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Vikram Appanna
College of Horticulture, Yalachahalli, Yelwala, Mysuru, Karnataka, India

Rashmi HB
College of Horticulture, Yalachahalli, Yelwala, Mysuru, Karnataka, India

Mamathalakshmi N
College of Horticulture, Yalachahalli, Yelwala, Mysuru, Karnataka, India

Sudarshan GK
College of Horticulture, Yalachahalli, Yelwala, Mysuru, Karnataka, India

Harish BS
College of Horticulture, UHSB Campus, GKVK, Bengaluru, Karnataka, India

Corresponding Author:
Vikram Appanna
College of Horticulture, Yalachahalli, Yelwala, Mysuru, Karnataka, India

Standardization and efficacy of ginger oil, turmeric leaf extract oil and eucalyptus oil in controlling the growth of spoilage microorganisms

Vikram Appanna, Rashmi HB, Mamathalakshmi N, Sudarshan GK and Harish BS

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Abstract
The aromatic plants namely ginger, turmeric and eucalyptus find widespread use in culinary applications for flavour, aroma, color, medicinal properties and industrial applications. Antimicrobials from Ginger, turmeric and eucalyptus plants have been widely used for controlling the growth of food spoilage microorganisms. However, the efficacy of the extracts and oils from these aromatic plants in controlling the growth of fruit and vegetable spoilage microorganisms has not been studied much. An attempt was made in this trial to study the efficacy of different concentrations of Ginger oil, Eucalyptus oil and Turmeric leaf extract oil in controlling the growth of fruit and vegetable spoilage microorganisms under *in vitro* conditions. Ginger oil, Eucalyptus oil and Turmeric leaf extract oil completely inhibited the growth of *Aspergillus* at all the tested concentrations (100, 50, 25, 12.5 and 6.25%) at both 7 and 14 days after incubation (DAI). Efficacy of different concentrations of Ginger oil and Turmeric leaf extract oil had a variable effect on the control of *Penicillium*. Eucalyptus oil completely inhibited the growth of *Penicillium* at all the tested concentrations at both 7 and 14 DAI. Efficacy of Ginger oil and Turmeric leaf extract oil reduced with the decrease in their concentrations. The current study has shown that different concentrations of Ginger oil, Eucalyptus oil and Turmeric leaf extract oil have been effective in controlling the growth of *Penicillium* and *Aspergillus* under *in vitro* conditions. The effective concentrations of these products should be checked for their efficacy in controlling the growth of *Penicillium* and *Aspergillus* by carrying out shelf life studies.

Keywords: Eucalyptus oil, antimicrobial, antifungal, inhibition

Introduction

The medicinal value and medicinal properties of Ginger oil, Eucalyptus oil and Turmeric leaf extract oil extract oil is very well known. Ginger (*Zingiber officinale*) is a medicinal plant that has been used for a wide array of ailments including arthritis, cramps, rheumatism, sprains, sore throats, muscular aches, pains, constipation, vomiting, hypertension, indigestion, dementia, fever and infectious diseases (Halvorsen *et al*., 2002; Ali *et al*., 2008; Panpatil *et al*., 2013) [13, 2, 18]. Ginger extracts have been earlier shown to possess antibacterial, anticonvulsant, analgesic, antiulcer, gastric antisecretory, antitumor, antifungal, antispasmodic, antithrombotic, hypocholesterolemic, antiallergic, antiserotonergic, anticholinergic and other beneficial activities (Tchombé *et al*., 2012; Panpatil *et al*., 2013) [24, 18]. Similarly, Turmeric (*Curcuma longa*) and turmeric oil have antimicrobial, antifungal, antioxidant, anti-inflammatory, anti-mutagenic, hepatoprotective, neuroprotective, anticancerous, and antidiabetic property (Dosoky and Setzer, 2018) [10]. Eucalyptus extracts have been shown earlier to possess antibacterial, antifungal, anti hyperglycaemic and antioxidant properties (Takahashi *et al*., 2004) [23]. The efficacy of ginger oil, eucalyptus oil and turmeric leaf extract oil in controlling the growth of food spoilage microorganisms has been done earlier. However, the efficacy of these oils in controlling the growth of fruit and vegetable spoilage microorganisms has not been studied much. The current trial was initiated with the objective to study efficacy of different concentrations of Ginger oil, Eucalyptus oil and Turmeric leaf extract oil in controlling the growth of fruit and vegetable spoilage microorganisms.

Materials and Methods

The microbial cultures used in this study were obtained from the Department of Agricultural Microbiology, College of Horticulture, Yalachahalli, Mysuru. Fruit and vegetable spoilage organisms like *Aspergillus* and *Penicillium* were used in this experiment. The current experiment was carried out at College of Horticulture, Yalachahalli, Mysuru. Ginger oil, Eucalyptus oil and Turmeric leaf extract oil used in this study were provided by Dr. Harish, Department of Plantation, Spices, Medicinal and Aromatic crops, College of Horticulture, GKVK, Bangalore.

Petri plates (90 mm) containing 20 ml of PDA were used to grow the fungi. In each agar plate, two 10 mm-diameter wells were made at equidistance from the center (30 mm) using a sterile cork-borer. 100 µL of the different concentrations of extracts were pipetted into each well. Control plates received 200 µL of sterile distilled water. Agar plugs of 5 mm diameter obtained from the edges of actively growing fungal cultures were inverted onto the centre of plates containing different concentrations of extracts. Plates were then sealed with Parafilm and incubated at room temperature. Radial mycelial growth measurements were recorded from treated and control plates at 7 and 14 days after incubation (DAI). Linear mycelial growth has been expressed as colony diameter in mm and the percent inhibitions were calculated using the following formula

Inhibition (%) = [(growth of the pathogen in untreated control plates-growth of the pathogen in treated plates)/growth of the pathogen in untreated control plates] ×100

The suitable concentration of Ginger oil, Turmeric leaf extract oil and eucalyptus oil in controlling the growth of both *Aspergillus* and *Penicillium* was assessed. Five different concentrations like 100, 50, 25, 12.5 and 6.25% of Ginger oil, Turmeric leaf extract oil and eucalyptus oil were tested in this trial. The experiment contained 16 treatments which was replicated twice.

The experiment contained 16 treatments which was replicated twice.

Treatment Number	Treatment details	Quantity used
T1	Control-Distilled Water	200 µl
T2	Ginger Oil (100%)	200 µl
T3	Ginger Oil (50%)	200 µl
T4	Ginger Oil (25%)	200 µl
T5	Ginger Oil (12.5%)	200 µl
T6	Ginger Oil (6.5%)	200 µl
T7	Eucalyptus Oil (100%)	200 µl
T8	Eucalyptus Oil (50%)	200 µl
T9	Eucalyptus Oil (25%)	200 µl
T10	Eucalyptus Oil (12.5%)	200 µl
T11	Eucalyptus Oil (6.25%)	200 µl
T12	Turmeric Leaf Extract Oil (100%)	200 µl
T13	Turmeric Leaf Extract Oil (50%)	200 µl
T14	Turmeric Leaf Extract Oil (25%)	200 µl
T15	Turmeric Leaf Extract Oil (12.5%)	200 µl
T16	Turmeric Leaf Extract Oil (6.25%)	200 µl

Results and Discussion

Among the treatments tested, all fifteen treatments completely inhibited the growth of *Aspergillus* at all the tested concentrations (100, 50, 25, 12.5 and 6.25%) at 7 days after treatment application (DATA). Similarly, ginger oil, Eucalyptus oil and Turmeric leaf extract oil completely inhibited the growth of *Aspergillus* at all the tested concentrations (100, 50, 25, 12.5 and 6.25%) at 14 DATA (Table 1). The antifungal and antibacterial activity of turmeric oil was tested by Gul and Bakht (2015) [12]. They found that turmeric oil was effective in controlling the growth of against *Candida albicans*, *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* under *in vitro* conditions. Similarly, Kapoor (1997) [15]; and Arora and Kaur (1999) [3] demonstrated the antifungal activity of turmeric oil against other molds like as *Aspergillus niger*, *Penicillium digitatum Aspergillus flavus*, *Penicillium javanium*, *Curvularia oryzae* and *Trichophyton mentagrophytes*. Methanol, chloroform and n-hexane extracts from turmeric showed distinct antimicrobial activity against *Candida albicans* (Mohammad *et al*., 2010; Gul and Bakht, 2015) [17, 12]. In another study petroleum ether, hexane, chloroform, acetone and ethanol extracts of *Curcuma zedoaria* and *Curcuma malabarica* exhibited antibacterial as well as antifungal activity against different bacterial and fungal pathogens (Wilson *et al*., 2005) [27]. Similarly, essential oil extracted from Ginger exhibited activity against food-borne pathogenic fungal and bacterial species (Singh *et al*., 2008) [22]. The reason for inhibition of the fungal pathogens can be attributed to due to cytoplasm granulation, cytoplasmic membrane rupture and inactivation and/or inhibition of intracellular and extracellular enzymes alone or in combination (Cowan, 1999) [7]. It is also reported that plant lytic enzyme act in the fungal cell wall causing breakage of β-1, 3 glycan, β-1, 6, glycan and chitin polymer (Brull and Coote, 1999) [6].

The efficacy of different concentrations of Ginger oil, Eucalyptus oil and Turmeric leaf extract oil had a variable effect on the control of *Penicillium*. Eucalyptus oil completely inhibited the growth of *Penicillium* at all the tested concentrations (100, 50, 25, 12.5 and 6.25%) at both 7 and 14 DAI (Table 2). *Eucalyptus globulus* essential oil could be used as a possible antifungal and antibacterial agent against foodborne and food spoilage microorganisms like *Candida albicans*, *C. parapsilosis*, *Staphylococcus aureus* and *Enterobacter sakazakii* (Boukhatem *et al*., 2020) [5]. The chemical composition of the different compounds characterizing the *Eucalyptus globulus* essential oil showed the predominance of a mixture of monoterpenes and oxygenated monoterpenes responsible for the microbial inhibitory effect against pathogens (Damjanović-Vratnica *et al*., 2011; Boukhatem *et al*., 2020) [8, 5].

The inhibitory effect of both Ginger oil and Turmeric leaf extract oil on *Penicillium* was highest at 100% concentration at both 7 and 14 DAI. The efficacy of both these oils reduced with the decrease in their concentrations (Table 2). The highest amount of percent inhibition of *Penicillium* at 7 DATA among various concentrations of Ginger oil was recorded by 100% ginger oil which was 79.5% while the lowest percent inhibition of *Penicillium* at 7 DATA among various concentrations of Ginger oil was recorded by 6.5% ginger oil. Similarly, the highest amount of percent inhibition of *Penicillium* at 14 DATA among various concentrations of Ginger oil was recorded by 100% turmeric leaf extract oil which was 73.9% while the lowest percent inhibition of *Penicillium* at 14 DATA among various concentrations of turmeric leaf extract oil was recorded by 6.5% turmeric leaf extract oil. The antifungal activity of turmeric oil and turmeric leaf extract oil have been tested *in vitro* earlier against various pathogens including *Aspergillus flavus*, *Fusarium oxysporum* and *Fusarium moniliforme* by several workers (Singh *et al*., 2002; Dhingra *et al*., 2007; Ferreira *et al*., 2013; Azhari *et al*., 2018) [20, 9, 11, 4]. They found out that the activity of these pathogens were severly inhibited in the presence of turmeric oil and turmeric leaf extract oil. They indicated that the active volatile compounds from turmeric called turmerones were responsible for the inhibitory capability of the essential oil on growth and morphology of the tested pathogens. Similar kind of results were also obtained by Vilela *et al*., 2009 [26] who compared the antifungal activity of the essential oil from *Eucalyptus globulus* with its major component (1.8-cineol) on the pathogens *A. flavus* and *A. parasiticus*.

The highest amount of percent inhibition of *Penicillium* at 7 DATA among various concentrations of turmeric leaf extract oil was recorded by 100% ginger oil which was 74.4% while the lowest percent inhibition of *Penicillium* at 7 DATA among various concentrations of turmeric leaf extract oil was recorded by 6.5% ginger oil. Similarly, the highest amount of percent inhibition of *Penicillium* at 14 DATA among various concentrations of turmeric leaf extract oil was recorded by 100% turmeric leaf extract oil which was 73.9% while the lowest percent inhibition of *Penicillium* at 14 DATA among various concentrations of turmeric leaf extract oil was recorded by 6.5% turmeric leaf extract oil. The efficacy in control of these microbial pathogens might have been due to various mechanisms in these aromatic plants namely ginger, turmeric and eucalyptus.

The efficacy in controlling *Aspergillus flavus* and other pathogens by turmeric leaf extract oil has been attributed to the presence of α-phellandrene, terpinolene, and p-cymene (Sindhu *et al*., 2011) [19]. The compounds namely α-phellandrene, 2-carene, o-cymene, camphor, 2-bornanone, curdione, eucalyptol, curzerenone, α-lemenone, longiverbenone, boldenone, and α-curcumene present in turmeric leaf extracts resulted in effective inhibition and control of various pathogenic microorganisms (Albaqami *et al*., 2022) [1]. Similarly, Jayaprakasha *et al*., 2001 [14] reported that the effective control of *Aspergillus flavus*, *Aspergillus parasiticus*, *Fusarium moniliforme* and *Penicillium digitatum* was achieved due to the presence of aromatic tumerone, tumerone, curlone and other oxygenated compounds in fractional distillate of turmeric oil.

In ginger too effective control of these microbial pathogens were achieved by the presence of monoterpenes and sesquiterpenes which alter the permeability and fluidity of the plasma membrane of microorganisms. Ginger also contains phenolic compounds like eugenol, shogaols, zingerone, gingerdiols and gingerols which have also been shown to be bringing about the inhibitory effect on the pathogens (Singh *et al*., 2005) [21]. It has been demonstrated earlier that the antifungal activity of essential oils from aromatic plants on *A. fumigatus* and *Trichophyton rubrum* has resulted in damage of cell wall and cytoplasmic contents (Khan and Ahmad, 2011) [16]. The lipophilic properties of essential oils will also allow them to penetrate the plasma membrane by causing polysaccharide accumulation under drought stress conditions and ultimately leading to plasma lemma breakage in fungal cells (Ultee *et al*., 2002) [25].

Table 1: Efficacy of different concentrations of Ginger oil, Eucalyptus oil and Turmeric leaf extract oil in controlling the growth of *Aspergillus* under *in vitro* conditions

Treatment Number	Treatment details	Observations
T1	Control-Distilled Water	Full growth of *Aspergillus* at 7 DAI and 14 DAI
T2	Ginger Oil (100%)	No growth of *Aspergillus* at 7 DAI and 14 DAI
T3	Ginger Oil (50%)	No growth of *Aspergillus* at 7 DAI and 14 DAI
T4	Ginger Oil (25%)	No growth of *Aspergillus* at 7 DAI and 14 DAI
T5	Ginger Oil (12.5%)	No growth of *Aspergillus* at 7 DAI and 14 DAI
T6	Ginger Oil (6.5%)	No growth of *Aspergillus* at 7 DAI and 14 DAI
T7	Eucalyptus Oil (100%)	No growth of *Aspergillus* at 7 DAI and 14 DAI
T8	Eucalyptus Oil (50%)	No growth of *Aspergillus* at 7 DAI and 14 DAI
T9	Eucalyptus Oil (25%)	No growth of *Aspergillus* at 7 DAI and 14 DAI
T10	Eucalyptus Oil (12.5%)	No growth of *Aspergillus* at 7 DAI and 14 DAI
T11	Eucalyptus Oil (6.25%)	No growth of *Aspergillus* at 7 DAI and 14 DAI
T12	Turmeric Leaf Extract Oil (100%)	No growth of *Aspergillus* at 7 DAI and 14 DAI
T13	Turmeric Leaf Extract Oil (50%)	No growth of *Aspergillus* at 7 DAI and 14 DAI
T14	Turmeric Leaf Extract Oil (25%)	No growth of *Aspergillus* at 7 DAI and 14 DAI
T15	Turmeric Leaf Extract Oil (12.5%)	No growth of *Aspergillus* at 7 DAI and 14 DAI
T16	Turmeric Leaf Extract Oil (6.25%)	No growth of *Aspergillus* at 7 DAI and 14 DAI

DAI: Days after incubation

Table 2: Efficacy of different concentrations of Ginger oil, Eucalyptus oil and Turmeric leaf extract oil in controlling the growth of *Penicillium* under *in vitro* conditions

Treatment Number	Treatment details	% Inhibition (7 DAI)	% Inhibition (14 DAI)
T1	Control-DW	-	-
T2	Ginger Oil (100%)	79.5	73.9
T3	Ginger Oil (50%)	37.5	29.5
T4	Ginger Oil (25%)	37.5	32.9
T5	Ginger Oil (12.5%)	43.2	27.3
T6	Ginger Oil (6.5%)	30.1	0.0
T7	Eucalyptus Oil (100%)	100.0	100.0
T8	Eucalyptus Oil (50%)	100.0	100.0
T9	Eucalyptus Oil (25%)	100.0	100.0
T10	Eucalyptus Oil (12.5%)	100.0	100.0
T11	Eucalyptus Oil (6.25%)	100.0	100.0
T12	Turmeric Leaf Extract Oil (100%)	74.4	73.9
T13	Turmeric Leaf Extract Oil (50%)	56.8	37.5
T14	Turmeric Leaf Extract Oil (25%)	60.2	37.5
T15	Turmeric Leaf Extract Oil (12.5%)	35.8	30.1
T16	Turmeric Leaf Extract Oil (6.25%)	31.3	28.4

DAI: Days after incubation

Conclusions

The current study has shown that by use of lower concentration of Eucalyptus oil efficient control of both *Aspergillus* and *Penicillium* were obtained under *in vitro* conditions. Similarly, effective control of these two pathogens were also recorded in the presence of various concentrations of ginger oil and turmeric leaf extract oil under *in vitro* conditions. Hence, an attempt can be made to test the efficacy of these three biologicals namely eucalyptus oil, ginger oil and turmeric leaf extract oil to control the growth of *Aspergillus* and *Penicillium* by carrying out shelf life studies on selected fruit and vegetable crops.

Conflicts of Interest

The authors declare that they have no conflict of interest

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