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Innovative Application of Botanical Essential Oils as Potent Agents for Managing Post-Harvest Fungal Pathogens

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ABSTRACT

This study explores the antifungal efficacy of essential oils (EOs) extracted from a diverse array of botanical sources against *Botrytis cinerea*, an ubiquitous phytopathogen responsible for post-harvest decay. Among the EOs assessed, ten exhibited pronounced fungicidal activity, including those derived from *Eucalyptus citriodora*, *Chenopodium ambrosioides*, *Ocimum canum*, *Ocimum gratissimum*, *Ocimum sanctum*, *Eupatorium cannabinum*, *Lawsonia inermis*, *Zingiber cassumunar*, *Zingiber officinale*, and *Prunus persica*. Each of these oils achieved complete inhibition of pathogen growth (100%). The study focused on EOs from *Ocimum sanctum*, *Prunus persica*, and *Zingiber officinale* due to their lower Minimum Inhibitory Concentration (MIC) values compared to other oils. Analytical standardization based on physicochemical properties and evaluation of antifungal potency revealed MICs of 200 ppm for *Ocimum sanctum* and 100 ppm for both *Prunus persica* and *Zingiber officinale*. At these concentrations, the oils exhibited fungistatic properties and thermostability. They also demonstrated broad-spectrum fungitoxicity against fifteen additional post-harvest fungal pathogens and maintained efficacy even under high inoculum pressure. Notably, the antifungal performance of these EOs exceeded that of conventional synthetic fungicides. In practical applications, managing gray mold in grapes during storage yielded promising outcomes. Grapes treated with *Ocimum sanctum* oil experienced an extended shelf life of up to 5 days, while those treated with *Prunus persica* oil showed a 4-day extension. Grapes treated with *Zingiber officinale* oil demonstrated an extended shelf life of up to 6 days, with no detrimental effects on the integrity of the fruit's epidermis. These findings suggest that EOs from *Ocimum sanctum*, *Prunus persica*, and *Zingiber officinale* have significant potential as sustainable, eco-friendly botanical fungicides. However, further comprehensive and long-term field trials are necessary to validate their efficacy under practical agricultural conditions.

Keywords: Essential Oil, EOs, Grey mould, Eco-friendly fungicides, Botanical fungicides.

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INTRODUCTION

Post-harvest diseases impose substantial economic burdens, particularly during the transportation and storage of perishable commodities (Kasso & Bekele, 2018). The intrinsic properties of these commodities, such as high water content, rich nutrient profiles, and specific pH levels, create an ideal environment for the proliferation of microorganisms (Preetha & Narayanan, 2020). Fruits, with their naturally low pH, are especially vulnerable to fungal pathogens (Barth et al., 2009), which not only cause spoilage through rot but also pose serious health risks via mycotoxin contamination (Sarrocchio & Vannacci, 2018). The global post-harvest losses for perishable foods are estimated to be between 40% and 50%, underscoring the critical need for effective mitigation strategies to enhance food security (Kiaya, 2014; Aslam et al., 2019; Alam et al., 2017).

Botrytis cinerea, or grey mould, represents a formidable challenge in viticulture, impacting grapevines both pre- and post-harvest (Elmer & Reglinski, 2006; Ahmed et al., 2018). This pathogen is notorious for causing extensive damage during the growing season and persisting through storage and transport, leading to significant economic losses (Hua et al., 2018). Post-harvest infections by *Botrytis cinerea* shorten the shelf life of grapes and compromise their market value by impairing visual appeal, quality, palatability, and texture, ultimately diminishing consumer demand (Achari & Devappa 2021). Effective management of *Botrytis cinerea* is crucial and involves meticulous cultural practices, such as stringent sanitation and targeted pruning, alongside chemical interventions like fungicide applications (Molitor et al., 2011). Additionally, optimizing storage conditions by controlling temperature and humidity can further reduce post-harvest fungal infestations (Sonker et al., 2016). Cutting-edge research continues to explore innovative control measures, including the use of biological agents (Zhang et al., 2020) and the breeding of genetically resistant grape varieties, advancing the development of sustainable and effective strategies to mitigate the detrimental effects of *Botrytis cinerea* on grape production (Poveda et al., 2020). The use of synthetic pesticides in agricultural plant protection has significantly boosted crop yield and stability (Saravi & Shokrzadeh, 2011). However, concerns persist regarding their environmental impact and associated health risks, particularly with the rise of pesticide-resistant pathogen strains (Hawkins et al., 2019). Fungicides used in post-harvest disease management have come under particular scrutiny due to their potential oncogenic risks (Sharma et al., 2012). In response, the global scientific community is increasingly exploring botanical-based alternatives as a more sustainable and safer approach (Bhagat et al., 2014; Dar et al., 2014; Koondhar et

al., 2024). Plant-derived biologically active compounds are especially promising, offering an ecologically favorable solution compared to synthetic chemicals (Chowdhury et al., 2023).

Essential oils (EOs) have emerged as a promising focus in the quest for sustainable post-harvest disease management strategies, attracting significant scholarly and practical interest (Alam et al., 2017; Kaya et al., 2024).. These hydrophobic liquids are characterized by a complex chemical composition, predominantly consisting of saturated and unsaturated hydrocarbons. Additionally, they contain a variety of oxygenated compounds, including alcohols, esters, ketones, aldehydes, and terpenes, which collectively contribute to their robust biological activity and functional versatility. The intricate blend of these compounds underscores the potential of EOs as a natural and eco-friendly alternative to synthetic pesticides, offering a sophisticated solution to enhance agricultural sustainability and ensure food security (Ali et al., 2015).

Extensive research has explored the natural fungicidal properties of essential oils (EOs), demonstrating their substantial potential in disease management, particularly in post-harvest contexts (Tabassum & Vidyasagar, 2013; Nazzaro et al., 2017). Derived from various plants, EOs are abundant in bioactive compounds that exhibit strong antifungal activities against a wide range of pathogens. This body of research has significantly deepened our understanding of the effectiveness of EOs in combating fungal diseases, reinforcing their role as viable and sustainable alternatives to conventional synthetic fungicides. These findings highlight the promise of EOs in contributing to more eco-friendly and effective disease management strategies (Tagne et al., 2008; Raveau et al., 2020).

The diverse array of plant sources available for essential oil (EO) extraction provides rich opportunities for continued research and innovative applications (El Asbahani et al., 2015). The effectiveness of EOs can differ markedly depending on the targeted pathogens, offering a flexible approach to tailoring disease management strategies (Hu et al., 2019). Extensive research confirms that EOs play a promising role in disease management, particularly in addressing the challenge of fungal pathogens in post-harvest settings. This potential highlights EOs as significant alternatives to conventional methods, contributing to the preservation of crop quality and safety (Tripathi et al., 2008; Plaza et al., 2004; da Costa Gonçalves et al., 2021). Translating scientific research into practical applications is crucial for developing effective food technology solutions. This study evaluates the efficacy of essential oils (EOs) from *Ocimum sanctum* (holy basil), *Prunus persica* (peach),

and *Zingiber officinale* (ginger) in extending the shelf life of grapes by inhibiting decay caused by *Botrytis cinerea*. The distinctive chemical compositions of these EOs, each exhibiting antifungal properties, make them viable candidates for enhancing post-harvest preservation and maintaining grape quality.

This research targets *Botrytis cinerea*, a critical challenge in grape storage and preservation. By assessing the efficacy of essential oils (EOs) in inhibiting or eradicating this pathogen, the study provides important insights into their potential as natural substitutes for synthetic fungicides in food technology. The findings aim to improve grape shelf life and quality while aligning with the increasing demand for sustainable and environmentally-friendly agricultural practices.

MATERIALS AND METHODS

A range of locally available aromatic angiosperms was collected for essential oil (EO) extraction to evaluate their antifungal potential. The following plant families are represented among the species used:

Rutaceae: Includes *Aegle marmelos* (Bael), *Murraya koenigii* (Curry Leaf Tree).

Zingiberaceae: Comprising *Ammomum subulatum* (Black Cardamom), *Elettaria cardamomum* (Cardamom), *Zingiber cassumunar* (Cassumunar Ginger), and *Zingiber officinale* (Ginger).

Meliaceae: Represented by *Azadirachta indica* (Neem).

Asteraceae: Encompassing *Caesulia axillaris* and *Eupatorium cannabinum* (Hemp Agrimony).

Myrtaceae: Includes *Callistemon lanceolatus* (Bottlebrush) and *Eucalyptus citriodora* (Lemon Eucalyptus).

Verbenaceae: Featuring *Lippia alba* (Lemon Verbena).

Lythraceae: Represented by *Lawsonia inermis* (Henna Tree).

Amaranthaceae: Including *Chenopodium ambrosioides* (Epazote).

Lamiaceae: Comprising *Nepeta hindostana* (Indian Catnip), *Ocimum basilicum* (Sweet Basil), *Ocimum canum* (Wild Basil), *Ocimum gratissimum* (Clove Basil), *Ocimum sanctum* (Holy Basil), and *Hyptis suaveolens* (Hyptis).

Rosaceae: Featuring *Prunus persica* (Peach).

This diverse selection highlights the rich potential of these plant taxa as sources of essential oils with promising antifungal properties.

Extraction of Essential Oils from Fresh Plant Material

For EO extraction, protocol described by Kandinsky & Musilova, 2010 was followed.

Isolation and Morphological Characterization of *Botrytis cinerea*

Isolation of fungus *Botrytis cinerea* was conducted from

contaminated fruits. This fungal species was recognized following the protocols outlined in the manual by Domsch et al. (1980).

Cultivation and Maintenance of Pathogen Cultures

Pathogens were cultured on Potato Dextrose Agar (PDA) medium, with the pH adjusted to approximately 5.6, and were aged for seven days prior to use. To evaluate the antifungal effectiveness of essential oils, the poisoned food technique described by Perrucci et al. (1994) was employed. Essential oils were mixed into the PDA medium, while control sets used sterilized distilled water instead of oils. After aseptic inoculation of the plates with assay discs containing the test fungus, the plates were incubated for six days. Observations were recorded on the seventh day, and the percentage of mycelial inhibition was calculated using the formula: (Sarker & Kumar, 2014).

Factors Affecting Fungitoxic Efficacy of Eos

The impact of increased fungal strain inoculum density on the fungicidal properties of the oils was investigated following the methodology outlined by Molyar & Pattisapu (1987). This approach involves varying the inoculum density of the fungal strains to assess how it influences the efficacy of essential oils in inhibiting fungal growth. The results provide insights into the effectiveness of the oils under different levels of pathogen challenge, offering a more comprehensive understanding of their potential as antifungal agents. Additionally, the influence of storage conditions and temperature on the fungitoxic efficacy of the essential oils (EOs) was evaluated using procedures outlined by Tripathi et al. (1978). The spectrum of fungitoxic activity was assessed across a range of concentrations, including both toxicant and hyper-toxicant levels, utilizing the poisoned food technique. This comprehensive evaluation helps determine how different storage conditions and temperatures impact the effectiveness of the EOs in inhibiting fungal growth.

Comparative Evaluation of Essential Oils and Synthetic Fungicides for Fungal Control Efficiency

The effectiveness of essential oils (EOs) was evaluated by comparing them to synthetic fungicides, including zinc dimethyldithiocarbamate (ziram), phenyl mercuric acetate (ceresan), diphenylamine (N-Phenylaniline), and benzimidazole (benomyl), using the conventional poisoned food technique. Grapes were treated with EOs according to standard protocols (Chandra, 1984; Sharma & Yadav, 1996) to determine their efficacy against grey mold. Pathogenicity testing of the fungus was conducted following the methodology outlined by Garcha & Singh (1980).

Evaluating the Efficacy of Essential Oils for Grey Mold Control in Grapes

Fruits were inoculated with a standardized spore suspension of *Botrytis cinerea*, derived from a seven-day-old culture. The spores were suspended in sterile distilled water with 0.03% Tween 80. Inoculation involved puncturing the fruits at multiple sites on each cluster with a pin, followed by applying the spore suspension (105 spores/ml) to each puncture. The inoculated fruits were then placed in desiccators, with four fruit clusters per desiccator. Essential oils (EOs) from *Prunus persica* (peach), *Zingiber officinale* (ginger), and *Ocimum sanctum* (holy basil) were introduced into the desiccators by placing saturated cotton pads with the EOs, resulting in final concentrations of 100 ppm, 100 ppm, and 200 ppm, respectively, based on their minimum inhibitory concentration (MIC) values. The development of fruit rot was monitored, with six replicates for both the treatment and control groups, to assess the efficacy of the EOs in controlling *B. cinerea* infection.

RESULTS

The findings presented in Figure 1 reveal the differential antifungal efficacy of twenty-five essential oils (EOs) against a diverse array of fungal strains, including *Chenopodium ambrosioides* L., *Eucalyptus citriodora* Hook., *Eupatorium*

cannabinum, *Lawsonia inermis*, *Ocimum canum*, *Ocimum gratissimum*, *Ocimum sanctum*, *Prunus persica*, *Zingiber cassumunar*, and *Zingiber officinale*. Notably, the tested compound achieved complete mycelial inhibition at a concentration of 500 ppm, indicating its potent fungicidal activity. Among the EOs, *Caesulia axillaris* and *Cymbopogon citratus* demonstrated the highest efficacy, with 95% inhibition of fungal growth. *Murraya koenigii* and *Ocimum basilicum* exhibited substantial inhibition at 80%. EOs from *Aegle marmelos* L., *Elettaria cardamomum*, *Azadirachta indica*, *Ammomum subulatum*, and *Nepeta cataria* provided moderate inhibition, ranging from 50% to 75%. In contrast, *Melaleuca leucodendron* and *Citrus reticulata* showed lower efficacy, with 40% and 45% inhibition, respectively. EOs from *Citrus medica* L., *Callistemon lanceolatus*, *Hyptis suaveolens* (L.) Poit., and *Lippia alba* exhibited the least effectiveness, with inhibition ranging from 20% to 30%. These results underscore the variable antifungal potency of different EOs, highlighting their potential for targeted application and warranting further exploration into their mechanisms of action and optimization for practical use in fungal disease management.

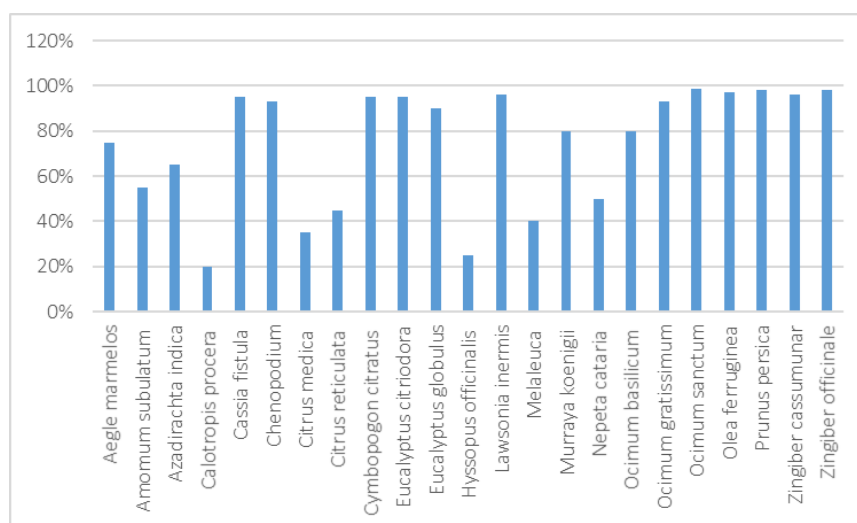


Figure 1: Percent inhibition of mycelial growth at 500 ppm

Comparative Evaluation of EOs and Synthetic Fungicides

Figure 2 confirms that the minimum inhibitory concentrations (MICs) of essential oils (EOs) are lower than those of synthetic fungicides, it indicates that EOs are more effective at inhibiting fungal growth at lower concentrations. This greater efficacy means that EOs achieve comparable levels of fungal inhibition with smaller quantities than the synthetic fungicides. Consequently, EOs

could be more efficient and potentially more cost-effective options for managing fungal pathogens.

Efficacy of Essential Oils as Sustainable Alternatives to Synthetic Fungicides

This study highlights the efficacy of essential oils (EOs) from *Ocimum sanctum*, *Prunus persica*, and *Zingiber officinale* in controlling grey mold on grapes caused by *Botrytis cinerea*. The EOs demonstrated superior fungitoxic activity with lower Minimum Inhibitory Concentrations

(MICs) compared to synthetic fungicides, indicating their effectiveness at lower concentrations (Figure 3).

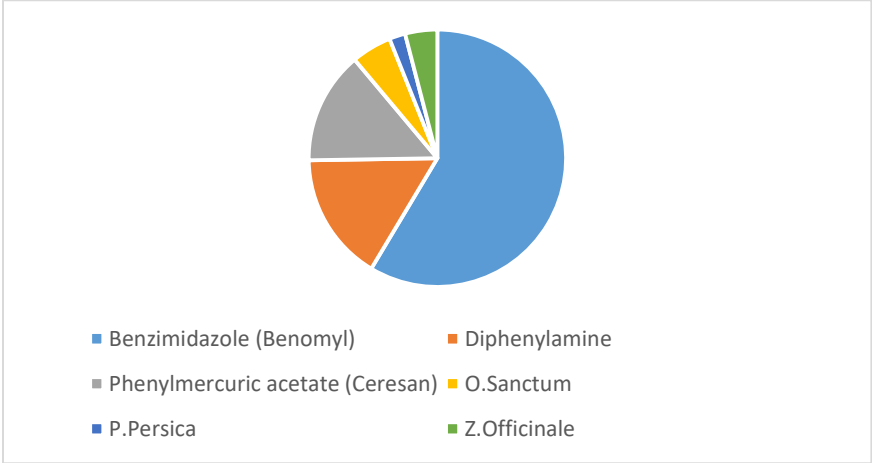


Figure 2: Minimum inhibitory Concentration against *B. cineria*

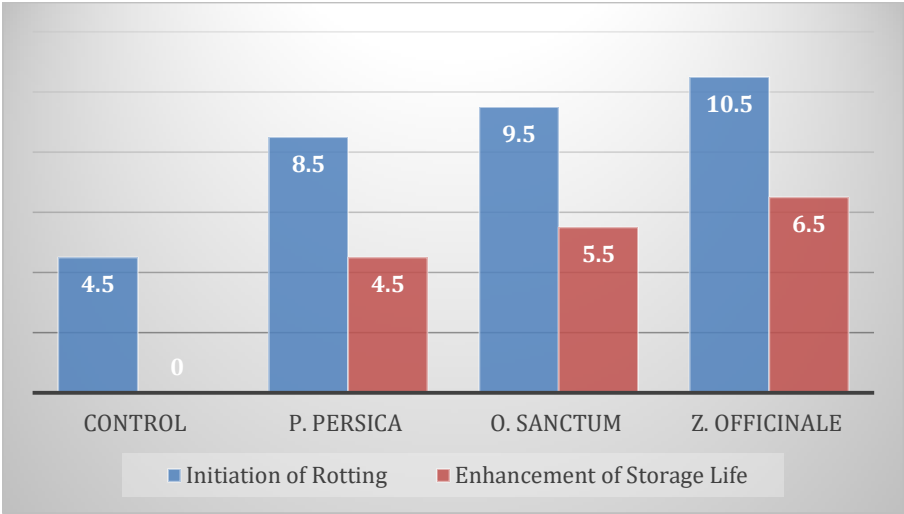


Figure 3: Efficacy of Essential Oil against Grey Mould of Grapes

These oils exhibited fungistatic properties, inhibiting fungal growth without cidal effects on the host tissues at MIC levels. Notably, the EOs extended the shelf life of grapes by up to five, four, and six days, respectively, while maintaining fruit quality. The findings underscore the potential of EOs as sustainable, biodegradable fungicides, offering a promising alternative to synthetic chemicals for post-harvest disease management and food preservation.

DISCUSSION

Essential oils (EOs) are thought to play a significant role in the plant's defense mechanism against phytopathogenic microorganisms. These natural compounds possess antimicrobial properties that help protect plants from pathogenic infections, acting as a chemical barrier or deterrent. By disrupting the growth and development of pathogens, EOs enhance the plant's resilience and reduce

the risk of disease (Raveau et al., 2020; Sil et al., 2020). Numerous studies have documented the inhibitory effects of essential oils (EOs) on post-harvest fungi in vitro. These investigations have demonstrated that EOs can effectively impede fungal growth and spore germination, showcasing their potential as natural antifungal agents. The findings support the application of EOs in controlling fungal pathogens and extending the shelf life of perishable commodities (López-Meneses et al., 2017; Grzesiak et al., 2016; Combrinck et al., 2011; Pawar & Thaker, 2006). Recent research has highlighted the efficacy of essential oils (EOs) as biodegradable and environmentally friendly fungitoxicans, emphasizing their potential as natural alternatives to synthetic fungicides. Their effectiveness in controlling fungal pathogens, combined with their sustainable and eco-friendly profile, makes EOs promising candidates for further exploration and development as

natural fungicides (Dwivedy et al., 2016; Maurya et al., 2021a; Maurya et al., 2021b).

In this study, essential oils (EOs) extracted from *Ocimum sanctum*, *Prunus persica*, and *Zingiber officinale* were selected for further investigation due to their relatively low Minimum Inhibitory Concentrations (MICs) compared to other fungitoxic oils. These oils were subjected to a thorough analysis of their physicochemical properties, fungitoxic efficacy, and practical applicability in controlling grey mold of grapes caused by *Botrytis cinerea*. Such comprehensive evaluations are crucial for assessing the potential of plant-based fungitoxic products, providing valuable data that can be used for recommending these EOs to agrochemical companies for formulation and commercial use.

These essential oils (EOs) exhibit fungistatic behavior at their respective Minimum Inhibitory Concentrations (MIC), indicating that they do not exert a cidal effect on the host tissues at these levels. This trait is advantageous as it implies that the oils possess fungicidal properties only at higher concentrations, thus minimizing the risk of adverse effects on the host tissues when applied at the MIC. A comparative analysis of the MICs of these oils against selected synthetic fungicides reveals that the EOs exhibit superior activity, with their MICs being lower than those of the synthetic alternatives, underscoring their enhanced efficacy (see Figure 2). Plant-derived fungitoxic compounds are typically considered safe for use on treated food items and have, in some cases, been shown to effectively extend the shelf life of these products. Specifically, the EOs from *Ocimum sanctum*, *Prunus persica*, and *Zingiber officinale* have demonstrated significant fungitoxic activity, effectively prolonging the shelf life of grapes by protecting them from grey mold. In the study, fruits were treated with the EOs at their respective MICs, resulting in an increased shelf life of up to five, four, and six days for grapes treated with *O. sanctum*, *P. persica*, and *Z. officinale*, respectively. Importantly, no adverse effects were observed on the fruit peel following the application of these oils.

CONCLUSION

This study highlights the efficacy of essential oils (EOs) from *Ocimum sanctum*, *Prunus persica*, and *Zingiber officinale* in controlling grey mold on grapes caused by *Botrytis cinerea*. The EOs demonstrated superior fungitoxic activity with lower Minimum Inhibitory Concentrations (MICs) compared to synthetic fungicides, indicating their effectiveness at lower concentrations. These oils exhibited fungistatic properties, inhibiting fungal growth without cidal effects on the host tissues at MIC levels. Notably, the EOs extended the shelf life of grapes by up to five, four, and six

days, respectively, while maintaining fruit quality. The findings underscore the potential of EOs as sustainable, biodegradable fungicides, offering a promising alternative to synthetic chemicals for post-harvest disease management and food preservation.

AUTHOR'S CONTRIBUTION

The idea was conceptualized by Dr. Amir Afzal. The project was conducted under the leadership of Dr. H.Z.U. Ghazali. The contributions of all team members are duly acknowledged.

CONFLICT OF INTEREST

Author showed no conflict of interest.

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