

Original Research Article

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Composition of Essential Oils (EOs) and their Antifungal Activity against the *B. cinerea*

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ABSTRACT

Keywords

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Selected essential oils namely clove bud oil, lemongrass oil, grapefruit oil, lemon oil and rosemary oils studied for their composition and antifungal activity against the *B. cinerea* a significant postharvest pathogen. The compositional analysis by FT-IR revealed the several characteristic peaks corresponding to the several aromatic compounds of essential oils. Major compounds were identified for each essential oil as eugenol in of clove bud oil, citralin lemongrass oil, β -myrcene in grapefruit oil, limonene in lemon oil and camphor in rosemary oil. Further antifungal activity of essential oils was studied by poison food technique at the concentration range of 500-1500 ppm. In-vitro antifungal assay proved that clove bud oil and lemongrass oil have better fungicidal ability at 500 ppm.

Introduction

Postharvest life of perishable goods undergoes heavy losses during the transport and storage due to the various biotic stresses. High moisture content, nutrients profile and pH makes them susceptible to the attack by microorganisms. Fruits having low pH are basically attacked by fungi and also contaminated by mycotoxins (Moss, 2002). *Botrytis cinerea* has been identified as remarkable post-harvest pathogens in fresh fruits and vegetables (Zhang *et al.*, 2014). It poses big challenge during storage, shipment and marketing because of its different modes of infection and its ability to develop under different conditions prevailing.

The synthetic fungicides are basic means of managing these fungal incidences (Narayansamy, 2006). But they have recently come under scanner for posing a potential risk (Tripathi *et al.*, 2008). Use of these chemicals has contaminated the commodities by fungicidal residues which have increased the public concern and also continuous use of fungicides has increased resistance in the pathogen populations (Tripathi and Dubey, 2004). The indiscriminate and excessive use of synthetic fungicides has been a prime cause for the development of resistant fungal pathogen populations, resulting in the use of even greater quantities of antifungal compounds in agriculture and the appearance of increased levels of toxic residues in food

products (Da Cruz Cabral *et al.*, 2013). Food safety is one of the striking concerns related to fresh fruit and vegetables (Antunes and Cavaco, 2010). The search for natural alternatives to synthetic fungicides is currently in the spotlight (Combrinck *et al.*, 2011). To meet the consumer requirement for the natural and safe fresh foods, plant based extracts might be a good alternative to the use of synthetic fungicides (Gachango *et al.*, 2012).

Plant essential oils (EOs) and extracts have been used for thousands of years in food preservation, pharmaceuticals, alternative medicine and natural therapies (Prabuseenivasan *et al.*, 2006). EOs, which is naturally synthesized in different plant organs as secondary metabolites, are characterized as oily fragrant liquids extracted from aromatic plant materials (El Asbahani *et al.*, 2015). These plant essential oils could provide an alternative to synthetic chemicals to control post-harvest phytopathogenic fungi on fruit (Aminifard and Mohammadi, 2013). These essential oils contain several major and minor components which are responsible for their wide spectrum of activity. Generally, percentage of major compound influences the antifungal activity (Ipek *et al.*, 2005). Some authors have reported their beneficial effect in controlling the postharvest diseases in fruits. Keeping this in view experiment was designed to evaluate the antifungal activity of some selected essential oils on significant postharvest fungi *B. cinerea*.

Materials and Methods

Clove bud oil, lemongrass oil, grapefruit oil, lemon oil and rosemary oil were purchased from the Allin exporters from the Uttar Pradesh, India. Fungal strain was obtained from the ITCC, Division of plant pathology, Indian Agricultural Research Institute, New delhi.

FT-IR analysis of essential oils

Samples were analyzed using a Fourier transform infrared spectrophotometer (Bruker ALPHA) equipped with a deuterated triglycine sulfate (DTGS) detector and ZnSe (Zinc Selenide) crystal single reflectance ATR. For each analysis, spectra scanning frequency ranged from 4,000 to 600 cm^{-1} . 24 scans were performed for each sample with resolution of 4 cm^{-1} at room temperature. Prior to each analysis, the background measurement was performed and ATR plate was cleaned using isopropyl alcohol. Data analysis was carried out using the OPUS 7.2 software (Bruker, U.K.).

Antifungal assay of EOs

Poisoned food technique (Grover and Moore, 1962) was used to evaluate the antifungal effect against molds. The 5 essential oils at various concentrations (500-1500 ppm) incorporated into the molten potato dextrose agar (PDA) and mixed well. Then, the PDA (20 mL/plate) is poured into Petri dishes.

The mycelial disc of 5 mm diameter size was placed at the center of the plate. Plates were sealed in sterile condition with para film strip. Three replications for each treatment were tested. Plates are incubated at 35°C, the diameters of fungal growth in control and sample plates were measured every alternate day and the antifungal activity is estimated by the following formula:

$$\text{Antifungal activity (\%)} = \frac{D_c - D_s}{D_c} \times 100 \quad \dots (3.4)$$

Where D_c is the growth in control plate and D_s is the growth in the plate containing tested essential oil. The effect was compared with carbendazim, commonly used systemic fungicide.

Results and Discussion

Fourier transform infrared spectroscopy (FT-IR) of essential oils

FTIR investigation of essential oil was done. The interpretation and identification spectrum was done by identifying intensity (High or low), shape (flat or sharp) and position (wavenumber) of source on the spectra (Hemalatha *et al.*, 2016). FTIR spectra of clove bud oil showed several peaks corresponding to volatile compounds (Fig. 1) and characterized by peaks at 3522 cm^{-1} because of O-H stretching, 2938 cm^{-1} due to C-H stretching, at 1510 cm^{-1} 1605 cm^{-1} , 1451 cm^{-1} because of stretching vibration of aromatic compound C=C, 1265 cm^{-1} C-O stretching vibration in phenolic hydroxyl and 1231 cm^{-1} is due to C-O bending were observed (Mohammed and Bayati 2009, Hasheminejad *et al.*, 2019). Peaks at 1638 cm^{-1} indicates C-H stretching vibration of benzene (Gao *et al.*, 2017). Peaks from 1100-1210 cm^{-1} range indicates asymmetric stretch of C-O-C linkage of ether function group (Stuart 2004; Rodriguez *et al.*, 2018).

FT-IR spectra of lemongrass is illustrated in the Fig. 2 in which peaks observed at 2980 cm^{-1} are of C-H stretching of alkanes (Munhuweyi *et al.*, 2017), sharp peaks at 2929 and 2856 cm^{-1} symmetric and asymmetric stretching of CH_2 were observed. At 1274 cm^{-1} bending of CH_3 group was found. Broad range from 1173 to 1016 cm^{-1} , stretching of C-O and vibrations of the CH were reported. The intense band observed at 1721 cm^{-1} corresponds to citral a common acyclic monoterpene is due to vibration of C=C (cis and trans isomers of citral), confirming the presence of conjugated double bonds (C=C-CHO) (Jamuna *et al.*, 2017). The peak at 1633 cm^{-1} indicated the stretching of C=O of aldehyde group (Natarajan *et al.*, 2015). Peaks at 1445 cm^{-1} methylene CH

bend, at 1366 cm^{-1} methyl C-H symmetrical bend was observed (Vazquez-Briones *et al.*, 2015). The peaks indicated the presence of cycloalkanes at 2500-3000 cm^{-1} , terpenoids, flavonoids, carbonyl compounds and ketones at 1500-2000 cm^{-1} and methyl group, sulfonic acid and ester at 400-1500 cm^{-1} (Wany *et al.*, 2014).

Rosemary oil spectrum was depicted in the Fig. 3 and found that peak observed at 3481.76 cm^{-1} are associated with C=C stretch of different alkenes, at 2937.68 cm^{-1} and 2880 cm^{-1} strongest peaks are due to stretching of methyl groups, 1732.79 cm^{-1} which corresponding to stretching vibration of carbonyl group C=O associated with camphor are observed (Fernade *et al.*, 2013). Peak at 1371.52 cm^{-1} indicates C-O bonding, at 1223 cm^{-1} asymmetric stretching of C-O-C and at 979 cm^{-1} wagging are associated with ether function from the epoxy ring of 1,8 cineole (Fernandes *et al.*, 2013). Observed peaks at 881.80 cm^{-1} and 845.99 cm^{-1} are due to C-H deformation (Rivera *et al.*, 2016).

FT-IR spectrum of grape fruit oil (Fig. 4) showed peaks at 1580 cm^{-1} indicating that a monoterpene with nonaromatic conjugated C=C double bond such as β -myrcene. At 1732 cm^{-1} indicates C-O mode of carboxyl group resonance with non-aromatic chain indicates the presence of different oxygen terpene such as citral. Peaks at 1271-1173 cm^{-1} antisymmetric and symmetric C-O-C stretching modes, indicates presence of esters such as linalyl acetate, observed peak at 2983 cm^{-1} corresponds to stretching mode of asymmetric CH_3 groups, at 1118 cm^{-1} shows presence of least one constituent with aromatic ring and methoxy substitute. Peaks at 1446.91 cm^{-1} and 1391 cm^{-1} shows the asymmetric and symmetric bending of CH_3 groups respectively, at 1366 cm^{-1} bending of CH_2 groups of β -myrcene were identified, at 1118-1014 cm^{-1} vibration of C-C and OH

groups, at 887 cm^{-1} out of plane bending of vinylidene group, was observed which is indicator of presence of limonene (Schulz *et al.*, 2002; Manaila *et al.*, 2016).

FT-IR analysis of lemon oil (Fig. 5) showed peaks at 2918.06 cm^{-1} stretching vibration of volatile terpenoids, at 1736.74 cm^{-1} C=O stretching vibration of aldehyde and ester carbonyl group (key aroma compounds octanol, citral), peaks at $1681.12\text{--}1643.81\text{ cm}^{-1}$

shows vibration of C=C occurring in the limonene molecules (Schulz *et al.*, 2002; Elzey *et al.*, 2016). Peaks at 1443.02 cm^{-1} and 1375.07 cm^{-1} indicates the deformation modes of CH_3 . At 1236.43 cm^{-1} , $1152.74\text{--}1019.24\text{ cm}^{-1}$ vibrational of C-O-C mode shows the presence of linalyl acetate (Fidalgo *et al.*, 2016). At 885.37 cm^{-1} cyclohexane vibration of α -Pinene, 792 cm^{-1} tri substituted double bond out of plane bending was observed from the spectrum.

Fig.1 FTIR spectrum of clove bud oil

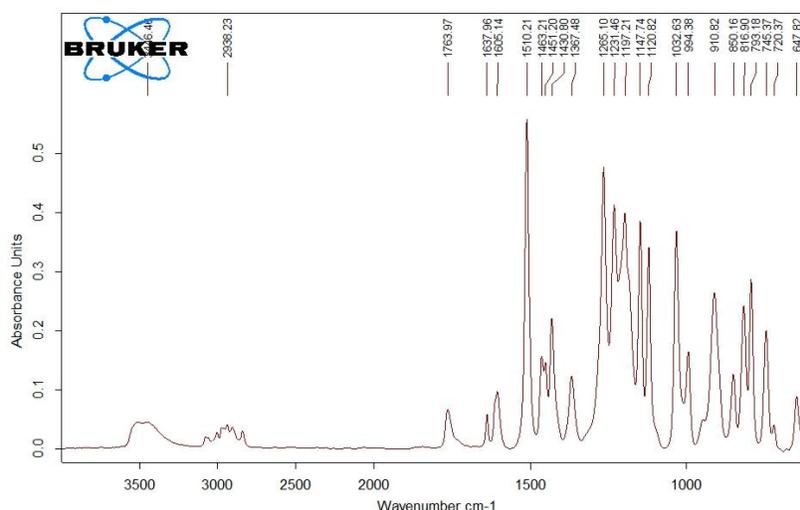


Fig.2 FT-IR spectrum of lemon grass oil

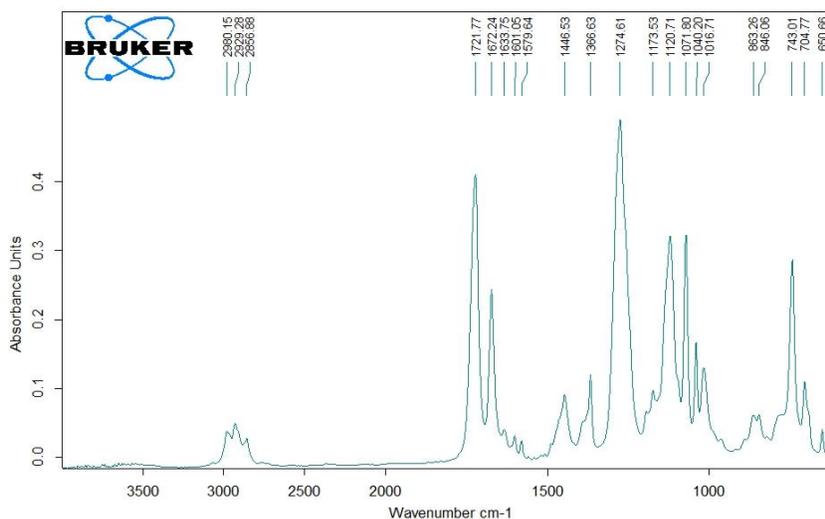


Fig.3 FT-IR spectrum of rosemary oil

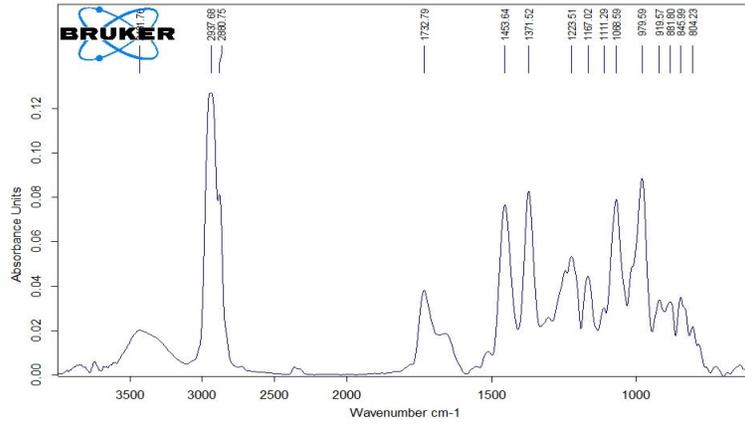


Fig.4 FT-IR spectrum of grape fruit oil

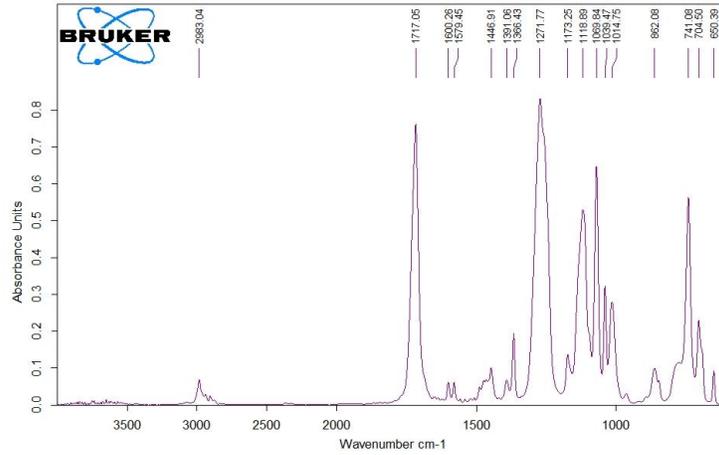


Fig.5 FT-IR spectrum of lemon oil

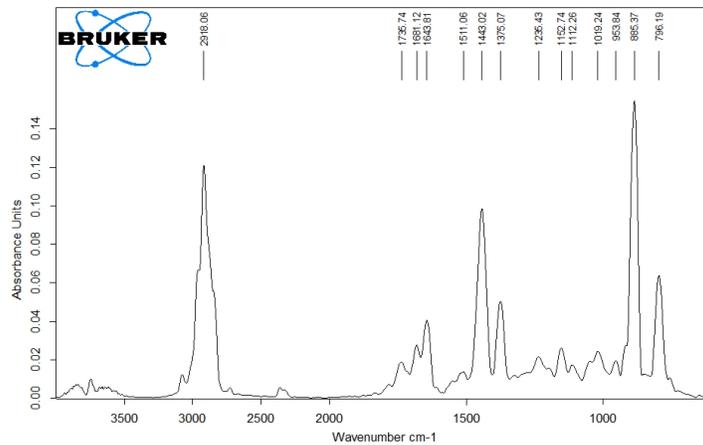
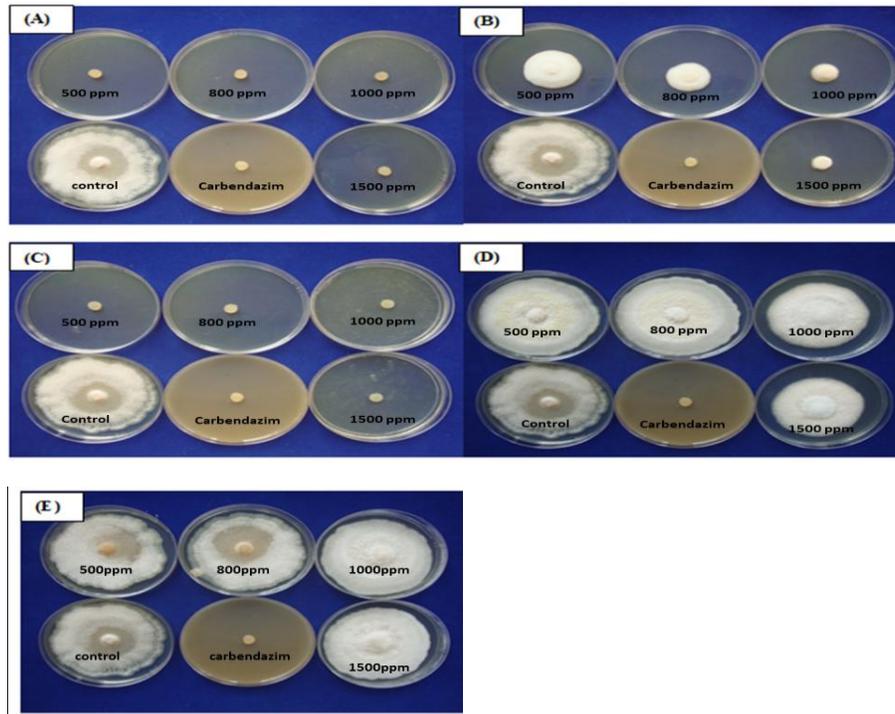


Fig.6 *In-vitro* antifungal activity of A) clove bud oil B) grape fruit oil C) lemongrass oil D) lemon oil and E) rosemary oil



Antifungal activity of the essential oils

Antifungal activity of the essential was studied by poison food technique. Effects of the essential oils on the radial mycelial growth are presented in the figure 6. Essential oils inhibited the radial mycelial growth on concentration dependent manner. During seven days of incubation period the radial mycelial growth was found rapid in case of rosemary oil and lemon oil. Grape fruit oil restricts the growth of mycelia based on the concentration used. Clove bud oil and lemongrass oil proved to be fungicidal at concentration of 500 ppm. In case of the rosemary oil the end of incubation period mycelial growth was 8.8-9 cm and for lemon oil 6.9-9cm at different concentrations. Grape fruit oil showed significant ($p < 0.05$) effect in controlling the radial mycelial growth of *B. cinerea*. This difference in the inhibitory activity of the essential oils is due to the

chemical structure of the individual components of EOs which in turn affect the particular mode of action and antifungal activity (Dorman and Deans, 2000). Antimicrobial property of the essential oils is also attributed to the phenolic nature of the essential oils (Bajpai *et al.*, 2012). In general the essential oil which exhibit high antimicrobial property contain the high percentage of the phenolic compounds (Lambert *et al.*, 2001; Burt, 2004).

Their mechanism of action appears to be predominantly on the fungal cell membrane, disrupting its structure causing leakage and cell death; blocking the membrane synthesis; inhibition of the spore germination, fungal proliferation and cellular respiration (Harris, 2002). The effective antifungal activity of the clove oil is attributed to its major components eugenol (Rana *et al.*, 2011). Cox and Markham (2007) reported that eugenol may

inactivate essential enzymes, react with the cell membrane or disturb the genetic material functionality thus resulting in death of microorganism. Antimicrobial activity of this oil can also be attributed to the presence of an aromatic nucleus and a phenolic OH group that are known to be reactive and can form hydrogen bonds with –SH groups in the active sites of target enzymes, resulting in the deactivation of enzymes in fungi (Vellutiet *al.*, 2003). The efficient antifungal activity presented by lemongrass oil is significantly associated with the citral. In our study, the antifungal property of LGEO could be due to two major mono-terpene aldehydes (geranial and neral). The literatures suggest that the antifungal action of lemongrass oils could be due to the existence of high amounts of oxygenated monoterpenes (Boukhatemet *al.*, 2014). Silva *et al.*, (2008) and Kuritaet *al.*, (1981) pointed out that the action of citral may be due to the ability of the compound to form a charge transfer complex with fungal cells electron donors, resulting in death of the fungus.

In conclusion the essential oils extracted from the natural plant sources have proved to meet the consumer preference for the safe and healthy fruits and vegetables. In the present study EOs displayed the potential to arrest the growth of the *B.cinerea*, a remarkable postharvest pathogen. Among the selected EOs the clove bud oil and lemongrass oil exhibited better fungicidal effect and grape fruit oil showed fungistatic effect on concentration dependent manner.

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