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## Biocontrol Potentials of Three Essential Oils Against Some Postharvest Pathogens

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**Abstract** Postharvest pathogens caused great economical losses to freshly harvested fruits and vegetables. Various pathogens both fungi and bacteria are responsible for postharvest diseases. Environmental pollution caused by excessive use and misuse of agrochemicals, as well as fear-mongering by some opponents to chemical fertilizers and pesticides, has led to considerable changes in people's attitudes towards the use of agrochemicals in agriculture. Alternative methods such as biocontrol technique to control pathogens have got worldwide appreciation. In the present study, postharvest pathogens of some vegetables and fruits were investigated and biocontrol potentials of three essential oils were evaluated against the pathogens causing rapid spoilage. Altogether, 45 fungal isolates representing 5 genera were isolated from diseased and rotten samples. The fungal genera that were mostly isolated are *Aspergillus*, *Mucor*, *Rhizopus*, *Geotrichum* and *Fusarium*. These pathogens were evaluated for their aggressiveness in *in-vitro*. Maximum rotting percentage was observed in lightly higher temperature than normal. Three essential oils were tested for their antifungal activity against the isolated pathogens. All the oil displayed antifungal activity in varying degrees. Among the oils, carrot oil was found to be most effective in inhibiting all the pathogens. *In-vivo* experiment to observe the efficacy of the three essential oils against *Geotrichum* was conducted in tomato. The result indicated all the oils have the ability to bring down the rotting percentage to considerable extent. The results of the present study suggest that essential oils could be potentially applied to control postharvest pathogens and in management of postharvest diseases.

**Keywords:** Postharvest pathogens, Essential oils, Antifungal activity, Biocontrol potentials

### Introduction

Vegetables and fruits are often infected by fungi after harvest resulting into significant losses. This loss can vary from an estimated 10-50% depending on the commodity both in developed and underdeveloped, tropical countries (Dennis, 1983; Wilson *et al.*, 1991). The reduction of losses in perishable food crops because of post-harvest diseases has become a major objective of international organizations (Kelman, 1989). Considerable effort has been done

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to diminish those losses by application of new technologies that control temperature and humidity during storage. But, still today post-harvest application of synthetic fungicides is the primary method of controlling these diseases. With public concern to restrict the use of synthetic fungicides for the control of post-harvest diseases of fruits and vegetables, there is an urgent need for safe and effective alternatives (Wisniewski and Wilson, 1992). So far biological control employing antagonistic microorganisms has proven to be effective against a variety of post-harvest diseases of fruits and vegetables (Wilson and Wisniewski, 1989; Fravel, 2005). However, at present attention is shifting towards other alternative that the consumers perceive as natural and in particular, plant extracts, including their essential oils and essences. Essential oils are concentrated hydrophobic liquid containing volatile aroma compounds derived from plants. They have been reported to exhibit exceptionally good antimicrobial effects against bacteria, yeasts, filamentous fungi, and viruses (Kalemba and Kunicka, 2003). There are some studies on effects of essential oils on post-harvest pathogens (Bishop and Reagan, 1997) and management of grey mould of grapes caused by *Botrytis cinerea* (Tripathiet *al.*, 2008). Nevertheless, use of essential oils and their derivatives for management of post-harvest pathogens are still not well documented.

The aim of this study was to isolate pathogens responsible for post-harvest rotting of vegetables and fruits and to evaluate biocontrol potentials of three essential oils (celery oil, carrot oil and clove oil) in management of these pathogens.

## **Materials and methods**

### ***Samples collection, isolation and identification of pathogens***

Diseased and infected vegetables and fruits samples were collected from local and storage market of Baripada, Odisha, India. The samples were collected separately for each vegetables and fruits in sterile polythene bags and brought immediately to the laboratory. For isolation of pathogens, infected rotten tissues with milky secretion and visible growth of fungal mycelium from the infected parts were gently scrape out with fine sterile needle and directly inoculated on Potato Dextrose Agar (PDA) medium. Infected disease parts were washed with sterile distilled water and surface sterilized by immersion in 70% ethanol for 1 min and 1% sodium hypochloride (NaOCl<sub>2</sub>) solution for 2 min and thoroughly rinsed with sterile distilled water. Surface sterilized diseased fragments were dissected out with sterile scalpel and transferred onto plates containing PDA medium. The PDA plates were supplemented with streptomycin sulphate (100 µgml<sup>-1</sup>) and incubated at 30 °C for 5 days. Fungi

growing out of the plated fragments were immediately transferred to PDA slant and stored at 4 °C for further study. The fungi were identified on the basis of morphological characters, viz. colony characters on media, mycelial characters, types of conidiophore and conidial characters with the help of manuals of Barnett and Hunter (1972) and Domsch *et al.* (1980).

### ***In-vitro testing for virulence***

The pathogens were test for their ability to cause rotting at specified incubation time and temperatures. The fungi were cultured in Potato dextrose broth (PDB) and incubated at 32 °C for a week till sporulation. Some healthy vegetables and fruits were taken and thoroughly washed, dried properly and then surface sterilized with 1% Ca(OCl)<sub>2</sub> for 3 min, 0.5% NaOCl<sub>2</sub> for 2 min and washed thoroughly with sterile distilled water. Then with help of sterile needle four wounds (3 mm deep and 3 mm wide) were made at the equatorial side for all the vegetables and fruits tested. The wounds were inoculated with 100 µL of spore suspensions (1x10<sup>5</sup> CFU) of each pathogen. One set was kept at room temperature (approx. 30 °C) and another set was kept in incubator at 35 °C for 3 days. After the incubation period wounds were examined and the percentage of rotting (R%) was determined as follows:

$$R (\%) = \frac{\text{Number of decayed wounds}}{\text{Number of total wounds}} \times 100$$

### ***Determination of antifungal activity of essential oils***

Virulent fungal pathogens were selected and three essential oils namely celery oil, carrot oil and clove oil were used to determine antifungal activity against the selected pathogens. The essential oils were obtained from Indian Institute of Chemical Technology (IICT) Hyderabad, India. The oils were dissolved separately in Dimethyl-Sulpho-Oxide (DMSO) to give a concentration of 1000 µl/ml and antifungal testing was carried out by disc diffusion assay. The pathogens were cultured in Potato Dextrose Broth and incubated for a week till sporulation. Lawns of pathogens were prepared by inoculating spore suspension of each pathogen onto PDA medium. Sterile discs, prepared from Whatman filter paper No.1 (7mm in diameter) were soaked in oils suspension separately. Each disc was then placed over PDA plates containing pathogens. The plates were incubated at 35 °C for 3 days and clear zone on inhibition developing on the plates as a result of the activity of the oils were measured and noted.

### ***In-vivo* biocontrol evaluation of essential oils in Tomato**

The essential oils were evaluated for their *in-vivo* biocontrol potentials against *Geotrichum* sp. The test was conducted in tomato, which was one of the most common vegetable that cause spoilage. *Geotrichum* sp. was selected among the pathogens because of its aggressiveness and ability to cause rapid spoilage in tomato and in other vegetables. Healthy tomato were selected and brought into the laboratory. It was washed with sterile phosphate buffer (0.05 M, pH 6.5) for 10 min with shaking and rubbing with a sterilized paint brush. It was then disinfected with 0.1% (v/v) sodium hypochlorite, rinsed three times in sterile distilled water and then air dried prior to wounding. Four wounds (3 mm deep and 3 mm wide) were made using a sterile needle at the equatorial side.

The set was prepared separately for the three oils and 30µl of the oils were pipetted into each wound site separately. At the same time one set of the wounds were inoculated with 50µl spore suspension of *Geotrichum* sp. After 24 h of incubation the treated sets were inoculated with 20µl of spore suspension of *Geotrichum* sp. The set was stored at 35 °C and at ~ 90% RH for 3 days. After the incubation period wounds were examined and the percentage of rotting (R%) was determined as follows:

$$R (\%) = \frac{\text{Number of decayed wounds}}{\text{Number of total wounds}} \times 100$$

### **Results**

A total of 45 fungal isolates were obtained from the collected samples, representing 5 genera of fungi (Table 1). The most frequently isolated fungal genera were *Aspergillus*, *Mucor*, *Rhizopus*, *Geotrichum* and *Fusarium*. More isolates of *Geotrichum* were obtained from tomato and carrot which was characterized by secretion of creamy and watery fluid. Among the pathogens, the genera of *Aspergillus* were isolated from almost all the samples. Species of *Fusarium* was isolated only from rotted potatoes and gingers. Similarly, *Rhizopus* and *Mucor* were isolated from rotten parts of potato, beet, pumpkin and grape respectively. These pathogens were obtained in pure culture and tested for their pathogenicity in *in-vitro* condition to incite rapid spoilage in room and incubation temperature. Healthy vegetables and fruits were inoculated with spores' suspension of respective pathogens and kept in room and incubation temperature and ability to cause rapid spoilage were observed. The result indicated that 73.33% of rotting occur at room temperature with rot

percentage ranging from 25% to 100% and 93.33% of rotting occur in incubation temperature with rot percentage ranging from 50% to 100% (Table 2). Among the pathogens, *Geotrichum*, *Aspergillus* and *Fusarium* could cause 100% rotting in incubation temperature on vegetables like tomato, beet and ginger and potato respectively. *Rhizopus* cause 100% rotting in lady's finger in room temperature but no rotting was observed, when inoculated with *Geotrichum* both in room and incubation temperature after 3 days. Similarly, *Aspergillus* and *Rhizopus* did not cause rotting in beans and potatoes respectively. In grapes, *Mucor* caused 50% rotting at incubation temperature and no rotting was observed at room temperature. Over all, rot percentages occur more at incubation temperature than in room temperature.

Antifungal activity of three essential oils namely celery oil, carrot oil and clove oil were studied *in-vitro* against the pathogens that caused rapid spoilage in vegetables and fruits. The result indicated that all the oils inhibited the postharvest pathogens to varying degrees (Table 3). Considerable antifungal activity was exhibited by celery and carrot oils against all the test pathogens but clove oil was not effective against *Mucor* sp. Carrot and celery oil showed highest zone of inhibition against *Mucor* with zone diameter of 33.0 mm and 31.5 mm respectively. Similarly, carrot oil also showed considerable activity against *Fusarium* with zone diameter of 30.0 mm. However, clove oil did not showed significant activity against *Aspergillus niger* which was characterized by very low zone of inhibition (8 mm). Over all, both celery and carrot oils were highly effectively in inhibiting all the tested pathogen but such activity was not observed in clove oil.

*In-vivo* biocontrol potentials of the three essentials oils were test against *Geotrichum* sp. frequently isolated from tomato and because of its ability to cause rapid spoilage. Healthy tomatoes were inoculated with spore suspension of *Geotrichum* in one set and in another set essential oils were loaded into the wounds that were pre-inoculated with spore suspension of the fungus. The sets up were kept at incubation temperature at 35 °C. The percentage of rots in inoculated set and treated set were calculated after three days of incubation. The result indicated that 100% rotting occur in inoculated set and percentage of rotting is minimum or no rotting take placed in the treated set (Table 4). All the oils showed ability to minimize rot (Fig. 1). Among the oils, celery and clove oil showed 75% reduction in rot, whereas, carrot oil showed 100% rot reduction indicating that naturally occurring essentials oil could be alternative method for biocontrol of postharvest pathogens.

**Table 1.** Post-harvest pathogens isolated from diseases samples

Pathogens	Isolation sources
<i>Aspergillus niger</i>	Potato, Beet, Lemon
<i>Aspergillus flavus</i>	Pumpkin, Bitter gourd
<i>Aspergillus</i> sp.	Bean, Banana
<i>Geotrichum</i> sp.	Tomato, Carrot, Ginger
<i>Fusarium</i> sp.	Potato, Ginger
<i>Rhizopus</i> sp.	Potato, Beet
<i>Mucor</i> sp.	Pumpkin, Grapes, Beet

**Table 2.** Pathogenicity test of selected pathogens on some fruits and vegetables

Pathogen	Host	% rots after 3 days of inoculation	
		R T (30 °C)	I T (35 °C)
<i>Geotrichum</i> sp.	Tomato	75	100
	Bitter gourd	25	50
	Lady's finger	--	--
	Carrot	50	75
	Beet	25	100
<i>Aspergillus</i> sp.	Bean	--	75
	Potato	25	50
	Ginger	75	100
	Bitter gourd	50	75
	Lemon	25	75
	Potato	--	75
<i>Rhizopus</i> sp.	Lady's finger	100	75
	Potato	25	100
<i>Fusarium</i> sp.	Ginger	25	50
<i>Mucor</i> sp.	Grapes	--	50

R T = Room Temperature; I T= Incubation Temperature

-- No rotting

**Table 3.** In-vitro antifungal activity of essential oils against some post-harvest pathogens

Pathogens	Isolation source	Essential oil (zone of inhibition in mm)		
		Cy	Ct	Cv
<i>Aspergillus niger</i>	Potato/Bean/Lemon	14.6	14.6	08.0
<i>Rhizopus</i> sp.	Potato/Beet	15.0	16.0	11.0
<i>Fusarium</i> sp.	Potato/Ginger	14.0	30.0	15.0
<i>Geotrichum</i> sp.	Tomato/Carrot	15.0	16.0	12.0
<i>Aspergillus</i> sp.	Carrot/ Bean	116	12.0	12.3
<i>Mucor</i> sp.	Grapes/Pumpkin/Beet	31.5	33.0	-

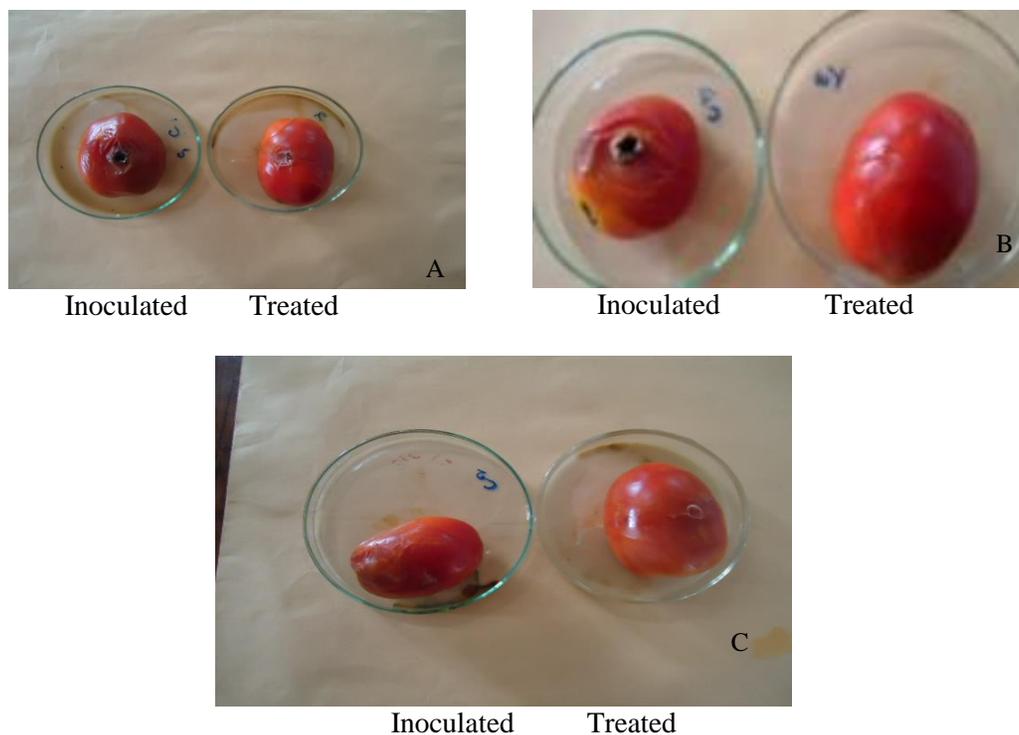
Cy= Celery oil; Ct= Carrot oil; Cv= Clove oil; - no inhibition

Values are mean of three replicates.

**Table 4.** In-vivo antifungal activity of essential oils against tomato pathogen (*Geotrichum* sp.)

Essential oil	% rots (after 3 days at 35 °C)	
	Inoculated	Treated
Celery oil	100	25
Carrot oil	100	--
Clove oil	100	25

-- no rotting



**Fig. 3.** Biocontrol potential of three essential oils against *Geotrichum* sp. on tomato. Tomato inoculated with spore suspension of *Geotrichum* sp. and treated with celery oil (A), carrot oil (B) and clove oil (C).

## Discussion

Postharvest losses are estimated to range from 10 to 30% per year despite the use of modern storage facilities and techniques (Harvey, 1978). Postharvest diseases affect a wide variety of crops particularly in developing countries which lack sophisticated postharvest storage facilities (Jeffries and Jeger, 1990). Infection by fungi and bacteria may occur during the growing season, at harvest

time, during handling, storage, transport and marketing, or even after purchase by the consumer (Dennis, 1983). The reduction of losses in perishable food crops because of postharvest diseases has become a major objective of international organizations (Kelman, 1989). The reality is that there is a portending food crisis that will require the concerted efforts of all who are involved in food production to double their efforts. To control the postharvest losses identification of pathogens causing spoilage/rots is very important.

Postharvest diseases of vegetables are caused by microscopic fungi and bacteria (Snowdon, 1992). Although it is commonly believed that vegetables are more prone to attack by bacteria due to their less acidic nature than fruits, but in many circumstances fungi are also equally responsible for rapid spoilage. Fungi produce numerous minutes' spores and these spores disseminate readily in the surrounding causing rapid spoilage to vegetables and fruits. In our present study, fungi causing postharvest rots were studied. Several fungi were isolated from diseased and infected parts. The fungi mostly isolated were *Aspergillus*, *Fusarium*, *Geotrichum*, *Rhizopus* and *Mucor*. In many instances these pathogens were described as serious postharvest pathogens causing rapid spoilage on vegetables and fruits (Northover and Zhou, 2002). Prevailing weather conditions while the crops are growing and at harvest contribute greatly to the possibility of decay. Certain cultivars are more prone to decay than are others to specific pathogens. In a recent study, it was found that resistance of major apple cultivars to the fungi that cause blue mold, graymold, bull's-eye rot, and *Mucor* rot was dependent on cultivar (Spottset *al.*, 1999).

Condition of the crop, as determined by fertilizer and soil factors, are very important in susceptibility of the crop to disease. Maturity of the crop at harvest, handling and type of storage has a great deal of influence on how long the crop can be stored without decay. Abundant inoculum and favorable conditions for infection during the season often result in heavy infection by the time the produce is harvested. This has been seen in our study where spore inoculation of pathogen could able to cause rapid spoilage in vegetables both at room and incubation temperature. Most of the rot fungi generally grow optimally at 25 to 35 °C. But temperature preference may vary among the organisms and prevailing environmental conditions. Humidity is also a major for growth and development of fungi. In our study were observed that maximum rot occur at incubation temperature (35 °C), than in room temperature. The maximum rotting at incubation temperature may due constant temperature and necessary relative humidity but at room temperature, fluctuation of temperature and humidity may be unfavourable for the fungi to cause rapid spoilage at short time. However, higher temperature may be used to control postharvest decay on crops that are injured by low temperatures such as

mango, papaya, pepper, and tomato (Spotts, 1990). In our study *Aspergillus* was found to cause several rots of vegetables. Similarly, several workers have reported *Aspergillus* species responsible for causing rots to vegetables (Kotan *et al.*, 2009). Furthermore, this pathogen is also one of the major fungi species producing aflatoxin, a group of toxic, carcinogenic compounds. Another pathogen frequently from tomato isolated was *Geotrichum*, characterized by secretion of creamy and watery fluid. The disease is commonly called sour rot of tomato. This pathogen has also been shown to cause sour rot of several vegetables and fruits (Cohen, 1989). In our present study also *Geotrichum* was found isolated from rotting carrot with similar disease symptoms to tomato.

Besides, *Rhizopus* and *Mucor* were also isolated from diseased vegetables like potato, beet and pumpkin. The disease is commonly called as *Rhizopus* rot and is reported as second most leading postharvest rots of vegetables. Fruit and vegetable diseased with *Rhizopus* quickly decompose. The skin of infected fruit readily slips. The flesh becomes brown, very soft, and soon collapses.

To control postharvest rots different parameters are taken into consideration like cold storage, chilling, sanitation and other physiological conditions. In spite of all these precaution roting occur due to microbial infection, which can overcome the storage parameters. Therefore, to ensure product safety and free from infection chemical are mostly used. Several fungicides are presently used as postharvest treatments for control of a wide spectrum of decay-causing microorganisms. However, increasing use of chemical inputs have causes several negative effects to living organisms and degrading the environment.

Moreover, the environmental pollution caused by excessive use and misuse of agrochemicals, as well as fear-mongering by some opponents to chemical fertilizers and pesticides, has led to considerable changes in people's attitudes towards the use of agrochemicals in agriculture (Gerhardson, 2002). Today, there are strict regulations on chemical use, and there is political pressure to remove the most hazardous chemicals from the market. Furthermore, the growing costs of chemical fertilizer and pesticides, particularly in developing countries of the world and consumer demand for chemicals free food has led to a search for substitute of these products. One of the most eco-friendly and effective methods to control postharvest pathogens is biological control, which is defined as use of natural occurring material to control pathogens. The naturally occurring materials range from use of microbial inoculants and other natural product such as plant extracts and essential oils. The former has been commonly used in managing postharvest diseases but there is little information on the latter in postharvest technology.

Several biological control agents have been developed in recent years, and a few have actually been registered for use on fruit crops. The first biological control agent developed for postharvest use was a strain of *Bacillus subtilis* (Pusey and Wilson, 1984). Other bacterial microorganisms that are being used against postharvest pathogens are strains of *Bacillus pumilus* and *Pseudomonas fluorescens*. Besides several yeast species are also reported and registered for control of postharvest decay on fruit and vegetables crops.

Recently there has been considerable interest in naturally derived compound in managing food borne and postharvest pathogens because of their non-toxic nature. Naturally occurring biologically active antimicrobial compounds from plant origins are generally assumed to be more acceptable and less hazardous than synthetic compounds and represent a rich source of potential disease-control agents. One of the potential plant-derived compounds used as antimicrobial are essential oils. Essential oils isolated from different aromatic plants are known to have a wide spectrum of antimicrobial activity against plant pathogens (Kalemba and Kunicka, 2003). They have been recognized for their antifungal activity and have been shown to inhibit mycelium growth of many fungal species. However, their applications for management of postharvest pathogens are less studied. In this study three essential oils were tested for their antifungal activity against some postharvest pathogens. All the oil displayed considerable antifungal activity against all the pathogens. Similarly, Tripathi *et al.* (2008), reported activity of some essential oils against postharvest *Botrytis cinerea* in management of grey mould disease.

*In-vivo* activities of the essential oils were tested against *Geotrichum* in tomato. Application of the oils in tomato prior to inoculation of the pathogen was found to reduce the rotting percentage to considerable extent. As essential oils are made up of many different volatile compounds and the make up of the oil quite often varies between species. It seems that the anti-fungal and anti-microbial effects are the result of many compounds acting synergistically which means that the individual components by themselves are may not be as effective as their mixer. Development of biodegradable and eco-friendly natural fungicides for the management of various plant pathogens has been the subject of increasing interest. Essential oils which have been registered as food additives are much easier to use for management of postharvest pathogens than new synthetic pesticides. The results of the present study suggest that naturally occurring plant-derived compounds such as essential oils could be potentially used in controlling postharvest pathogens and in postharvest technology. Different methods should also be worked out for effective control and management of postharvest pathogens.

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