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USING ESSENTIAL OILS TO PROTECT PEACHES FROM POST-HARVEST ROT CAUSED BY *RHIZOPUS* SPECIES

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ABSTRACT

Rhizopus is an important post-harvest pathogen that causes rot in fruits and vegetables. During 2022, a considerable incidence of post-harvest rot of peaches was observed in local markets in Bahawalpur, Pakistan. The symptoms appeared as whiskery moldy growth on the surface of the fruit. The pathogen was isolated and identified as *Rhizopus* species based on morphological features. Under the microscope, sporangiospores of *Rhizopus* were observed as light brown in color. The colony was very fast-growing and spreading. The color of *Rhizopus* mycelium was greyish. The pathogenicity was confirmed to satisfy Koch's postulates. The pathogen caused lesions on the surface of peach fruit. The current study also assessed the antifungal effects of essential oils on the growth of *Rhizopus*. Two essential oils were used: clove oil and cinnamon oil at concentrations of 0%, 25%, 50%, 75%, and 100%, respectively. The disease lesion with a diameter of 1.6 cm was treated with clove oil, 1.3 cm with 100% concentrated cinnamon oil, and 2.4 cm with the control (inoculated with *Rhizopus*). Cinnamon oil gave the maximum inhibition of *Rhizopus* on peach fruit compared to clove oil. The loss in fruit weight was minimum (2 g) in cinnamon-treated fruit compared to clove oil (3 g), control (2 g), and the infected one without any treatment (7 g). In conclusion, the study provides important information about *Rhizopus* as a significant post-harvest pathogen causing rot in fruits and vegetables, with a specific focus on peach fruit in Bahawalpur, Pakistan. The findings suggest that cinnamon oil could be a potential natural antifungal agent to mitigate post-harvest rot caused by the fungus in peaches.

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INTRODUCTION

Peaches (*Prunus persica* L.), having a delicious flavor and excellent health benefits, are among the most popular fruits. Due to their distinctive flavor and rich nutritional components, such as minerals, carbohydrates, and amino acids, peaches are consumed frequently (Hussain et al., 2021). Peaches have a variety of antioxidant substances, such as vitamins, phenolic compounds, volatiles, carotenoids, and organic acids (Bento et al., 2022). Being climacteric fruits, peaches have a short shelf life and are susceptible to rot because they produce ethylene while being stored, and their respiration rate also increases (Busatto et al., 2017). Furthermore, fungi that cause diseases such as brown rot (*Monilinia* spp.), grey mold (*Botrytis* spp.), blue mold (*Penicillium* spp.), and soft rot (*Rhizopus* spp.) can infect the soft tissue and thin pericarp of the peach during its postharvest storage (Obi et al., 2018; Ahmed et al., 2018; Aslam et al., 2019).

Due to its high incidence and quick penetration, soft rot caused by *Rhizopus* spp. is a frequent postharvest disease that reduces the quality of peaches after harvest and results in latent infection during storage. The maximum development rate of *Rhizopus* spp. in peaches is reached at a temperature of 25°C, with symptoms only delayed by a few days at lower temperatures of 0, 4.4, and 18°C. Peaches infected with *Rhizopus* spp. show water-soaked decay and grey stripe mycelia surrounding the disease lesions, causing them to lose their economic value (Zhou et al., 2019). As a result of post-harvest losses, the fruit supply reduces, which also increases the price per unit for marketing and transportation. Postharvest losses ultimately affect consumers and producers equally due to high pricing and decreased availability (Murthy et al., 2009).

Chemical fungicides are used as an effective way to manage *Rhizopus* rot, but they also have a major adverse effect on the environment and human health (Iqbal and Mukhtar, 2020a). For the management of *Rhizopus* rot, several fungicide groups, such as iprodione, boscalid, fludioxonil, and tebuconazole have been extensively used. Pathogens develop resistance to the targeted chemicals due to the stable and widespread use of synthetic fungicides and pesticides in packinghouses, leading to the development of resistant strains. These challenges underscore the need for alternative techniques to control postharvest diseases in fruits and vegetables, particularly the *Rhizopus* rot of peach fruits (Zhang et al., 2008; Iqbal and Mukhtar, 2020b; Ahmed et al., 2021; Azeem et al., 2021; Mukhtar et al., 2021, 2023; Haq et al., 2022; Saeed et al., 2021; Shahbaz et al., 2023; Shakoor et al., 2015).

Using natural chemicals and plant-derived products, instead of synthetic fungicides, for postharvest treatments is an environmentally favorable choice. The avoidance of post-harvest fruit disease by using essential oils is a promising method of control (Nabila and Soufiyan, 2019; Sales et al., 2016; Yazdani et al., 2011). The objective of the current study was therefore to determine post-harvest rot of peach disease and explore its management with essential oils.

MATERIALS AND METHODS

Isolation and identification of the fungus

From 15 diseased samples of peach fruit showing soft rot, a total of 10 isolates with similar morphologies were identified. Slices from the infected areas were cut into tiny pieces (1-2 cm) and disinfected with 1% NaOCl for 2 minutes after being washed with distilled water. These sterilized slices were then placed on Potato Dextrose Agar (PDA) medium and incubated at 32°C in the dark. To ensure pure fungal isolation, a single spore culture was obtained. The shape of the fungus was confirmed by examining eight-day-old mycelium under a light microscope at 100× magnification. To confirm the symptoms of the disease, pathogenicity test was performed, following the protocol described by Cui et al. (2019) and Saqib et al. (2020). For the pathogenicity test, each fruit was injured with a 3 mm deep and 3 mm wide wound using an inoculating needle. The identified pathogens were then injected into each wound using 10 µl of spore suspension (1×10^5 spore ml^{-1}). After inoculation, the fruits were incubated at 20°C for 5 days. After the 5-day incubation period, the incidence of the disease and the width of the lesions (in cm) were noted. For expressing the decay incidence and lesion diameter, the protocol described by Shao et al. (2013) was followed. Each test was replicated three times, with five fruits used in each treatment as per the methodology presented by Xu et al. (2021).

Determination of weight loss

All samples were weighed before and ten days after the treatments, during which they were stored at 4°C, to determine weight loss. The weight loss percentage was calculated using the given below formula:

$$\mathrm{W1} = \frac{(\mathrm{W0} - \mathrm{Wt})x}{\mathrm{W0}} \times 100$$

Where W1 represents the weight loss, W0 is the initial weight, and Wt is the post-treatment weight. The Mean SD values for all treatments across three replications were analyzed using ANOVA. Significant differences between all treatments were examined using Tukey's test with a p-value of 0.05 (Saqib et al., 2020).

Evaluation of essential oils for controlling postharvest disease

Various concentrations of essential oils of cinnamon, and clove (0%, 25%, 50%, 75%, and 100%) were prepared by mixing them with Tween 20 surfactant (0.05 ml) and combining the mixture with 1 L of water while ensuring the correct concentrations. The fruits were submerged in this treatment solution for one minute. For the treatments with artificial inoculation, mycelium discs (7 mm) of *Rhizopus* spp. were introduced by damaging the fruit with a needle after the fruits had been dipped. Subsequently, the fruits were dried on sterilized paper. Only post-harvest treatments with essential oils were applied to fruits that had not been artificially inoculated, to determine the impact of latent infections. The fruit was placed in 36×20 cm^2 plastic trays with a clear lid with a diameter of 50 mm and surrounded by 2 cm high plastic polyvinyl chloride (PVC) rings. To maintain humidity, the fruit was placed on a paper towel saturated with distilled water, and was left on the bench for 5 days at a carefully regulated temperature of 25°C (Fontana et al., 2021).

Statistical analysis

All the recorded data were subjected to statistical analysis by using the suitable statistical package and differences were compared using LSD (least significant differences) (Steel et al., 1997).

RESULTS AND DISCUSSION

Colony morphology and microscopic identification

Under the microscope, *Rhizopus* was observed to contain sporangiospores. The aerial stolons, along with sporangia containing sporangiospores, were clearly visible. The color of both Rhizopus mycelium and spores was light brown (Figures 1 and 2).

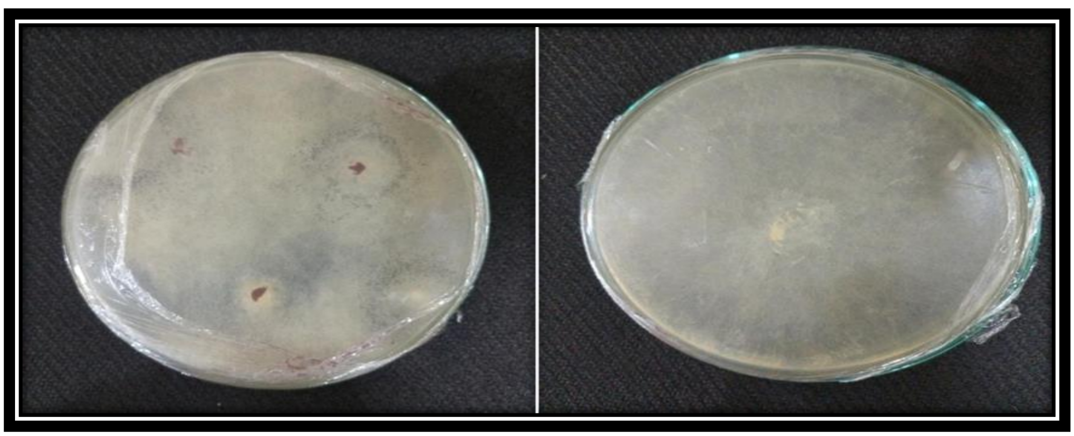

Figure 1: (A) Colony of *Rhizopus* on PDA plate (Isolation Plate), (B) Pure cultured plate.

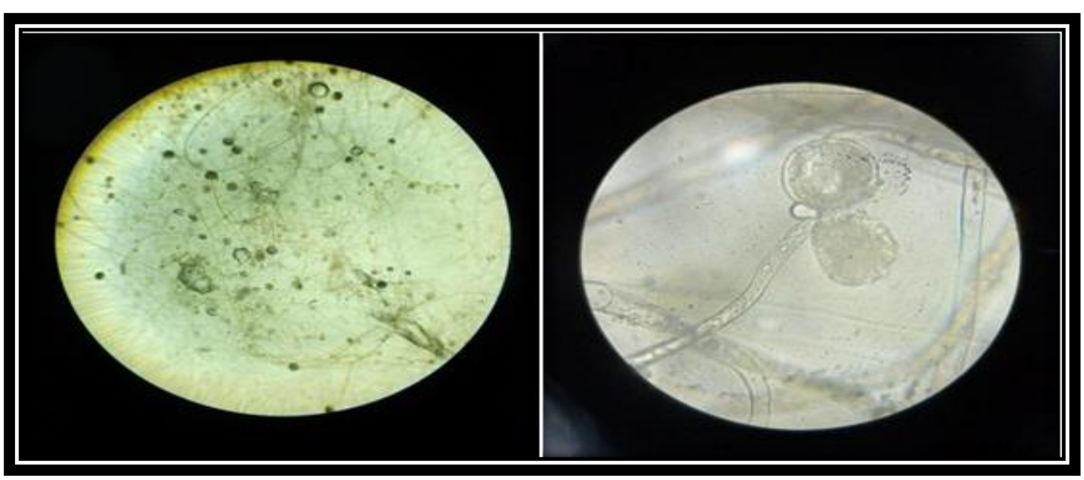

Figure 2: Identification of *Rhizopus* under 40× and 100× magnification.

Pathogenicity test

The pathogen caused lesions on the surface of the fruit with visible aerial growth of mycelium. The diameter of the lesions on infected fruits was 8.2 cm, while on control fruits; it was 0 cm (Figure 3, Table 1).

Effect of essential oils against *Rhizopus* rot of peach

Two essential oils, cinnamon oil, and clove oil, were tested for their potential to inhibit fungal growth on peach fruits. Cinnamon oil exhibited a higher inhibition of fungal growth compared to clove oil. The diameter of lesions on fruits treated with clove oil was 1.6 cm, while on fruits treated with cinnamon oil, it was 1.3 cm. For infected control fruits, the lesion diameter was 2.4 cm, and for healthy control fruits, it was 0 cm (Figures 4, 5 and 6, Table 2).

Numerous essential oils have been found to have antifungal properties against a variety of pathogens, and these properties were primarily influenced by the major antimicrobial components of essential oils. The main aromatic compounds present in clove oil and cinnamon oil, respectively have broad-spectrum anti-microorganism action, protecting food from fungal spoilage (Nostro et al., 2012). According to an *in vitro* research (Ochoa-Velasco et al., 2018), it might prevent the growth of *Botrytis cinerea*, *Phytophthora cactorum*, *Cryphonectria parasitica*, and *Rhizopus* rot. In the current investigation, essential oils showed strong antifungal efficacy against *Rhizopus* rot of peach under *in vitro* conditions (Wang et al., 2017).

Figure 3: Infected (left two) and control (right two) peach fruit to confirm pathogenicity.

Table 1: Lesions diameter of *Rhizopus* on peach fruit.

Sr. No	Treatment	Lesion diameter (cm)
1	Infected (without treatment)	3.4
2	Control	0.0

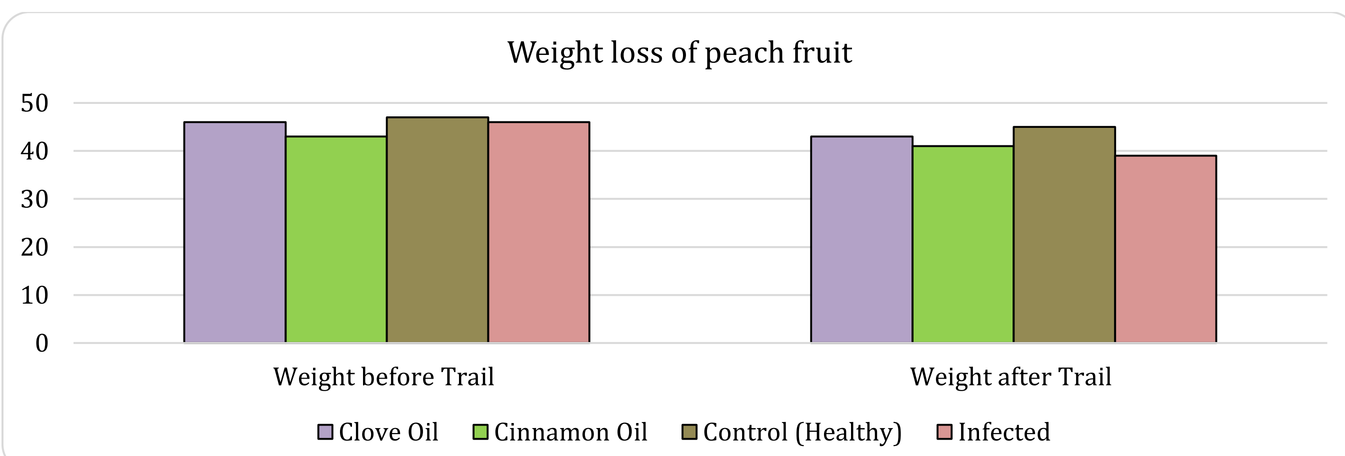

Figure 4: Weight loss of peach fruit before and after treatments.

Figure 5: Infected control of peach fruit.

Figure 6: Peach fruit treated with essential oils.

Table 2. Effect of essential oils on the development of *Rhizopus* rot on peach

Sr. No	Treatment	Lesion diameter (cm)
1.	Clove oil	1.6
2.	Cinnamon oil	1.3
3.	Infected control	3.4
4.	Healthy control	0

The continued evolution of microorganisms, the resistance of fungal isolates to treatments being used, and the need for contamination-free food has made it crucial to find effective solutions for managing fruit diseases. As a result, the effectiveness of the essential oils investigated in this work can reduce the use of natural fungicides. Additionally, scientists are now more interested in utilizing essential oils to treat fruits and vegetables since they are categorized by the FDA (Food and Drug Administration) as GRAS (Generally Recognized as Safe) (González-Aguilar et al., 2008; Fontana et al., 2021).

Based on observation and results presented in this experiment, it is confirmed that essential oils are suitable to use for controlling fungal diseases as well as reduces water loss without causing any damage to fruit, human beings as well as the environment.

CONCLUSION

It is concluded that essential oils of clove and cinnamon show promise in controlling peach fungal disease, reducing water loss and promoting sustainable agriculture. Future research should test essential oils in combination with other biocontrol agents against fungal diseases of other fruits and vegetables.

AUTHORS' CONTRIBUTIONS

TM and AM came up with the concept for the study; ARK, AM, and GA collaborated on the study design; TM, KA, and BK were responsible for collecting diseased samples; ARK and AM assisted in the isolation and identification of the fungi; TM, KA, and BK conducted the essential oil screening trial; TM and ARK performed statistical data analysis; TM took the lead in writing the manuscript; AM and ARK provided proofreading and editorial support for the manuscript; AM supervised the entire project.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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