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## Sandalwood Essential Oil (SEO) Readily Inhibits *Colletotrichum gloeosporioides*-Mediated Anthracnose in Post-Harvest Stored Mango (*Mangifera indica* L. cv. 'Keitt')

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**ABSTRACT:** Mango (*Mangifera indica* L. cv. 'Keitt') is one of the core fruit delicacies produced by China. During the post-harvest storage span, the fungal pathogen *colletotrichum gloeosporioides* readily invades the fruits and leads to a significant overall yield loss. In recent years of development, the exploitation of naturally occurring fungitoxic compounds such as Sandalwood Essential Oil (SEO) has been useful in tackling various fungal species. This study demonstrates the potential of SEO as part of a storage protection strategy against *C. gloeosporioides*-induced post-harvest anthracnose. SEO displayed a relatively higher mycelial growth inhibition rate when compared to various other essential oils. Furthermore, the Minimal Inhibitory Concentration (MIC), Minimum Fungicidal Concentration (MFC), and EC<sub>50</sub> (Half maximal effective concentration) of SEO were determined to be 2000, 2500, and 610.38 µL/L, respectively. Moreover, the chitosan glutamate-SEO emulsion controlled the anthracnose spread for several days by multiple folds at ½ MIC, MIC, and 2 MIC concentrations. These results strongly support the potential for large-scale production and application of SEO emulsions by agrochemical firms and post-harvest storage facilities handling Keitt mangoes.

**KEYWORDS:** Essential oils; sandalwood essential oil; *Colletotrichum gloeosporioides*; anthracnose; fungitoxic compounds

### 1 Introduction

China is the titan of the global fruit production sector, and it's no surprise that it is also the 2nd largest mango (*Mangifera indica* L.) producer in the world as well [1]. According to the latest statistics, China produces around 2.4 million metric tons of mangoes yearly on average, and that number is projected to rise thanks to modern advancements in fruit production research [2]. In this research field, one of the main



vertices deals with safe prolonged storage, especially tropical fruits such as mangoes. It is estimated that due to a lack of precise post-harvest handling practices and robust storage, 20%–30% of the total fruit becomes spoiled [3]. Microbial and fungal activity is considered the primary cause of such extensive post-harvest fruit loss [4]. Among the primary fungal pathogens responsible for mango spoilage is *C. gloeosporioides*. The post-harvest anthracnose (fruit rot) mediated by this particular species is the leading cause of storage decay losses in more than 100 plant species [5]. Quiescent infection is characterized as the most damaging phase of the *C. gloeosporioides* post-harvest attack, especially when the fruit just starts to ripen up [pre-climacteric phase]. When the ripening processes start, the fungus also starts to gradually resume its growth [6]. In mango cultivation, disease management has traditionally relied on synthetic chemical fungicides, but their repeated use often drives pathogen resistance [7–9]. Moreover, these chemicals pose considerable risks to human health and the environment: many are acutely toxic, teratogenic, or carcinogenic, persist as pollutants, and degrade only very slowly [10–13]. Due to these apparent negative effects of synthetic fungicides, alternate approaches in controlling *C. gloeosporioides* infections are needed.

Recent field developments reflect a growing trend in the fruit-research community toward applying naturally derived products such as essential oil extracts to postharvest fruit preservation [14,15]. The use of these extracts has proven to be very useful in tackling these devastating infections in multiple fruit species through enhanced decay control and improved storage span [16–18]. Recent investigations into essential oils have shown superior results [19–22]. These findings were evident in both *in vitro* and *in vivo* tests for anti-fungal activity [23–26]. This specific fungal-inhibition property makes them more desirable for their applications in the safe storage of post-harvest fruit crops. One of these well-researched, naturally-occurring products is SEO. SEO has demonstrated consistently strong anti-fungal activity across diverse studies, both through direct application on post-harvest fruit crops and in controlled laboratory experiments [27–31]. Essential oils like SEO primarily inhibit fungal growth by sequentially damaging proliferating fungal cells. This progressive assault irreversibly denatures critical cellular components, yielding a sustained and elevated rate of mycelial growth inhibition [32–35]. The vapor-phase bioactivity of essential oils such as SEO is another property that makes them excellent fumigants for stored fruit stocks [36]. Moreover, a growing body of research demonstrates that essential-oil extracts can substantially fortify the intrinsic defense systems of the plant, markedly improving resistance against phytopathogenic microorganisms [37–39].

This study builds upon the existing body of research on SEO and post-harvest fungal control. Presently, we have methodically demonstrated the *in vivo* anti-fungal efficacy of chitosan-based SEO coatings in suppressing the *C. gloeosporioides*-mediated anthracnose infection of the stored mango fruits. The findings serve as a valuable technical reference for future investigations into SEO-based antifungal coatings aimed at preventing anthracnose and enhancing the storage longevity of post-harvest fruit crops.

## 2 Materials and Methods

### 2.1 Preparation of Essential Oil Dilutions

All essential oils under testing, including wormwood, pine needle, rose essential oil, lemon, sandalwood essential oil, citronella, lavender, and perilla essential oil, were purchased from Shanghai Macklin Biochemical Co., Ltd. (Pudong, Shanghai, China). Each essential oil was diluted to 200 µL/L using a precisely measured volume of Tween-80 as the emulsifying agent [40]. The emulsification after the subsequent dilution process was performed using the Sonics Materials™ Ultrasonic Processor VCX130 (Newtown, CT, USA) [41].

## 2.2 Formulation of Coating Films

To make the SEO coating films, chitosan glutamate was used as the film base by dissolving it in 0.1% concentration of acetic acid ( $\text{CH}_3\text{COOH}$ ) up to the final concentration of 1% on the whole [42]. Subsequently, the Tween-80-emulsified SEO was incorporated in accurately measured volumes and stirred for 20 min to prepare a range of concentrations for application on the fruit samples.

## 2.3 Culturing of Fungal Samples

The potent *C. gloeosporioides* samples were kindly gifted to us by Dr. He Zhang (Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences). The isolates were cultured on Potato Dextrose Agar (PDA) or in Potato Dextrose Broth (PDB) at 28°C using a horizontal shaker set to 200 rpm [43]. After 4 days, the fungal conidia were collected from the subsequent suspension cultures, followed by filtration and centrifugation at 4000 rpm for 5 min. The acquired pellets were then thoroughly washed twice and resuspended at a final concentration of  $1 \times 10^6$  spores/mL [44].

## 2.4 Calculation of Mycelial Growth Inhibition Rate

To assess the mycelial growth inhibition rate, the PDA-containing 90 mm Petri dishes were inoculated with 2.5  $\mu\text{L}$  of spores each in the dead center [45]. The inoculated Petri dishes were then incubated in darkness at 28°C for 7 days. After incubation, the colony diameter ( $cd$ ) was calculated, and the final percentage of the mycelial growth inhibition rate was calculated following the upcoming equation [46].

$$\text{Mycelial growth inhibition rate (\%)} = \frac{cd \text{ on control} - cd \text{ on treatment}}{cd \text{ on control}} \times 100$$

## 2.5 Observation of Mycelial Morphology

The mycelial proliferative morphology was keenly observed using the ZEISS Smartzoom 5 Automated Digital Microscope (Carl Zeiss Microscopy, LLC—White Plains, New York, NY, USA) [47].

## 2.6 Inoculation of Fruit Subjects

The fresh indigenous fruits of *Mangifera indica* cv. 'Keitt' at the mature green stage was collected from the natively occurring marketplaces. The collected fruits were then subjected to disinfection soaping using the 1% Sodium hypochlorite ( $\text{NaClO}$ ) solution for 2 min before further treatment [48]. Fruits were inoculated by piercing to a 3 mm depth using a sterilized 1 mm needle, followed by the injection of 4  $\mu\text{L}$  of spore suspension into each wound [49]. The post-harvest plant defense response and other crucial fruit analyses, i.e., weight loss, respiratory rate, hardness, total soluble solids, total acids, Vitamin C (Ascorbic Acid— $\text{C}_6\text{H}_8\text{O}_6$ ) content, Catalase activity [CAT], Malondialdehyde content [MDA], Superoxide dismutase activity [SOD], and Polyphenol oxidase activity [PPO] were also performed using the standard protocols [50,51].

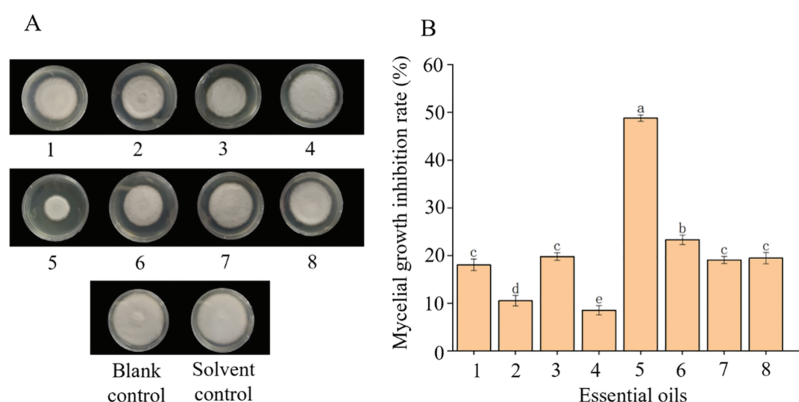
## 2.7 Statistical Analyses

All experiments were biologically replicated three times using groups of 6–8 fruits each to ensure statistical robustness. Furthermore, all the statistical tests and analyses viz. one-way ANOVA, linear regression, Goodness-of-Fit ( $R^2$ ), and  $p$ -value were executed by feeding all data into Minitab 22.1.0 (State College, PA, USA) [52].

### 3 Results

#### 3.1 SEO Inhibits the Mycelial Growth of *C. gloeosporioides*

A series of naturally extracted essential oils were evaluated for their relative mycelial growth inhibition against inoculated *C. gloeosporioides* spores on standard 90 mm Petri dishes (oil concentration: 500  $\mu\text{L/L}$ ; medium: standard PDA). The essential oils under testing include wormwood, pine needles, rose essential oil, lemon, sandalwood essential oil, citronella, lavender, and perilla essential oil. Blank and the standard Tween-80 served as the control groups for subsequent testing. As far as visual observation goes, the SEO outperformed all other essential oils under testing considerations by extreme comparative margins. On statistical scales, a significant relative difference was observed between different essential oil treatments in terms of mycelial growth inhibition of *C. gloeosporioides* spores, see Fig. 1 below. Notably, SEO exhibited a mycelial growth inhibition rate approaching 50%. On the other hand, the next contender *viz.* citronella oil, showed a rate reaching just above the 20% mark. This says a lot about the relative efficacy of SEO in inhibiting proliferative mycelial growth. The other essential oils that did not differ significantly in results include wormwood, rose essential oil, lavender, and perilla essential oil, with an inhibition rate barely reaching 20% individually. This means that these two oils in particular just performed marginally better when compared to the blank and solvent controls. Interestingly, the pine needle and lemon oil did not perform any better than the four aforementioned oils with an inhibition rate hardly approaching 10%. This testing procedure highlights the potential applications of SEO as a potent anthracnose inhibitor.



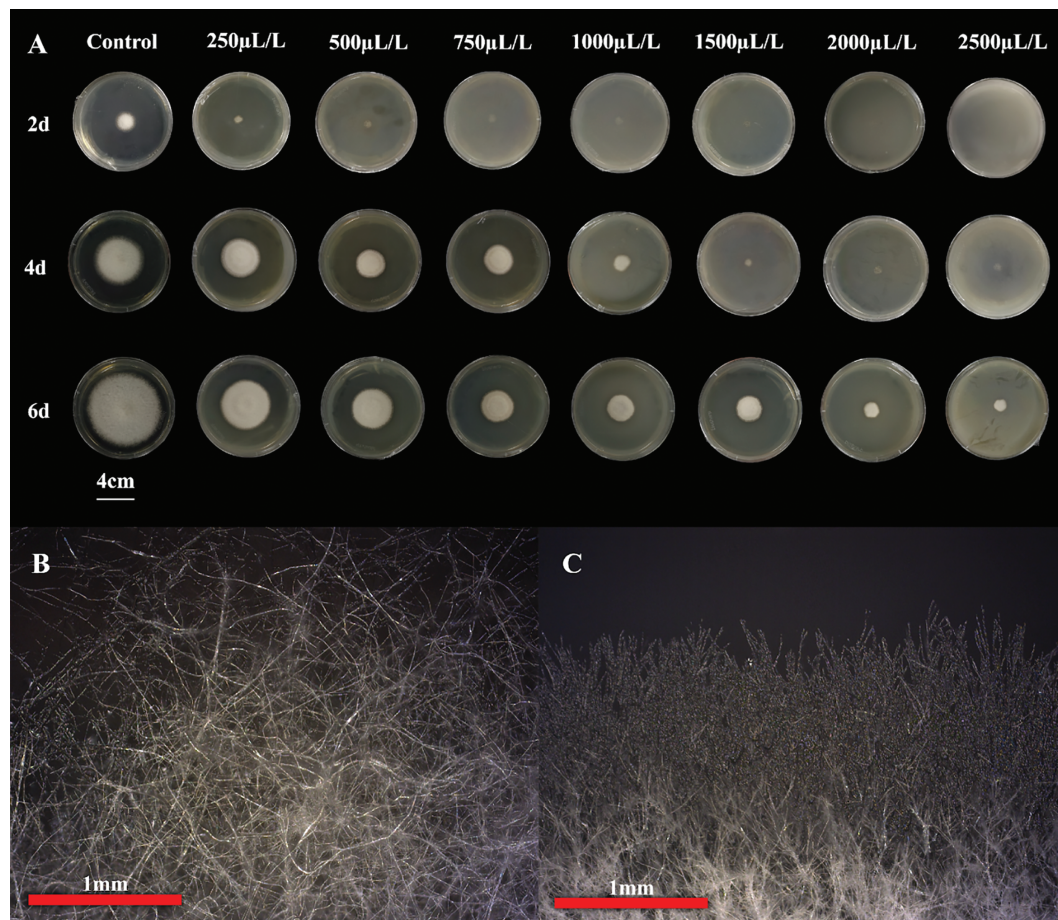
**Figure 1:** The effect of different essential oils on inhibition of mycelial growth of *C. gloeosporioides*. (A) Spores of *C. gloeosporioides* were inoculated on the standard PDA medium and 90 mm Petri dishes each with 500  $\mu\text{L/L}$  of essential oil respectively. (B) Mycelial growth inhibition rates (%) of different essential oils. Different lower-case letters (a–e) on the top of the bars indicate significant differences among treatments ( $p < 0.05$ ). Note: 1 = wormwood oil, 2 = pine needle oil, 3 = rose essential oil, 4 = lemon oil, 5 = sandalwood essential oil, 6 = citronella oil, 7 = lavender oil, and 8 = perilla essential oil. Controls = blank and solvent (Tween-80)

#### 3.2 SEO Inhibits Mycelial Growth at Multiple Concentrations and Periods

In another series of experimental iterations, the effect of different concentrations of SEO was quantified using the colonial morphology of *C. gloeosporioides* inoculated PDA plates, see Fig. 2A below. The mycelial growth inhibition rate was measured at fixed 2-day intervals following treatment with each concentration. At an emulsion concentration of 250  $\mu\text{L/L}$ , the inhibition effect was constantly significant relative to the control group, see Table 1 below. At 500 and 750  $\mu\text{L/L}$  concentrations, the inhibition rate was significant and comparatively the same, statistically. Furthermore, the inhibition rate spurts greatly over the course at concentrations of 1000 and 1500  $\mu\text{L/L}$ . The two aforementioned concentrations have a 2-fold statistical



difference among their subsequent rates of inhibition. Following this, when the concentrations of 2000 and 2500  $\mu\text{L/L}$  were approached, a completely significant inhibition [ $100.00 \pm 0.00$ ] was achieved during the 2 days. Thus, 2000  $\mu\text{L/L}$  served as the MIC while the Minimum Fungicidal Concentration MFC *viz.* Total Inhibitory Concentration was statistically plotted to be greater than the baseline of 2500  $\mu\text{L/L}$  to achieve maximum efficacy. However, when the two aforementioned concentrations were coursed through the 4 days, a slightly significant decrease in the inhibition rate was also observed with it becoming more and more significant as the period reached 6 days. Moreover, the relationship between the concentration of SEO [x] and the logarithm of the fungal growth inhibition rate [y] was described using the linear regression equation. The  $\text{EC}_{50}$  value for mycelial growth inhibition was calculated to be approximately 610.38  $\mu\text{L/L}$ , representing the concentration at which SEO inhibits 50% of mycelial growth, see Fig. 2B,C below.



**Figure 2:** Mycelial growth inhibition effect of SEO emulsion on *C. gloeosporioides*. (A) Colonial morphology of *C. gloeosporioides* on PDA plates at different concentrations. (B) Blank control (Red bar = 1 mm). (C) Mycelial morphology with 610.38  $\mu\text{L/L}$  [ $\text{EC}_{50}$ ] emulsion (Red bar = 1 mm)

**Table 1:** Correlation between procedural SEO concentrations and mycelial inhibition rate

Concentration [μL/L]	Inhibition rate (%)			MIC [μL/L]	MFC [μL/L]	Toxic regression equation	EC <sub>50</sub> [μL/L]
	2d	4d	6d				
250	41.88 ± 2.30 <sup>e</sup>	39.14 ± 1.01 <sup>f</sup>	34.04 ± 1.13 <sup>e</sup>	2000	>2500	y = 0.8489x + 2.6353 [R <sup>2</sup> = 0.9638]	610.38
500	51.42 ± 0.87 <sup>d</sup>	50.08 ± 0.56 <sup>e</sup>	48.08 ± 0.43 <sup>d</sup>				
750	54.02 ± 0.87 <sup>d</sup>	53.72 ± 0.49 <sup>d</sup>	51.91 ± 0.43 <sup>d</sup>				
1000	69.64 ± 1.74 <sup>c</sup>	78.68 ± 1.48 <sup>c</sup>	56.81 ± 2.10 <sup>c</sup>				
1500	88.72 ± 0.87 <sup>b</sup>	88.78 ± 1.48 <sup>b</sup>	59.15 ± 1.95 <sup>c</sup>				
2000	100.00 ± 0.00 <sup>a</sup>	91.87 ± 0.74 <sup>a</sup>	64.68 ± 1.53 <sup>b</sup>				
2500	100.00 ± 0.00 <sup>a</sup>	94.39 ± 0.56 <sup>a</sup>	72.55 ± 1.11 <sup>a</sup>				

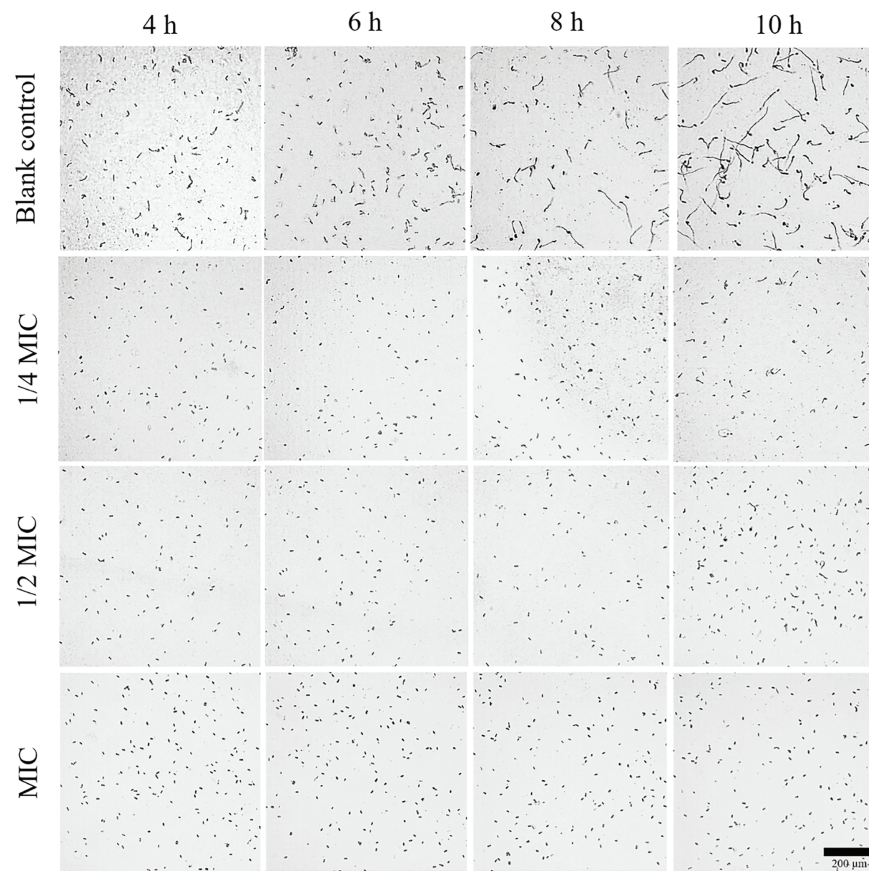
Note: **MIC** = Minimal Inhibitory Concentration; **MFC** = Minimum Fungicidal Concentration. Different alphabets (a–f) display the significant difference between subsequent treatment concentrations.

### 3.3 SEO Hinders the Germination of *C. gloeosporioides* Spores

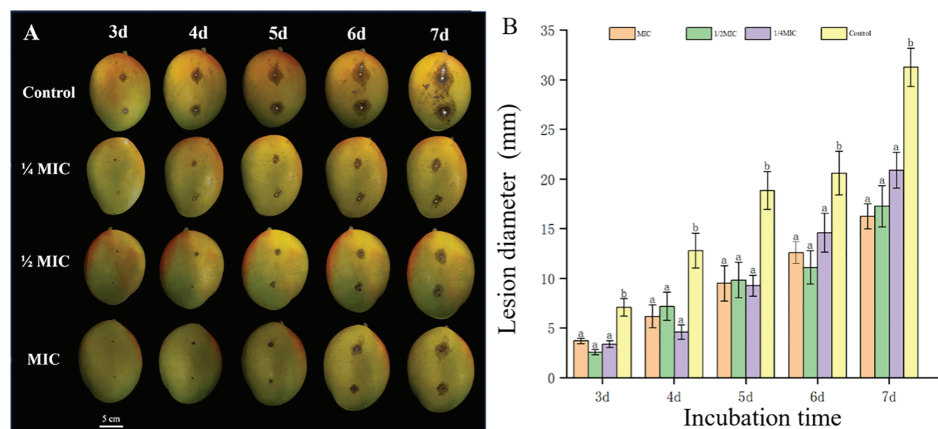
To further solidify the direct inhibition effect of SEO, inoculation of *C. gloeosporioides* spores was treated with ¼ MIC, ½ MIC, and MIC iterations throughout 4-, 6-, 8-, and 10-h standard to be observed for germination progression, see Fig. 3 below. In about 4 h, the blank water control showed the most germination out of all treatments. The same conditions were also observed when the time mark reached 6 h with the blank water control still leading the pack in terms of germination. However, when 8 h passed, the ¼ MIC began presenting with significant signs of germination of spores. In the same period, ½ MIC and MIC groups also presented with min spore germination. At the time point of 10 h, the blank water control group displayed the most spore germination of all followed by ¼ MIC and ½ MIC group. At this stage, the absolute MIC was the only iteration with the significant and least amount of spore germination. A clear negative correlation between increasing SEO concentration and decreasing spore germination was observed, indicating a substantial inhibitory effect.

### 3.4 SEO Maintains Fruit Morphology and Resists Anthracnose Progression

The subsequent phases of fruit morphology under active *C. gloeosporioides* infection were also observed under ¼ MIC, ½ MIC, and MIC sets with water as a control group, see Fig. 4A below. Each fruit was soaked in the treatment solution for 2 min immediately after inoculation and prior to incubation. The diameter of the spots of active infection were penned down during time intervals of 2, 3, 4, 5, 6, and 7 days, respectively, under the aforementioned treatment sets, see Fig. 4B below. As expected, the infection progressed rapidly in the control group with visible hyphae from the spots of infection starting to appear after just 3 days post-inoculation. The ¼ MIC and ½ MIC sets resisted the proliferation of infection up to 4 and 6 days respectively, with hyphae just starting to appear only in ¼ MIC iteration after 4 days post-inoculation. The iteration with the absolute MIC concentration resisted infection proliferation the most with almost no visible hyphae during the whole course of experimentation. On the whole, the results were critically significant relative to the disease progression in the control group with statistically similar resistance potential in all other treatment groups, i.e., ¼ MIC, ½ MIC, and MIC, respectively. This experiment demonstrates that brief exposure to SEO, through a 2-min soaking, effectively enhances anthracnose resistance in mango fruits.



**Figure 3:** Effects of SEO on the *C. gloeosporioides* spore germination [MIC = 2000 µL/L]. The extent of germination was observed over the time intervals of 4-, 6-, 8-, and 10-h standard with blank water control, ¼ MIC, ½ MIC, and MIC sets each simultaneously (Black bar = 1 mm)

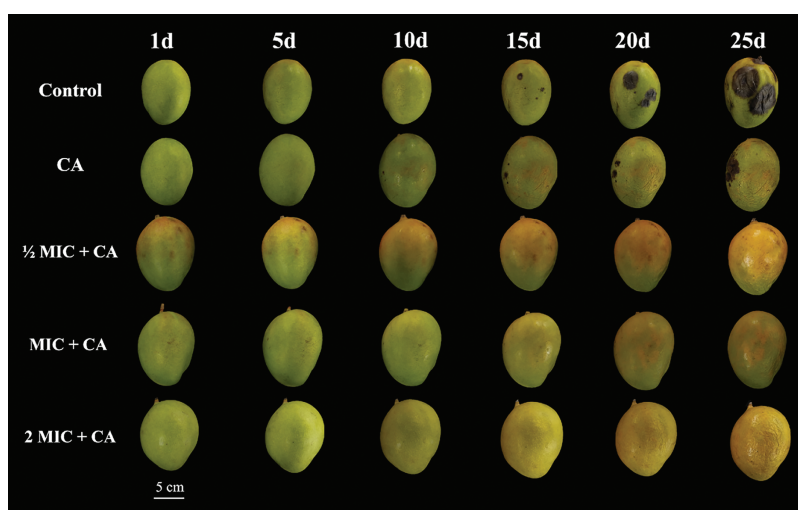


**Figure 4:** Fruit morphology and anthracnose progression under different iterations of MIC. (A) Mango fruit morphological phases under the infection of the inoculated *C. gloeosporioides* and different subsequent treatment concentrations (¼ MIC, ½ MIC, and MIC sets and water as control group with a 2-min soaking period each) (White bar = 5 cm). (B) Graph of the diameter of infection spots on fruit surface during incubation periods (3, 4, 5, 6, and 7 days respectively). Different lower-case letters (a, b) on the top of the bars indicate significant differences among treatments ( $p < 0.05$ )



### 3.5 SEO Coating Films Halt Anthracnose Progression During Storage and Maintain Post-Harvest Fruit Quality

To evaluate SEO-mediated resistance during active storage, mango fruits were treated with chitosan glutamate (CA),  $\frac{1}{2}$  MIC + CA, MIC + CA, and 2 MIC + CA, alongside a blank control group, see Fig. 5 below. The morphology of the mango fruit in all test groups was closely observed over the constant-interval periods of 1, 5, 10, 15, 20, and 25 days. The initial observations after 1 day were more or less the same in all test groups with no significantly detectable change in fruit morphology whatsoever. When the 5-day mark was reached, the subjects in the control group began displaying yellow surface signs from the top edge of the fruit while all other test groups remained the same in terms of morphology. After 10 days, the yellowish surface of the control group started to proliferate to the rest of the fruit while the coating agent CA group began showing min signs of anthracnose on one side of the fruit. All other test groups remained the same in morphological assessment. After the 15-day mark, the control group now also had visible anthracnose spots rapidly spreading in all directions. After 20 days, both the blank control and coating agent CA group were visibly infested with anthracnose but the former had a more rapid proliferation rate relative to the latter one. There are still no signs of anthracnose in any of the remaining test groups at this point. After the final 25-day mark, the test subjects in the control group now had more than half of their surface covered with rotting disease spots while the coating agent CA group had just 1/5th of its surface covered comparatively. At this stage, all other test groups were still anthracnose-free with relatively insignificant variations in the fruit color and appearance on a whole. Significant findings from additional post-harvest analyses included weight loss, respiratory rate, firmness, total soluble solids, total acids, Vitamin C content, CAT, MDA, SOD, PPO activities, decay rate, and disease index [Supplementary Tables S0–S12].



**Figure 5:** Differences in mango fruit appearance during storage. The treatment conditions include a blank control, coating agent CA (Chitosan glutamate),  $\frac{1}{2}$  MIC + CA, MIC + CA, and 2 MIC + CA sets over an observation time interval of 1, 5, 10, 15, 20, and 25 days each simultaneously. All fruits were subjected to an overall 2-min soaking period (White bar = 5 cm)

Post-harvest quality assessments revealed that SEO significantly mitigated fruit weight loss compared to the blank control, even after 25 days. The respiratory rates of the fruit in all the iterations, i.e., after 5, 10, 15, 20, and 25 days remained substantial with SEO. The fruit hardness was retained partially significantly up to 3.44 N/cm<sup>2</sup> at CA +  $\frac{1}{2}$  MIC after the same period. Similarly, the content of total soluble solids and total

acids also remained moderately substantial when compared to the control group with sequential percentage increase and decline, respectively. On the other hand, the Vitamin C content faced a steep decline in the blank control relative to the SEO iteration of CA + 2 MIC. Also, the level of the enzymes of the antioxidant defense system, i.e., CAT, MDA, SOD, and PPO also remained relatively and consistently higher in the SEO-treated groups in almost all iterations after the 25 days. Moreover, both the percentage of decay rate and the disease index number were found to be highly correlated with the concentrations of SEO used under subsequent MIC groups at the same periods. The combination of all the aforementioned factors displays the efficacy of SEO in mediating and maintaining the quality parameters of the post-harvest mango fruit. Furthermore, the SEO-enhanced antioxidant enzyme system employs complex mechanisms that help maintain mango freshness and protect against *C. gloeosporioides*-induced anthracnose, thereby extending storage life.

#### 4 Discussion

Decades of research have consistently demonstrated that essential oils effectively protect various plant species from diverse types of fruit rot, especially during post-harvest storage [53–57]. These aforementioned researches consistently show that essential oils exhibit potent antifungal, antibacterial, and antioxidant activities, which together extend fruit shelf life. By suppressing pathogen proliferation and mitigating oxidative stress, these natural compounds preserve both the quality and safety of stored produce [58–61]. The integration of essential oils in post-harvest treatment protocols offers a sustainable and eco-friendly alternative to synthetic chemicals, aligning with the growing demand for natural and organic solutions in agricultural practices. In the present research, SEO has come forward as one of the distinguished and potent members of the essential oil family in terms of resistance against *C. gloeosporioides*-mediated anthracnose in post-harvest stored mango. Our findings highlight the remarkable efficacy of SEO in curbing the incidence and severity of anthracnose, a prevalent and destructive fungal disease in mangoes in controlled trials, SEO exhibited robust antifungal activity, markedly reducing anthracnose symptoms compared with untreated controls. This not only underscores SEO's potential to enhance post-harvest mango quality but also highlights its promise for managing fruit-rot diseases in a wide range of susceptible crops. Indeed, its powerful antifungal effects have been extensively documented in earlier studies across multiple plant species [62–64]. These investigations have proven crucial in establishing the efficacy of SEO as a fungitoxic agent. These kinds of research provide essential insights for scaling up the production of essential oil emulsions by large agrochemical firms, ensuring optimal physicochemical formulation values. Such strategic formulation improves the stability and efficacy of SEO-based treatments, while emphasizing the critical role of scientific validation for their successful commercial deployment against fungal diseases in agriculture.

Several factors are sequentially involved in determining the quality of the essential oil emulsions being produced such as acid number, refractive index, thermostability, specific gravity, phenolic content, saponification value, optical rotation, ester value, and organic solvent solubility [65]. Generally, essential oils emulsions such as chitosan-based SEO nano-coatings are composed of multiple fungicidal components working in a synergistic combination to provide maximum efficacy against fungal infection [66,67]. Synergistic effects are amplified when two or more essential oils are combined into a single emulsion, significantly boosting antifungal efficacy [68,69]. Since a single fungicidal compound acts on a specific biochemical pathway, fungal populations can develop resistance over time. Combining multiple fungicidal essential-oil constituents not only mitigates resistance development but also enhances economic feasibility. Moreover, in MIC assays, SEO exhibited partially significant yet stochastic effects on fruit hardness, total soluble solids, and titratable acidity. These quality parameters may improve further when broad-spectrum blends of essential oils or multiple potent fungicidal components are applied simultaneously.

Comparative analyses demonstrate that SEO significantly surpasses commonly used essential oils including wormwood, pine needle, rose, lemon, sandalwood, citronella, lavender, and perilla in antifungal efficacy. However, broader investigation into synergistic blends of multiple potent essential oils is warranted to inform the formulation of multi-component emulsions that optimize efficacy while minimizing any off-target effects. Moreover,  $EC_{50}$  was calculated and tested to a concentration of 610.38  $\mu\text{L/L}$  while the MIC is far much higher at 2000  $\mu\text{L/L}$ , therefore the MFC must be greater than 2500  $\mu\text{L/L}$  for the formation of an effective emulsion. The multiple variations of MIC value were also effective to a statistically significant and correlative extent. However, anything less than the MFC concentration of 2500  $\mu\text{L/L}$  might not be as effective as intended while using more concentrated emulsions might not be as economically feasible at a large scale. When specifically dealing with *C. gloeosporioides*-mediated anthracnose at this level, the MIC of 2000  $\mu\text{L/L}$  and MFC of 2500  $\mu\text{L/L}$  might also not yield expected exponentially upscaled results. Consequently, comprehensive and rigorous calculations must be performed in advance, explicitly accounting for all potential error sources that could derail the intended outcomes. The emulsion concentrations tested in this study were carefully optimized to maintain antifungal effectiveness for up to one month after harvest. However, it's important to recognize that results observed under controlled laboratory conditions may differ when scaled up to real-world applications. Moreover, the effects might also not be fully duplicated in other fruit plant species affected by *C. gloeosporioides*-mediated anthracnose with the same MIC and MFC values due to a large number of unknown variables to account for the equation. The protective storage efficacy and fungitoxic potential of SEO can also be a topic of research for future studies to come.

## 5 Conclusion

This study underscores the significant potential of essential oils recognized for their efficacy against fruit rot in a wide range of plant species. Decades of research have documented their potent antifungal, antibacterial, and antioxidant properties, which are vital for prolonging shelf life and safeguarding both the quality and safety of fresh produce. SEO, highlighted in this research, emerges as a potent agent against *C. gloeosporioides*-mediated anthracnose in mangoes, demonstrating substantial efficacy in reducing disease severity. The effectiveness of SEO demonstrated in this study, positions it as a promising tool for sustainable post-harvest management in agriculture, aligning with the shift towards eco-friendly practices. Moreover, the research emphasizes the synergistic potential of essential oil combinations, enhancing their fungicidal effectiveness while minimizing the risk of resistance development. Challenges related to formulation optimization and economic viability highlight the necessity for ongoing research into scalable production methods and practical application techniques. Although our controlled experiments yielded encouraging antifungal outcomes, real-world applications may produce variable results. Therefore, additional studies are essential to assess efficacy of SEO and adaptability across diverse fruit species afflicted by anthracnose under practical post-harvest conditions.

The findings endorse SEO as a viable natural alternative to synthetic treatments in post-harvest protocols, offering promising prospects for enhancing agricultural sustainability and securing food supply chains. Future research should focus on refining formulation strategies, assessing broader applicability, and addressing practical challenges to maximize its protective benefits in agricultural settings. To meet the demands and production criteria of large-scale agrochemical firms such as in China, expansive-scale trials of SEO efficacy against *C. gloeosporioides*-mediated anthracnose in post-harvest stored mango fruit should be conducted. Large-scale trials will comprehensively evaluate legality, safety, and cost-effectiveness, ensuring the approach meets regulatory standards and economic viability. Expectantly, then SEO can be used commercially in mango fruit storage facilities with maximal effectiveness both in efficacy and economically.



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**Availability of Data and Materials:** The authors confirm that the data supporting the findings of this study are available within the article and its Supplementary Materials.

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**Supplementary Materials:** The supplementary material is available online at <https://www.techscience.com/doi/10.32604/phyton.2025.065065/s1>. Complementary methodology and tentative generated data on fruit post-harvest weight loss, respiratory rate, hardness, total soluble solids, total acids, Vitamin C content, Catalase [CAT] activity, Malondialdehyde [MDA] content, Superoxide dismutase [SOD] activity, Polyphenol oxidase [PPO] activity, decay rate, and the disease index.

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