±0.00	3.30±0.50	5.00±0.10	5.60±0.20	6.20±0.10
			Botrytis cinerea			
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.00±0.00	0.00±0.00	2.6±0.25	3.35±0.05	3.90±0.00	4.50±0.10
7th	0.00±0.00	0.00±0.00	3.25±0.6	4.05±0.20	4.50±0.15	5.90±0.00
9th	0.00±0.00	0.00±0.00	3.25±0.6	4.15±0.10	5.15±0.25	6.50±0.10
11th	0.00±0.00	0.00±0.00	3.25±0.6	5.00±0.30	5.25±0.20	6.80±0.10
13th	0.00±0.00	0.00±0.00	3.3±0.55	5.00±0.30	5.60±0.20	6.80±0.10
15th	0.00±0.00	0.00±0.00	3.3±0.65	5.00±0.30	5.60±0.20	7.00±0.10
			Fusarium oxysporum			
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.00±0.00	0.00±0.00	1.75±0.05	2.00±0.00	2.10±0.10	4.00±0.00
7th	0.00±0.00	0.00±0.00	2.15±0.05	2.35±0.05	2.50±0.10	4.50±0.10
9th	0.00±0.00	0.00±0.00	2.45±0.05	2.75±0.05	2.90±0.10	4.70±0.10
11th	0.00±0.00	0.00±0.00	2.90±0.00	2.75±0.05	2.90±0.10	5.40±0.10
13th	0.00±0.00	0.00±0.00	3.35±0.05	3.50±0.00	3.60±0.10	6.30±0.10
15th	0.00±0.00	0.00±0.00	3.35±0.05	4.00±0.00	4.25±0.25	7.00±0.10
			Penicillium expensum			
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.00±0.00	0.00±0.00	1.20±0.10	3.40±0.20	3.75±0.05	3.60±0.10
7th	0.00±0.00	0.00±0.00	2.05±0.05	3.80±0.20	4.00±0.00	4.50±0.10
9th	0.00±0.00	0.00±0.00	2.55±0.25	4.10±0.10	4.35±0.05	5.00±0.10
11th	0.00±0.00	0.00±0.00	2.55±0.25	4.10±0.10	5.05±0.05	5.50±0.10
13th	0.00±0.00	0.00±0.00	2.75±0.25	4.35±0.15	5.05±0.05	5.80±0.10
15th	0.00±0.00	2.05±0.05	2.75±0.25	4.35±0.15	5.15±0.05	6.00±0.10

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

Period			*Alternaria alternata*			
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.00±0.00	4.05±0.25	4.15±0.05	4.20±0.10	4.25±0.05	2.60±0.00
7th	0.00±0.00	4.45±0.55	4.70±0.10	4.75±0.15	5.20±0.40	3.20±0.10
9th	0.00±0.00	4.70±0.80	4.95±0.25	4.95±0.05	6.10±0.40	4.90±0.10
11th	4.10±0.90	4.75±0.75	5.00±0.20	4.95±0.05	6.10±0.40	5.60±0.10
13th	4.10±0.90	4.75±0.75	5.00±0.20	4.95±0.05	6.10±0.40	6.00±0.10
15th	4.10±0.90	4.75±0.75	5.00±0.20	4.95±0.05	6.10±0.40	6.20±0.10
			Botrytis cinerea			
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.00±0.00	4.05±0.25	4.30±0.20	3.95±0.15	3.95±0.65	4.50±0.10
7th	0.00±0.00	4.70±0.80	4.70±0.10	5.25±0.45	4.10±0.80	5.90±0.00
9th	0.00±0.00	4.95±1.05	4.95±0.25	6.10±0.40	4.20±0.70	6.50±0.10
11th	0.00±0.00	4.95±1.05	4.95±0.25	6.10±0.40	4.20±0.70	6.80±0.10
13th	4.10±0.90	4.95±1.05	4.95±0.25	6.10±0.40	4.20±0.70	6.80±0.10
15th	4.10±0.90	4.95±1.05	4.95±0.25	6.10±0.40	4.20±0.70	7.00±0.10
			Fusarium oxysporum			
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.00±0.00	1.10±0.10	1.45±0.05	2.10±0.10	2.25±0.25	4.00±0.00
7th	0.00±0.00	1.10±0.10	1.90±0.10	2.50±0.10	2.80±0.20	4.50±0.10
9th	1.15±0.05	1.60±0.10	2.20±0.10	2.95±0.05	3.30±0.30	4.70±0.10
11th	1.50±0.10	1.90±0.10	2.55±0.05	3.50±0.10	3.65±0.15	5.40±0.10
13th	1.75±0.05	2.20±0.10	2.95±0.05	3.90±0.10	4.05±0.15	6.30±0.10
15th	2.05±0.05	2.45±0.05	3.25±0.25	4.25±0.05	4.50±0.10	7.00±0.10
			Penicillium expensum			
Days	**5000ppm**	**1000ppm**	**500ppm**	**250ppm**	**125ppm**	**Control**
5th	0.00±0.00	1.65±0.05	2.00±0.10	2.30±0.10	3.05±0.05	3.60±0.10
7th	0.00±0.00	1.95±0.05	2.35±0.05	2.55±0.05	3.65±0.65	4.50±0.10
9th	2.20±0.10	2.25±0.05	2.75±0.05	2.90±0.10	3.95±0.75	5.00±0.10
11th	2.60±0.10	2.65±0.05	3.05±0.05	2.90±0.10	4.25±0.75	5.50±0.10
13th	3.05±0.05	3.00±0.10	3.05±0.05	3.15±0.15	4.50±0.50	5.80±0.10
15th	3.05±0.05	3.05±0.05	3.25±0.05	3.15±0.15	4.50±0.50	6.00±0.10

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

Essential oils of plants	MIC of oils against fungi			
	Phytopathogenic fungi			
	A. alternata	***B. cinerea***	***F. oxysporum***	***P. expansum***
Acorus calamus	250ppm	500ppm	500ppm	500ppm
Artemisia nilagerica	5000ppm	5000ppm	Higher Conc.	5000ppm
Erigeron canadensis	Higher Conc.	Higher Conc.	Higher Conc.	Higher Conc.

Table 5. Toxicity nature of Essential oils on phytopathogenic fungi

Essential oils	*A. alternata*	*B. cinerea*	*F. oxysporum*	*P. expansum*
Acorus calamus	Fungicidal at 500ppm	Fungicidal at 500ppm	Fungicidal at 1000ppm	Fungicidal at 500ppm
Artemisia nilogirica	Fungicidal at 500ppm	Fungicidal at 500ppm	Fungistatic	Fungicidal at 5000ppm
Erigeron canadensis	Fungistatic	Fungistatic	Fungistatic	Fungistatic

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%), 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 (Wijesekara *et al.,* 1997). Radusiene (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 *Sclerotium rolfsii* and *Rhizoctonia bataticola*. 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. nilagerica* 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-terpineol (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%), 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.,* (2014) 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.

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 (limomene 57.2%), camphene (2.5%) α and β-pinenes (1.9 % & 2.1%) and sesquiterpenoids (caryophyllene (6.7%), germacrene D (4.9%) and α-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 acitivity.

It can be concluded on the basis of present findings 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.*

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