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Assessing the efficacy of essential oil fumigation in mitigating *Botrytis cinerea* infection in cherry tomato

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Abstract

Cherry Tomato (*Solanum lycopersicum L. var. cerasiforme)* believed to be the progenitor of modern cultivated tomatoes, is one of the world's most cultivated and widely consumed vegetables. However, it is very susceptible to fungal (*Botrytis cinerea*) infection, which decreases shelf life, and increases post-harvest loss. Postharvest loss and lower quality of fruit decrease the economic value of the crop or may make it unsuitable for human consumption and affect farmer profit. Therefore, an effective method is needed to reduce tomato's post-harvest loss. This study aims to assess the efficacy of *Zingiber officinale oil* and *Piper nigrum oil* in controlling fungal disease in tomato fruit. The antifungal activity of essential oil was evaluated using the essential oil fumigation method. The result showed that essential oil significantly inhibits *Botrytis cinerea* growth as compared to the control. Therefore, essential oil can effectively control the fungal diseases of cherry tomatoes against *Botrytis cinerea* fruit rot and reduce the post-harvest loss of tomatoes.

Keywords: Cherry Tomato*, Botrytis cinerea,* essential oil, fumigation treatment, post-harvest disease.

1 Introduction

Botrytis cinerea, commonly referred to as gray mold, is a destructive fungal pathogen that affects a wide range of horticultural crops [1]. It stands out as the predominant fungal pathogen responsible for the post-harvest decay of *S.lycopersicum L. var. cerasiforme* [1]. *Botrytis cinerea* is a ubiquitous pathogen that easily grows in diverse environmental conditions, which significantly affects the production of fruit and vegetables worldwide [2]. The importance of combating post-harvest fungal diseases in cherry tomatoes cannot be overstated. Post-harvest losses due to fungal infections result in economic losses for farmers and contribute to food waste and insecurity. The impact of *Botrytis cinerea* on cherry tomatoes manifests as necrotic lesions, which result in reduced fruit quality and shelf life, ultimately affecting marketability and profitability, and rendering them unfit for human consumption. Farmers can extend the shelf life of cherry tomatoes by focusing on post-harvest fungal diseases, such as gray mold, improving marketability, and reducing food waste along the supply chain.

Traditionally, chemical fungicides have been the primary means of controlling *Botrytis cinerea* infections. However, the excessive use of synthetic fungicides has led to environmental pollution, pesticide resistance, and concerns regarding human health and food safety [3,4]. As a result, chemical preservatives are being replaced by natural antimicrobials such as essential oils which are safe and healthier for consumers [5].

In recent years, essential oils derived from various plant sources have emerged as promising approaches for disease control [6]. These natural compounds possess diverse bioactive properties, including antifungal, antibacterial, and antioxidant activities, making them potential alternatives to synthetic fungicides [7]. Essential oils are also known for their low toxicity and biodegradability, making them environmentally sustainable alternatives for disease management. The potential of essential oils for disease control is gaining interest due to their sustainable and eco-friendly properties [5, 6, 7]. The utilisation of novel essential oils derived from plants such as Cinnamon (*C. verrum*), Peppermint (*M. peperita*), and Citronella (*C. nardus*) demonstrates a promising approach for exploring natural alternatives to traditional chemical treatments [8]. The study aimed to assess the potential utilisations of essential oils in mitigating fungal infections. Other studies have shown that essential oils isolated from plant sources, such as *Origanum vulgare* have antifungal activity at 250 mg/L, which inhibits cherry tomato deterioration by 96.39%. Furthermore, thymol and carvacrol at 125mg/L fully eliminated the grey mould [9].

These findings underscore the potential of essential oils as biopesticides and antifungal agents for fumigation applications, emphasising their role in sustainable pest management strategies for crops like cherry tomatoes affected by pathogens like *Botrytis cinerea*. This research aims to assess the antifungal efficacy of essential oil through fumigation treatment in mitigating *Botrytis cinerea* in cherry tomato plants.

2 Materials and Methods

2.1 Fruit materials, essential oils, and fungal strains

Fresh cherry tomatoes with uniformity in colour and size and smooth surface with no spots were Purchased from a supermarket. The plant pathogenic fungus spores used in this study were collected from the Institute of Agricultural Sciences, CSIC, Madrid, Spain. The essential oils utilized in this research were sourced from the commercial market in India.

2.2 Antifungal assay in vitro

First, stock solutions of essential oils were prepared in ethanol, with a concentration of 4 mg/mL. 5 µL of a 1 x 10^6 / mL solution for *Botrytis* spores was inoculated onto Potato Dextrose Agar (PDA) in multi-celled petri plates. Cellulose disks, measuring 2 cm in diameter, contain 250µL of the essential oil (from stock solutions) and are placed on the centre cell of the Petri plate. A disk containing 250µL of ethanol was used as a control. Ethanol was used as a positive control because essential oil was diluted in it to check its effect on fungal growth. The plates are sealed with parafilm and incubated at 25°C for three days or until the control reaches a specified diameter. The growth of each fungus is measured. The inhibition of fungal growth is calculated using the formula,

$$\text{Inhibition (\%)} = 100\text{-}\frac{Gt}{Gc}\text{x}100$$

where,
Gt: Diameter of fungal growth using essential oil (cm)
Gc: Diameter of fungal growth using Ethanol (cm)

2.3 Antifungal assay in vivo

The essential oil showing the highest antifungal activity in vitro study was selected for in vivo study. Cherry tomato fruits are washed and disinfected with sodium hypochlorite (1%), and alcohol (75%), rinsed with sterile distilled water for a few minutes and dried before treatment with essential oil. Fruits were punctured (approximately 0.3cm), and each wound was then inoculated with 10uL of a spore solution (2 x 106 /mL spore concentration). The experiment was set up for in-vivo experiments i.e. blank (untreated), control (ethanol) treatments 1 (*Zingiber officinale* oil) and treatment 2 (*Piper nigrum* oil). The experiment was performed in duplicates and three tomato fruits were used for each replicate. A disk containing 250µL of essential oils was applied for treatment and 250µL ethanol was for control for the fumigation treatment. The discs were attached to the jar lid and incubated at 25°C for 1 week and observation was taken every day [10].

2.4 Weight loss percentage

The weight of cherry tomatoes in each jar was measured on the first and last day of the experiment. The average weight loss across all trials was calculated and presented as a percentage of the initial weight.

2.5 Total Soluble Solids

The TSS (Total Soluble Solids) content of the cherry tomatoes was determined by blending the fruit to extract the juice. Using a refractometer, measurements were taken first and last day of the experiment to assess any variations.

2.6 pH

The pH level was also measured using a pH meter, on the first and last day of the experiment.

2.7 Statistical Analysis

All statistical analysis was done using Microsoft Excel and IBM SPSS version 29.0.2.

3 Results and Discussion

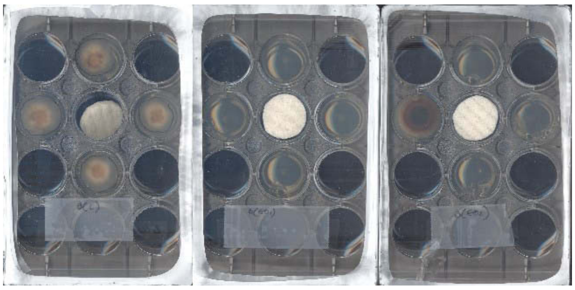
3.1 Antifungal efficacy of essential oils in vitro

Zingiber officinale oil and *Piper nigrum* oil both demonstrated promising results in the in vivo study, showing 100% inhibition of *Botrytis cinerea* growth. This indicates that the selected essential oils can control the infection caused *by B. cinerea* and that the quantity and concentration of oils used in the treatment are an optimal standard for inhibiting fungal growth in cherry tomatoes.

3.2 Antifungal efficacy of essential oil in vivo

In in-vivo study essential oils of *Zingiber officinale* and *Piper nigrum* completely inhibited the growth of *B. cinerea.* The Fruit treated with *Zingiber officinale* oil showed higher weight loss (0.76%), while fruit treated with *Piper nigrum oil* and ethanol (control) showed similar weight losses i.e. 0.64% and 0.63% respectively.

The conclusion drawn from the physical analysis of the fumigant treatment in vivo indicates that there was a negligible weight loss observed in both the essential oil and ethanol treatments when compared to the control group. There was no significant difference between weight loss percentage of fruit using ethanol and essential oil treatments which can be due to antifungal properties of ethanol [11, 12]. Total soluble solids (TSS) increased during storage and the high weight loss of tomatoes was observed with storage which may be due to fruit ripening or deterioration (Fig. 3 & Table 1). There is no significant change in the pH of the fruit during storage which indicates that there is the slow deterioration of the fruit in the presence of essential oil. Previous literature also reported that essential oils like thyme and oregano oil significantly inhibited the growth of *B. cinerea* and *Fusarium* species [13, 14]. Vitoratos et al. [15] reported that lemon essential oil at 0.05µL completely inhibited (100.0%) the growth of grey mould (*B. cinerea*) on strawberries and 39.0% inhibition of *B. cinerea* on cucumber using lemon (*Citrus limon L.)* essential oil.



a b c

Fig. 1. Antifungal efficacy of essential oil fumigation treatment after 7 days in vitro (a) Control (b) treatment with *Zingiber officinale* oil (c) treatment with *Piper nigrum* oil.

Fig. 2. Antifungal efficacy of essential oil fumigation treatment after 7 days in vivo (a) Control (b) Control (ethanol) (c) treatment with *Zingiber officinale* oil (d) treatment with *Piper nigrum* oil.

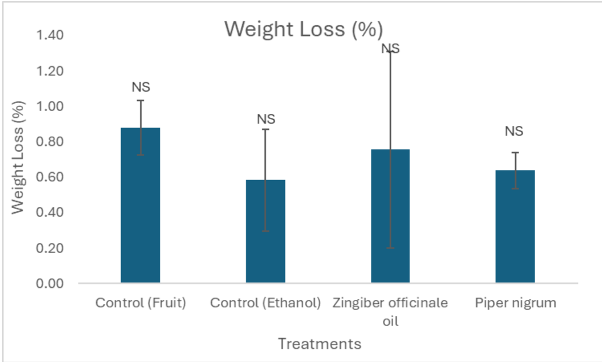


Fig. 3. Effect of *Zingiber officinale* oil and *Piper nigrum* oil fumigant treatment on the weight loss of Cherry tomatoes. NS indicates no significant difference between the treatments ($p>0.05$)

Table 1. Effects of *Zingiber officinale* oil and *Piper nigrum* oil fumigation treatment on the quality of the cherry tomatoes.

Treatments	pH Pre-treatment	pH Post-treatment	TSS Pre-treatment (°Brix)	TSS Post-treatment (°Brix)
Control	$5.63±0.0^{NS}$	$4.8±0.0^{c}$	$4.5±0.0^{NS}$	$5.4±0.0^{c}$
Control (Ethanol)	$5.63±0.0^{NS}$	$5.22±0.0^{b}$	$4.5±0.0^{NS}$	$4.5±0.0^{b}$
Zingiber officinale oil	$5.63±0.0^{NS}$	$5.28±0.0^{a}$	$4.5±0.0^{NS}$	$5.0±0.0^{a}$
Piper nigrum oil	$5.63±0.0^{NS}$	$5.30±0.0^{a}$	$4.5±0.0^{NS}$	$5.0±0.0^{a}$

Note: Different lowercase letter indicate significant difference between the treatments ($p<0.05$) in which a is highest. NS indicates no significant difference between the treatments ($p>0.05$)

4 Conclusion

The diseases in cherry tomatoes caused by *Botrytis cinerea* infection lower the quality of fruit, decrease the economic value of the crop, and make it unsuitable for human consumption. The application of essential oil from the natural source could be a potential approach to control fungal infection in tomatoes. In this study, both *Zingiber officinale* and *Piper nigrum* oils showed antifungal activity against *Botrytis cinerea*. The physical analysis indicated that spoilage of fruit was slow in the presence of essential oils.

Therefore, essential oils could be a potential alternative to chemical pesticides which effectively control the fungal diseases in tomato fruits.

Conflict of interest

The authors declare no conflict of interest.

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