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(Research Article)

Assessment of antifungal treatments in the post-harvest of oranges (*Citrus sinensis*)

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Abstract

The aim of this study was to evaluate the effectiveness of mineral oil, sodium bicarbonate, sodium hypochlorite, distilled water, and the fungus *Trichoderma harzianum* as alternatives for fungal control in oranges during postharvest. One hundred and twenty oranges were used, distributed in groups of five fruits per treatment. Each orange was subjected to one of the following treatments: *T. harzianum* (1×10^9 spores/mL), sodium bicarbonate (1%), sodium hypochlorite (1%), mineral oil, or distilled water, immersed for two minutes. Twenty additional oranges received no treatment and served as a control. The oranges were stored for 14 days at room temperature in plastic containers sealed with absorbent paper. The study was conducted in quadruplicate to determine fungal growth or other alterations. Growth of the fungus *Penicillium digitatum* was observed, being more frequent in oranges treated with distilled water, with 30% growth. In the treatments with sodium hypochlorite, *T. harzianum*, and sodium bicarbonate, growth reached only 20%. The control oranges showed 30% growth, like the distilled water treatment. Meanwhile, in oranges treated with mineral oil, fungal growth occurred in only 5% of the fruit, showing differences between treatments and demonstrating the greatest efficacy in postharvest fungal control. These results suggest that mineral oil is the most effective alternative for fungal control in oranges during postharvest. *P. digitatum* was identified by observing its colonial and microscopic morphology.

Keywords: *Citrus sinensis*; *Trichoderma harzianum*; *Penicillium digitatum;* Mineral oil; Sodium bicarbonate; Sodium hypochlorite.

1. Introduction

Citrus fruits are widely consumed because of their distinctive organoleptic qualities and their substantial nutritional contribution to the daily diet. They are a major source of vitamin C and provide readily digestible sugars [1].

Fruit quality depends not only on harvesting but also on post-harvest handling, which includes transport, turning over, washing, waxing, packaging, and storage. Harvesting, typically performed manually, must be carried out carefully to avoid damaging the fruit or exposing it to conditions that encourage contamination, such as direct sunlight [2]. Depending on the fruit and supply chain, days or even months may pass between harvesting and consumption. During this period, the fruits continue to ripen and senesce, undergoing physiological and biochemical changes that influence their organoleptic properties and may promote microbial growth [3].

The genus *Citrus* comprises more than 20 species, all producing edible fruits that are rich in vitamin C, flavonoids, and essential oils. The orange (*Citrus sinensis*) is a small, round fruit with a thin to semi-thick peel, abundant juice, and oval

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seeds. Its pith surrounds juicy, sweet pulp, and the fruit is distinguished by its characteristic aroma. Oranges are among the most widely consumed citrus fruits [4]. The main post-harvest diseases affecting oranges include green mold (caused by *Penicillium digitatum*), blue mold (*Penicillium italicum*), sour rot (*Geotrichum citriaurantii*), gray mold (*Botrytis cinerea*), and necrotic lesions caused by *Alternaria alternata*. Citrus fruits are typically infected through wounds sustained before, during, or after harvest, which can lead to irreversible infections [5].

The chemical control of fungi and rots in fruits and vegetables through post-harvest fungicides has been widely practiced. However, their use is limited due to negative consequences such as toxicity, environmental contamination, and the development of pathogen resistance. Consequently, natural alternatives with antimicrobial properties are being explored to reduce losses both in the field and during storage. Among these alternatives, biological control using bacteria, yeasts, and filamentous fungi, as well as the application of plant extracts and mineral salts, has proven particularly effective against citrus rots caused by fungi such as *Penicillium digitatum* and *Penicillium italicum* [6].

This study evaluated the effects of chemical coatings, mineral salts, oils, and biological agents, including *Trichoderma harzianum*, on phytopathogenic fungi and identified the species that affect orange quality during storage, aiming to determine the most effective approach to minimize losses.

2. Material and methods

2.1. Experimental design

A completely randomized design was used, consisting of six treatments with four replicates per treatment. Each replicate contains five orange fruits. In total, 120 orange fruits will be used, divided into 30 experimental units for processing.

2.2. Treatments

Six treatments were evaluated on oranges, using five fruits per treatment. The procedures for each treatment were as follows:

- *Trichoderma harzianum* treatment. Unwashed oranges were immersed for 2 minutes in a suspension of *Trichoderma harzianum* spores in distilled water at a concentration of 1×10^9 spores/mL. The suspension was prepared from 8-day-old cultures grown on PDA (Potato Dextrose Agar). Sterile distilled water was added to the culture surface, which was then gently scraped with a sterile glass rod to release the spores. The resulting suspension was transferred to Erlenmeyer flasks and diluted with distilled water to achieve the desired concentration.
- Sodium hypochlorite treatment. Oranges were immersed for 2 minutes in a 1% (w/v) sodium hypochlorite solution.
- Sodium bicarbonate treatment**.** Oranges were immersed for 2 minutes in a 1% (w/v) sodium bicarbonate solution prepared in distilled water, with the pH adjusted to 8.3.
- Mineral oil treatment. The oranges were coated with a thin layer of mineral oil using a brush.
- Plain water treatment. The oranges were submerged in plain water for 2 minutes.
- Control group. The oranges were placed directly in a plastic container lined with absorbent paper. This treatment was used to monitor post-harvest fungal growth in the oranges under study.

After the oranges underwent the various treatments, each group was individually placed in a plastic container lined with absorbent paper and kept at 27 °C and 90% humidity for the 14 days of the experiment. At the end of this period, the presence of post-harvest diseases was assessed.

2.3. Phytopathological analysis of fungi in oranges

The treatments were evaluated every three days, and any observed mycelium, spots, or rot were analyzed microscopically for identification. The adhesive tape method was used to determine the different types of fungi present; this involved placing a piece of tape on the affected area and examining it under a microscope.

3. Results and Discussion

3.1. Treatments

The efficacy of different treatments to inhibit postharvest fungal growth in orange fruit was evaluated using a controlled phytopathological analysis. Before the start of the experiment, the fruit was examined macroscopically, confirming the absence of visible fungal structures on the surface.

The trial was conducted over 14 days at room temperature, with evaluations performed on days 3, 6, 9, 12, and 14. The experimental design was carried out in quadruplicate. The treatments tested were 1% sodium bicarbonate, 1% sodium hypochlorite, mineral oil, *Trichoderma harzianum* at concentration of 1×10^9 spores/mL, distilled water, and an untreated control.

At the conclusion of the experiment, the number of fruits exhibiting fungal growth was recorded, allowing for comparison of the efficacy of each treatment. The results indicated that mineral oil was the most effective, achieving 95% inhibition of fungal growth across all four replicates. In contrast, the distilled water treatment showed the lowest efficacy (70%), with the highest incidence of fruit deterioration and rot, similar to the control group. The treatment with *Trichoderma harzianum* demonstrated 80% inhibition, comparable to the results obtained with 1% sodium hypochlorite and 1% sodium bicarbonate (Table 1, figure 1).

Table 1 Fungal growth on the surface of oranges.

Treatment	Fungal growth	Percentage of fungal growth inhibition
Mineral oil	1/20	95
Trichoderma harzianum	4/20	80
1% Sodium hypochlorite	4/20	80
1% Sodium bicarbonate	4/20	80
Distilled water	6/20	70
Control	6/20	70

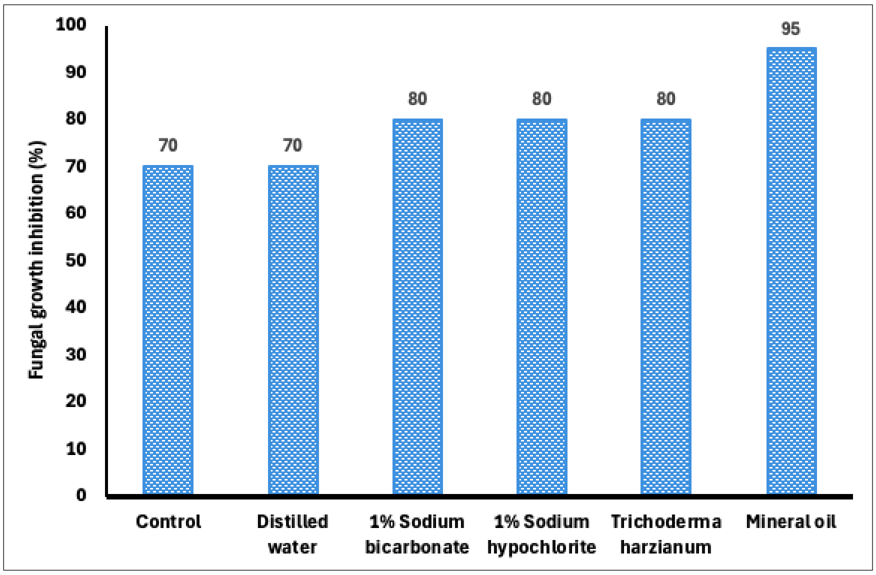

Figure 1 Treatment efficacy. A comparison is shown between the treatments evaluated in the study and the control group.

3.2. Microscopic Identification

The fungus isolated from the oranges was identified as *Penicillium digitatum*. Macroscopic identification was based on the olive-green color of the colonies covering the fruit peel, with a circular morphology and predominantly white mycelium. The colony surface, once the conidia formed, appeared velvety, cottony, or filamentous. Microscopic confirmation revealed structures typical of *P. digitatum*, with conidia arranged in brush-like branches that terminate in conidiogenous cells (phialides). The conidia are irregular, large, biverticulate, and green, with nearly cylindrical spores and rough, cylindrical phialides [7], as shown in figure 2.

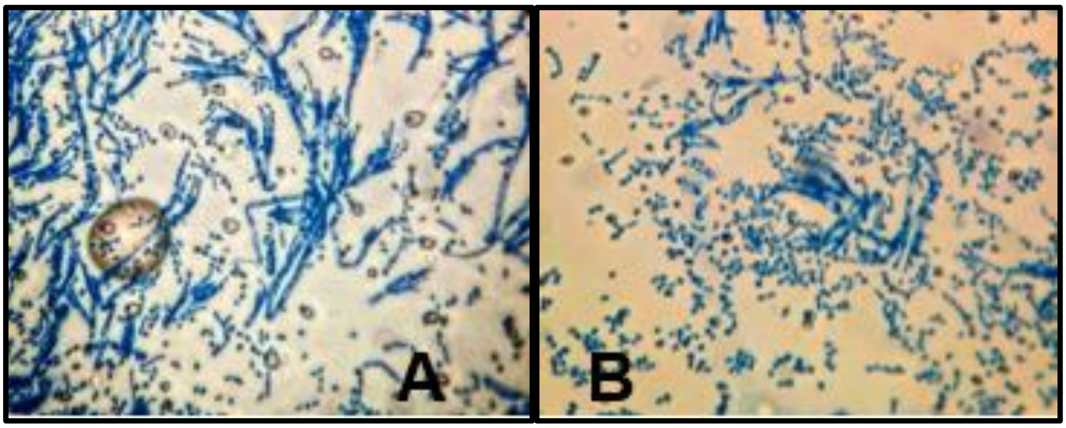

Figure 2 Microscopic colonial morphology of *P. digitatum*. The fungus is observed with (A) 40x and (B) 10x objectives, stained with methylene blue.

Mineral oil has demonstrated both insecticidal and acaricidal activity by forming a thin film on the surface of oranges, which coats and suffocates insect spores, thereby preventing colonization. Its effectiveness in pest control depends on physicochemical properties such as viscosity, the number of paraffinic carbons, distillation temperature, and saturated hydrocarbon content. These same properties also contribute to its ability to inhibit fungal growth [8].

In contrast, the distilled water treatment exhibited the lowest preventive effect, with the highest number of fruits showing fungal growth and rot. These results suggest that water creates a high-humidity environment that favors microbial development. While it can remove some surface contaminants, it may also cause adverse effects such as pigment discoloration and oxidation of compounds, including vitamin C. Additionally, the increased humidity promotes peel softening and facilitates pathogen proliferation, thereby accelerating fruit deterioration [9].

The fungus *Trichoderma harzianum* exhibits multiple mechanisms of action in phytopathological control, including competition for substrates, deactivation of pathogen enzymes, and antibiosis. Its widespread use is attributed to its ease of isolation, rapid growth, and ability to develop on diverse substrates. Additionally, it produces antifungal metabolites and hydrolytic enzymes that induce cellular alterations in pathogens, such as vacuolization, cytoplasmic disintegration, and cell lysis [10]. Its tolerance to a range of environmental conditions enhances its effectiveness as a biocontrol agent; however, its efficacy may be reduced by prior application of conventional fungicides [11]. In the present study, *T. harzianum* demonstrated moderate inhibition of postharvest fungal growth in oranges, likely due to its capacity to colonize the fruit surface, compete for nutrients, and adapt to varying environmental conditions [6].

Sodium hypochlorite and sodium bicarbonate, both 1%, demonstrated 80% efficacy in controlling postharvest fungal growth, whereas the control treatment showed approximately 10% lower inhibition, as presented in table 1 and figure 1. Sodium bicarbonate acts by altering the pH and reducing the water activity of the medium, creating unfavorable conditions for pathogen development and exerting a fungicidal effect due to its alkaline nature [12]. Sodium hypochlorite, widely used as a disinfectant in the food industry, reduces microbial load through its bactericidal and fungicidal properties; however, it is generally more effective against bacteria than against fungi [13].

The control lots were selected through macroscopic inspection, with fruits showing damage or visible pathogens being discarded. Despite this, 30% of the oranges in the control group exhibited fungal growth (Table 1), a value similar to that observed with the distilled water treatment. However, the control fruits showed a lower incidence of rot, suggesting that the additional moisture in the water treatment contributes to accelerated fruit deterioration.

Citrus infections typically originate at early stages, either on the tree, during harvest, or in transit. The most common symptoms include discoloration associated with mycelial growth and the appearance of olive-green spores (*Penicillium digitatum*) or blue spores (*Penicillium italicum*), which can lead to losses of up to 40% of production [14]. Several factors influence fruit susceptibility, including environmental conditions, agronomic management, the physiological state of the tissue, the presence of wounds, humidity, temperature, and inoculum load. Additionally, human practices during harvesting, postharvest handling, transport, and storage can promote infection and negatively affect product quality [15].

The most common postharvest fungi in citrus fruits are *Penicillium digitatum* and *Penicillium italicum*, which cause green and blue mold, respectively. These pathogens lead to significant fruit depreciation, often preventing marketability. Infection primarily occurs through wounds in the peel, highlighting the importance of minimizing mechanical damage during harvesting, transport, and storage. Nevertheless, these preventive measures are not always sufficient, so postharvest treatments are employed to reduce losses. The development of these fungi is influenced by factors such as humidity, environmental conditions, inoculum load, and the physiological condition of the fruit tissue. Under favorable conditions, these microorganisms become active, causing visible signs of deterioration that compromise product quality [7,16].

Green mold, caused by *Penicillium digitatum*, is characterized by the appearance of circular green spots on the rind, which rapidly expand to cover large portions of the fruit with an olive-green hue. Blue mold, caused by *Penicillium italicum*, is less common and forms bluish-gray colonies with similar growth characteristics. Both diseases lead to soft rot that progresses until the fruit surface is covered by a layer of dry spores, which detach easily and facilitate the spread of the pathogen [7,17].

Alternatives for the prevention and control of postharvest diseases in oranges include the use of natural products and compounds that are safe for consumers while exhibiting strong antimicrobial activity. Among the most employed strategies are biological control, plant extracts, mineral salts, oils, and disinfectants. Biological control, particularly with *Trichoderma harzianum*, is widely used due to its low cost, ability to colonize fruit surfaces, and antagonistic effects against pathogens. Similarly, mineral salts, such as sodium bicarbonate and sodium carbonate, inhibit the development of fungal diseases by altering pH and other environmental conditions [5,18].

Sodium hypochlorite is commonly used as a disinfectant to reduce microbial load during pre-storage washing, whereas distilled water primarily serves to remove surface contaminants, albeit with lower preventive efficacy. Mineral oils, in contrast, form a protective film on the fruit surface, limiting fungal colonization by suffocating spores [19]. The results obtained in this study align with previous reports, confirming the variable effectiveness of these treatments in controlling postharvest pathogens.

4. Conclusion

Mineral oil was the most effective treatment for inhibiting postharvest fungal growth in oranges, providing protection against a range of microorganisms and enhancing fruit preservation. Immediate application after harvest is crucial to minimize losses during transport, storage, and marketing.

Trichoderma harzianum and 1% sodium bicarbonate, as well as 1% sodium hypochlorite, showed moderate efficacy, reducing fungal growth while offering environmentally friendly or widely accepted alternatives. Distilled water exhibited the lowest preventive effect, highlighting the importance of combining proper hygiene with more effective treatments. Overall, these results underscore the value of selecting appropriate postharvest strategies to maintain fruit quality and reduce economic losses.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that do not have any conflict of interest.

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