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Essential Oils as Alternatives to Synthetic Fungicides for Managing *Alternaria* Fruit Rot in Pomegranate (*Punica granatum* L.)

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A B S T R A C T

Pomegranate (*Punica granatum* L.), an economically and nutritionally valuable fruit crop, faces significant post-harvest losses due to fungal pathogens, notably *Alternaria* spp., which cause black spot and fruit rot diseases. With growing concerns over fungicide resistance and residue toxicity, eco-friendly alternatives such as essential oils are being explored for plant disease management. This study aimed to evaluate the in vitro antifungal efficacy of seven essential oils rose, lemon, cardamom, citronella, clove, ginger, and eucalyptus against *Alternaria alternata* and *A. burnsii* isolated from diseased pomegranate fruits. Using the Poisoned Food Technique, essential oils were tested at a concentration of 1500 ppm on potato dextrose agar (PDA). Results revealed that rose oil exhibited the highest antifungal activity, inhibiting mycelial growth by 91.47% and 94.44% against *A. alternata* and *A. burnsii*, respectively. Lemon oil also demonstrated strong efficacy, particularly against *A. burnsii*, with 94.44% inhibition. In contrast, eucalyptus oil showed minimal antifungal activity against both species. The observed differences in sensitivity between *Alternaria* species suggest a species-specific response to the bioactive compounds in essential oils. These findings underscore the potential of rose and lemon essential oils as promising botanical fungicides and support their further evaluation in integrated and sustainable disease management strategies for pomegranate cultivation.

Introduction

Pomegranate (*Punica granatum* L.) is a fruit of growing global importance due to its high nutritional value, therapeutic properties, and economic potential. It is cultivated extensively in tropical and subtropical regions, especially in countries such as India, Iran, Afghanistan, Turkey, and various Mediterranean nations. The fruit is valued for its rich antioxidant profile, particularly in polyphenols, anthocyanins and tannins which contribute to its medicinal uses in cardiovascular health, diabetes management, and cancer prevention (Holland *et al.,* 2009; Jurenka, 2008). Owing to its wide application in the food, pharmaceutical, and cosmetic industries, the commercial cultivation of pomegranate has expanded significantly in recent decades. However, this increased cultivation has also made the crop more susceptible to various biotic stresses, among which fungal diseases pose a major threat. One of the most important and destructive pathogens affecting pomegranate is *Alternaria* spp. a necrotrophic fungus known to cause fruit rot, and black spot diseases.

The symptoms typically include small, dark, sunken lesions on leaves and fruits that enlarge over time, leading to premature leaf fall and fruit decay. Infected fruits often become unmarketable, causing substantial economic losses both pre- and post-harvest (Khanzada *et al.,* 2005; Mirzaei-Najafgholi *et al.,* 2017). The disease is favored by warm and humid conditions, under which the pathogen can rapidly spread and infect large portions of the crop.

To manage *A. alternata*, conventional practices rely heavily on chemical fungicides. Although fungicides provide effective control, their repeated and unregulated use has led to several serious concerns. These include the development of fungicide-resistant fungal strains, disruption of beneficial microbial populations, accumulation of toxic residues on fruits, environmental contamination, and potential health hazards to consumers (Brent and Hollomon, 2007; Singh and Dwivedi, 2010).

Furthermore, the increasing consumer preference for chemical-free, organic produce has intensified the need for safer, sustainable alternatives to synthetic agrochemicals.

In this context, natural plant-derived compounds such as essential oils have emerged as promising bio-rational alternatives in plant disease management. Essential oils are volatile, aromatic secondary metabolites extracted from various parts of medicinal and aromatic plants. These oils are rich in biologically active compounds like terpenes, phenolics, aldehydes, and ketones that have been shown to possess potent antimicrobial, antifungal, and antioxidant properties (Bakkali *et al.,* 2008; Cowan, 1999). Several studies have reported the efficacy of essential oils from plants such as *Thymus vulgaris*, *Ocimum sanctum*, *Cymbopogon citratus*, and *Eucalyptus globulus* against a broad spectrum of phytopathogenic fungi, including *Fusarium spp.*, *Aspergillus niger*, *Rhizoctonia solani*, and *Botrytis cinerea* (Daferera *et al.,* 2003; Nene and Thapliyal, 1993; Prakash *et al.,* 2012).

Despite a growing body of evidence supporting the antifungal activity of essential oils, limited research has been directed towards their specific application against *Alternaria alternata* in pomegranate. Most available studies are either preliminary or focused on other host-pathogen systems. There is, therefore, a significant knowledge gap regarding the effectiveness of essential oils in controlling *A. alternata* under controlled conditions in pomegranate cultivation.

Given this background, the present study was undertaken to evaluate the in vitro antifungal efficacy of selected essential oils against *Alternaria* spp. isolated from diseased pomegranate tissues.

The Poisoned Food Technique was employed to assess the inhibitory effects of different oils on fungal mycelia growth. This investigation aims to identify potential plant-based fungicidal agents that can serve as eco-friendly alternatives to synthetic chemicals. The findings of this study may contribute to the development of integrated disease management strategies that are both environmentally safe and economically viable for pomegranate growers.

Materials and Methods

The antifungal efficacy of selected essential oils against *Alternaria* spp. was evaluated *in vitro* using the Poisoned Food Technique (Nene and Thapliyal, 1993) with potato dextrose agar (PDA) as the basal medium. Prior to the experiment, the test pathogens were cultured on PDA medium in Petri plates and incubated at 28 ± 2°C for 15 days to obtain active cultures.

To prepare the poisoned medium, essential oils were first emulsified using the surfactant Tween-80, and the desired concentrations of each oil were incorporated into molten PDA (cooled to approximately 45–50°C). Each Petri plate (90 mm in diameter) was poured with 20 ml of the amended PDA medium and allowed to solidify under aseptic conditions. Control plates without any essential oil served as untreated checks. A 5 mm mycelial disc from the actively growing margin of the fungal culture was cut using a sterile cork borer and centrally placed on each treated and control plate. Each treatment, including the control, was replicated three times. The plates were incubated in an inverted position at 28 ± 2°C. Radial mycelial growth of the fungal colonies was measured at 24-hour intervals. Observations continued until the fungal growth in the control plates completely covered the Petri dish. The percentage inhibition of mycelial growth was calculated using the formula (Vincent, 1947),

$$\textbf{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Where,

C= Mycelial growth in control plate,

T= Mycelial growth in treated plate

Results and Discussion

Seven different essential oils were evaluated at a concentration of 1500 ppm for their in vitro antifungal efficacy against two species of *Alternaria Alternaria alternata* and *Alternaria burnsii* using the poisoned food technique. The results revealed that, except for eucalyptus oil, all tested essential oils exhibited significant antifungal activity against both fungal species compared to the untreated control.

Efficacy Against *Alternaria alternata*

The radial mycelial growth of *A. alternata* was significantly suppressed by rose oil, which was found to be the most effective treatment, showing a mean colony diameter of just 7.66 mm compared to 90.00 mm in the control. This corresponded to the highest percentage inhibition of 91.47%. Cardamom oil followed with a colony diameter of 15.66 mm and 82.59% inhibition.

Other oils showed moderate to low antifungal activity in the following order of efficacy: lemon oil (52.66 mm, 41.47%), citronella oil (77.66 mm, 13.70%), clove oil (78.00 mm, 13.33%), and ginger oil (83.33 mm, 7.40%). Eucalyptus oil exhibited the least antifungal activity with 88.00 mm radial growth and only 2.22% inhibition, statistically similar to the control.

Efficacy Against *Alternaria burnsii*

Similar trends were observed in the case of *A. burnsii*. Rose oil and lemon oil were found to be equally effective, each limiting fungal growth to 5.00 mm, with a corresponding inhibition of 94.44%. Cardamom oil followed with 29.00 mm colony growth and 67.77% inhibition. Citronella, clove, and ginger oils showed moderate activity with growth diameters of 62.33 mm, 62.66 mm, and 63.33 mm, respectively. Their percentage inhibitions were 30.73%, 30.36%, and 29.62%, respectively. Eucalyptus oil again recorded the least inhibition (8.14%), with colony growth measuring 82.66 mm.

Comparative Analysis Between *A. alternata* and *A. burnsii*

The study revealed that while rose oil was most effective against *A. alternata*, both rose and lemon oils were equally effective in completely arresting the growth of *A. burnsii*. Eucalyptus oil consistently showed poor performance against both species, indicating limited or no fungitoxic properties at the tested concentration.

The variations in sensitivity between the two fungal species could be attributed to differences in their physiology, cell wall composition, or metabolic activity, which may influence their response to the bioactive constituents of the essential oils.

Correlation with Previous Studies

These findings are in agreement with earlier research. For instance, Gadhi *et al.,* (2020) reported significant antifungal activity of several essential oils including sesame, coconut, and clove oils against *A. alternata* at various concentrations. Sesame oil showed the highest inhibition (80%), followed by coconut (77.04%), henna (72.59%), and mint (66.07%). Clove oil, tested in both the current and previous studies, showed moderate inhibitory effects, affirming its partial efficacy against *Alternaria* spp.

This consistency across studies underlines the potential of essential oils as viable bio-fungicides. However, efficacy varies based on the oil type, fungal strain, and application method.

The superior performance of rose and lemon oils observed in the current investigation could be due to the presence of potent antifungal compounds such as citronellol, geraniol, and limonene, which have been reported to disrupt fungal cell membranes and inhibit spore germination (Bakkali *et al.,* 2008; Burt, 2004).

The present study provides strong evidence for the antifungal potential of rose and lemon essential oils against *Alternaria* spp. infecting pomegranate.

Table.1 *In vitro* evaluation of different essential oils on *Alternaria* spp.

Tr.No	Essential oils	*Alternaria burnsii*		*Alternaria alternata*	
		Colony Dia.*(mm)	% Inhibition	Colony Dia.*(mm)	% Inhibition
T_1	Clove (*Syzygium aromaticum*)	62.66	30.36 c (33.43)	78.00	13.33 d (21.41)
T_2	Rose (*Rosa damascena*)	05.00	94.44 a (76.36)	07.66	91.47 a (73.01)
T_3	Citronella (*Cymbopogon nardus*)	62.33	30.73 c (33.66)	77.66	13.70 d (21.72)
T_4	Eucalyptus (*Eucalyptus globulus*)	82.66	8.14 d (16.58)	88.00	02.22 f (08.56)
T_5	Ginger (*Zingiber officinale*)	63.33	29.62 c (32.97)	83.33	07.40 e (15.78)
T_6	Lemon (*Citrus limonum*)	05.00	94.44 a (76.36)	52.66	41.47 c (40.08)
T_7	Cardamom (*Elettaria cardamomum*)	29.00	67.77 b (55.40)	15.66	82.59 b (65.33)
T_8	Control	90.00	00.00 e (00.00)	90.00	00.00 e (00.00)
SE(m)±		**1.07**	**1.19**	**1.32**	**1.46**
C.D.		**3.29**	**3.66**	**4.04**	**4.49**

* Mean of three replications, Dia.: Diameter
Figures in parentheses are arcsine transformed values.
Note : Same alphabets denotes that the treatments were not significantly different at 0.01

Their high efficacy, particularly in comparison to synthetic treatments and poorly performing oils like eucalyptus, highlights their promise as natural, eco-friendly alternatives in integrated disease management programs.

Further studies, including in vivo evaluations and formulation development, are recommended to validate their field-level effectiveness and commercial application.

Author Contributions

R. R. Chavan: Investigation, formal analysis, writing—original draft. S. V. Kolase: Validation, methodology, writing—reviewing. T. K. Narute:—Formal analysis, writing—review and editing. P. E. More: Investigation, writing—reviewing. K. S. Raghuwanshi: Resources, investigation writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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