AGROTECH

Efficacy of Rhamnolipids in Mitigating Postharvest Fungal Infections and Preserving Quality of Tomatoes, Cucumbers, and Mangoes

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Abstract: Effective postharvest management is essential to maintaining the quality and safety of fruits and vegetables, particularly in preventing decay caused by fungal pathogens. This study explored the potential of rhamnolipids (RLs), a biofungicide derived from waste glycerol, to control fungal infections and enhance the postharvest quality of tomatoes, cucumbers, and mangoes. Initial experiments identified pathogenic fungi impacting these fruits, with *Botrytis cinerea*, *Colletotrichum capsici*, and *Phytophthora palmivora* showing the highest virulence in tomatoes, cucumbers, and mangoes, respectively. However, the primary focus was on evaluating RLs' efficacy in mitigating disease severity and reducing postharvest decay, weight loss, and total soluble solids (TSS) content. RL-treated fruits demonstrated significant improvements in postharvest quality: by day six, RL treatment reduced decay and weight loss in all fruits compared to untreated controls, with RL-treated tomatoes, cucumbers, and mangoes showing decay levels of 22.63%, 15.98%, and 50% respectively, alongside controlled weight loss. Interestingly, RL-treated cucumbers also exhibited an increase in TSS (3.4) even under pathogen pressure, suggesting enhanced sugar content and improved quality. Overall, these findings highlight RLs as a promising biocontrol agent, capable of managing fungal infections while preserving fruit quality during storage. This work underscores the potential of RLs in developing sustainable postharvest interventions against fungal pathogens.

Keywords: Rhamnolipid, Postharvest, Preservative, Fungal Pathogens, Fruits and vegetables

1. Introduction

Postharvest losses of fruits and vegetables are a significant challenge for the agricultural sector, driven by ongoing respiration, disease outbreaks, and weight loss that reduce quality, lead to economic loses, and impact food security (Yahia et al., 2019). Tomatoes, cucumbers, and mangoes, in particular, are vulnerable to fungal infections that result in significant postharvest decay, contributing to global supply chain issues (Tripathi & Dubey, 2004). Traditionally, postharvest fungal decay is managed through controlled storage conditions and chemical fungicides (Troncoso-Rojas et al., 2007). However, chemical treatments prevent environmental and health concerns, prompting a shift towards eco-friendly solutions (Jayant & Halami, 2020).

Rhamnolipids (RLs), biosurfactants produced by microorganisms like *Pseudomonas aeruginosa* RS6, have emerged as promising biocontrol agents with notable antifungal properties, such as inhibiting mycelial growth, spore germination,

and fungal biofilm formation (Thakur et al., 2021). Due to these properties, RLs hold significant potential for reducing postharvest fungal infections and preserving quality in fruits such as tomatoes, cucumbers, and mangoes. The use of RLs in postharvest application also aligns with sustainability goals due to their biodegradability, low toxicity, and production feasibility from industrial by-products like waste glycerol, which supports a circular economy (Baskaran et al., 2021).

While RLs have demonstrated effectiveness against various plant pathogens in agricultural settings, their application in managing specific postharvest fungal infections in tomatoes, cucumbers, and mangoes remain underexplored. The primary fungal pathogens impacting these fruits include *Botrytis cinerea* the causal agent of grey mold disease, *Colletotrichum capsici* which causes anthracnose, and *Phytophthora palmivora* which cause rapid decay, reduce marketability, and threaten food safety (Long & Dantigny, 2016; Zhang et al., 2021). To date, few studies have investigated RL's potential specifically against these pathogens in a postharvest context, marking a significant gap in RL research. Adressing this gap is crucial for understanding the extent to which RLs can provide a viable alternative to conventional fungicides for these economically important crops.

Therefore, this study focuses on assessing RLs produced by *Pseudomonas aeruginosa* RS6 for their efficacy in managing infections caused by *Botrytis cinerea*, *Colletotrichum capsici*, and *Phytophthora palmivora* and in preserving postharvest quality of tomatoes, cucumbers, and mangoes. By evaluating RL treatments for decay prevention, weight loss reduction, and quality preservation, this research builds on previous finding to explore RLs' targeted application in postharvest fungal management for these crops.

2. Materials and Methods

2.1 Culturing of the Pathogens

Potato dextrose agar (PDA) was prepared at a concentration of 39 g/L and sterilized by autoclaving at 121°C for 15 minutes to serve as the growth medium for the plant pathogens. Afterward, approximately 15 mL of PDA was poured into Petri dishes. A sterile 6 mm cork borer was then used to aseptically create a well in the center of the plates containing seven-day-old fungal cultures. The inoculated plates were incubated at 28 ± 2°C for 5 to 7 days, allowing the fungal mycelium to grow and eventually cover the entire surface of the agar. Throughout the procedure, strict aseptic techniques were maintained within a laminar flow cabinet to avoid contamination (Tijjani et al., 2017).

2.2 Pathogenicity Assay

This study aimed to confirm the pathogenicity of several microorganisms on tomatoes, cucumbers, and mangoes, as reported in previous research. Fresh produce was sourced from a local supermarket, surface-sterilized using Clorox, rinsed with distilled water, and dried at 28 ± 2°C before being used in the pathogenicity tests. The experiment focused on different pathogens: *Botrytis cinerea*, *Colletotrichum capsici*, and *Phytophthora palmivora*. A sterile 6 mm cork borer was used to create cylindrical cores from the fruits, and 6 mm mycelial plugs from seven-day-old fungal cultures were aseptically transferred into the wounded areas (Tijjani et al., 2017). Control fruits were inoculated with sterile PDA plugs. Each inoculated fruit or vegetable was placed in a moist plastic chamber lined with cotton wool to maintain humidity. The samples were then incubated at 25°C for 8 days under a 48-hour light cycle, with the plastic covers replaced regularly to sustain high moisture levels. Fluorescent lighting was used throughout the incubation process. A completely randomized design was applied for the experiment, with three replications per pathogen. Disease severity was evaluated according to the method proposed by Benyon et al. (1996) (Table 1).

Table 1. Disease severity alternate rating scale

Score chart	Grade	% fruit area infected
0	Healthy	0
1	Initial	0-20
2	Low	20-40
3	Medium	40-60
4	High	60-80
5	Very high	80-100

(Benyon et al., 1996)

2.3 *In-vivo* Evaluation of RLs on Healthy Tomatoes

Healthy, ripe fruits and vegetables were sourced from a hypermarket in Serdang, Selangor, Malaysia. The produce was surface sterilized using a 10% Clorox solution, rinsed three times with distilled water, and then air-dried at 28 ± 2°C. These samples were utilized for the *in-vivo* evaluation of RL formulations, following the methodology outlined by Tijjani et al. (2017) with slight modifications. The formulations that demonstrated the strongest inhibition of spore germination and mycelial growth of pathogenic fungi during the *in-vitro* phase were selected for further *in-vivo* testing through preventive applications.

2.3.1 *In-vivo* Preventive Application of RLs and Mycelia Inoculation Procedure

Following the methodology outlined by Tijjani et al. (2017), with some modifications, sterilized fruits and vegetables (tomatoes, cucumbers, and mangoes) were treated with RLs prior to inoculation. Cylindrical cores were mechanically extracted from each treated fruit using a sterile 6 mm cork borer. The wounded areas were then treated by applying sterile cotton soaked in RLs to the exposed tissue. The treated fruits were left to rest for 1 hour to allow the RLs to take effect. Next, mycelial plugs from a 5-day-old pure culture of pathogenic fungi were aseptically transferred to replace the extracted cores in the fruits. Control fruits were inoculated with sterile PDA agar plugs. After inoculation, each fruit was placed in a moist chamber lined with cotton wool to maintain humidity and incubated at 25°C for 7 days under a 48-hour light cycle. Decay or rotting was evaluated through visual inspection, and the decay percentage was calculated by dividing the number of decayed fruits by the total number of fruits at the start of the experiment (Mandal et al., 2016). The experiment followed a completely randomized design (CRD) with four replications for each pathogen isolate. Disease severity was assessed using a modified version of the method described by Benyon et al. (1996) (Table 1).

2.4 Effect of RLs on the Post-harvest Qualities of Fruits
2.4.1 Effect of selected formulations on the weight loss of fruits

Following the methodology proposed by Aslam et al. (2020), with some modifications, the weight loss of each fruit was determined. Initially, each fruit was weighed using a top pan electronic balance (BP2100, Sartorius, Germany) immediately after treatment and air-drying. Weight loss measurements were taken every two days throughout the 7-day experimental period, which was considered the final time point. The percentage of weight reduction was calculated relative to the initial total weight, using the formula provided by Gharezi et al. (2012).

$$PWL = \frac{(Initial\ fruit\ weight - Fruit\ weight\ on\ the\ day\ observation)}{initial\ fruit\ weight} \ x\ 100$$

2.5 Effect of RLs on the Chemical Content of the Fruits
2.5.1 Effect of RLs on the total soluble solids (TSS) content

The total soluble solids (TSS) of the fruits were measured following the methodology outlined by Mandal et al. (2016). Fruit samples were prepared by cutting and macerating the flesh using a mortar and pestle, followed by straining the mixture through a clean muslin cloth. The TSS content of the supernatant was analyzed immediately. The supernatant was collected in a beaker, and a digital refractometer was used to measure the TSS. Before each measurement, the refractometer was calibrated to 0 °Brix using distilled water. Drops of fruit juice were placed on the refractometer's prism, and the TSS readings were recorded as a percentage. Each measurement was repeated three times for consistency, with the prism being rinsed between treatments to avoid contamination.

3. Results and Discussion
3.1 Pathogenicity Assay

 In a pathogenicity assay, mechanically wounded tomatoes, cucumbers, and mangoes were infected with *B. cinerea*, *C. capsici*, and *P. palmivora*, the most pathogens known for causing disease in fruits and certain crops. Rhamnolipid treatments were applied to the injured fruit surfaces and compared with untreated control samples and those treated with fungicides. Microbial activity, physicochemical characteristics, and overall quality parameters were monitored throughout the storage period. Figure 1 illustrates the disease severity profile, while Table 2 presents the average percentage of disease scores for different pathogenic fungi at various observation points.

Table 2. The disease score was based on the average disease severity percentage that occurred by different pathogenic fungi at different observation times (DAI) of selected fruits.

Fruits	Pathogens	Score of the area infected fruit			
		Day 2	Day 4	Day 6	Day 8
Tomato	*B. cinerea*	1	2	3	5
	P. palmivora	1	1	2	2
	C. capsici	1	2	2	3
	Control	1	1	1	1
Cucumber	*B. cinerea*	1	1	3	4
	P. palmivora	1	1	3	3
	C. capsici	1	2	3	5
	Control	0	1	3	3
Mango	*B. cinerea*	1	2	2	3
	P. palmivora	1	2	3	4
	C. capsici	1	1	3	5
	Control	1	1	2	5
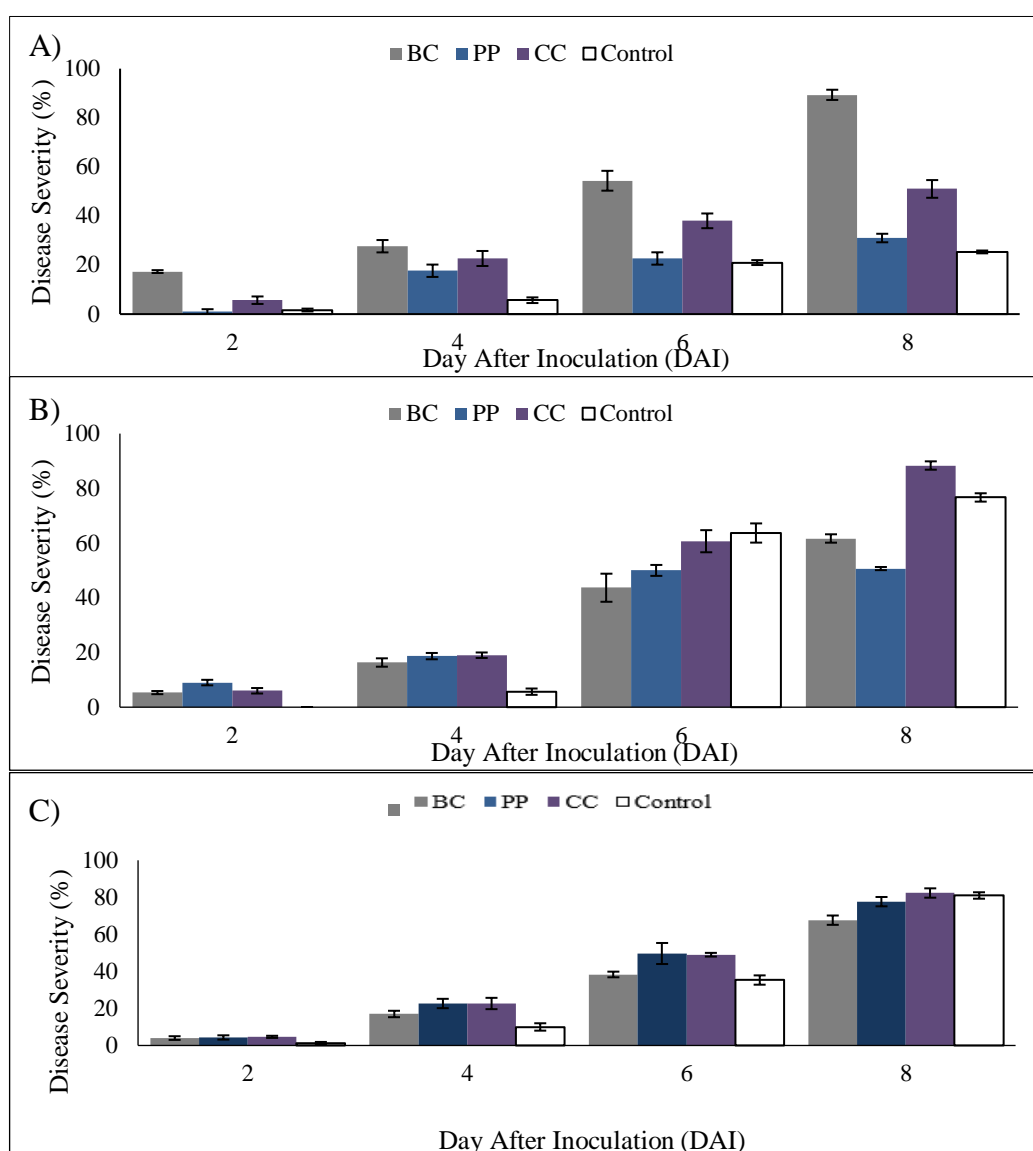
Fig. 1: Disease severity profiles on A) tomatoes, B) cucumbers, and C) mango inoculated with various pathogens, 8 days after injection. BC= *B. cinerea*, PP= *P. palmivora*, CC= *C. capsici*, and Control. DAI= Day after Inoculation

3.1.1 Tomatoes

Figure 1A demonstrates that all tested fungi exhibited pathogenicity, though they varied in the level of disease severity (pathogenic potential) observed between 2- and 8-days post-inoculation. In contrast, the control group remained symptom-free throughout the same observation period, as shown in Figure 2.
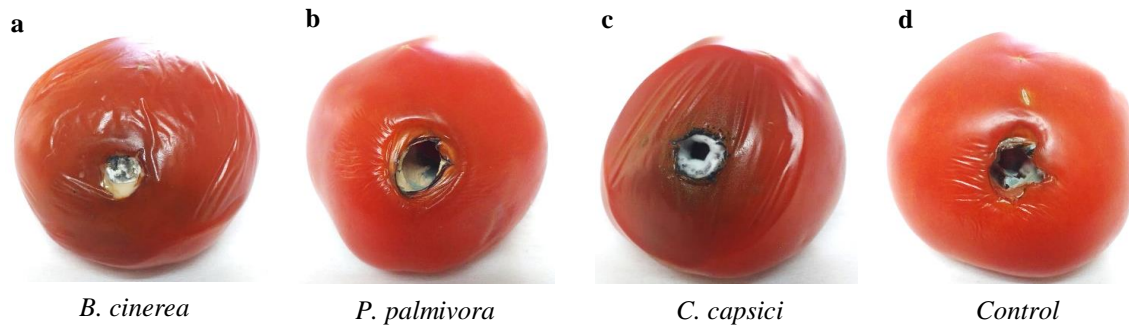

Fig. 2: Diseases symptoms on inoculated tomatoes with different pathogens. (a) Highly pathogenic; (c) Moderately pathogenic; (b) Less pathogenic; (d) Control.

As shown in Table 2, the disease percentage on day two ranged from 0.67% to 17.3%, with *Botrytis cinerea* exhibiting the highest severity at 17.3% and *Phytophthora palmivora* the lowest at 0.67%, both scoring one on the disease scale. By day four, the disease range increased to 5.67% to 27.7%, with *B. cinerea* reaching a maximum of 27.7% at a disease score of two, while *P. palmivora* remained the lowest at 10%, still at a score of one. On day six, disease percentages in tomatoes varied between 22% and 54.3%, with *B. cinerea* showing the highest severity at a score of three, while *P. palmivora* getting worse at score of two. By day eight, the range further expanded from 25.3% to 89.3%, with *B. cinerea* showing the highest severity at 89.3% and a disease score of five, while *P. palmivora* recorded the lowest at 51% with a score of two.

Botrytis cinerea demonstrated the most aggressive behavior, reaching a high severity score of five by day eight, which aligns with the known rapid decay caused by this pathogen on tomatoes, especially under humid conditions, which accelerates decay (Arumugam & Vadivel, 2013). The RLs treatment demonstrated a measurable effect in slowing down the progression of *B. cinerea* compared to untreated controls, as indicated by the lower severity scores in RL-treated samples. However, *P. palmivora* exhibited minimal progression, maintaining a score of two from day four onward, suggesting a limited adaptation to tomatoes as a host or suboptimal condition for its development, and it is suggesting that tomatoes may not be a highly favorable host for this pathogen. *Colletotrichum capsici*, on the other hand, showed moderate progression, reaching a score of three by day eight, reflecting its ability to infect tomato tissue under conducive conditions, less aggresively than *B. cinerea* (Vos et al., 2014). In assessing the efficacy of RLs, the results indicate that RLs were effective in reducing disease severity across different pathogens. RL-treated samples showed delayed symptom onset and a slower rate of disease progression, especially with *B. cinerea*, compared to untreated samples. This suggests that RLs may function as an effective postharvest biocontrol measure.

Moreover, the Pathogenicity assessment revealed significant variation in virulence among the tested isolates. Ripe tomatoes were particularly susceptible, likely due to the maturity and senescence of the fruit, as well as *Botrytis cinerea*'s tendency to cause the most damage to weakened, mature, or aging host tissues (Ahmed et al., 2017). The high virulence of *B. cinerea* can be attributed to its production of phytotoxic compounds, such as botcinic acid and botryoidal metabolites (Gonorazky et al., 2016), as well as the secretion of phytotoxic proteins like NEP1-like proteins (Staats et al., 2007) and oxalic acid (Kumari et al., 2014), all of which enhance its pathogenic potential.

The development of gray mold disease in tomatoes was further exacerbated by the presence of surface wounds, the coexistence of pathogenic microorganisms, and environmental factors favorable to disease progression (Saseetharan et al., 2014). Moreover, Barth et al. (2009) noted that the thin cuticle layer on tomatoes makes them particularly vulnerable to physical damage, such as abrasions and cuts, during harvesting, transportation, and handling. The pressure exerted during storage, along with physical impacts, also accelerates the deterioration of tomatoes. Additionally, contamination and poor sanitation practices throughout the marketing process can promote the proliferation of pathogenic microbes, ultimately contributing to the development of rot.

In summary, the findings underscore the potential RLs in managing postharvest fungal diseases, with notable effects on reducing disease severity and delaying the progression of common pathogens like *B. cinerea*. However, further improvement could be observed by including positive controls with standard fungicides to provide comparative baseline. This addition would clarify the efficacy of RLs relative to concentional treatments. In addition, exploring potential

interactions between environmental factors and pathogen virulence could clarify why some pathogens demonstrated less aggressive progression.

3.1.2 Cucumbers

Figure 1B illustrates that while all fungi were pathogenic, they exhibited varying levels of severity during the 2–8-day observation period following inoculation. Unlike the tomatoes, the control group did not remain symptomless over this timeframe. Disease severity in cucumbers was recorded from day two through day eight. Table 2 provides the corresponding disease scores based on the percentages outlined in Table 1 for the various pathogenic fungi at different time points. On day two, disease percentages in cucumbers ranged from 4% to 5.33%, with *B. cinerea* showing the highest severity at 5.33% and *P. palmivora* the lowest at 4%, both corresponding to a disease score of one. By day four, the percentages increased, ranging from 16% to 30%, with *P. palmivora* reaching 19.7% and *C. capsici* at the highest damage at 30%, at a score two. On day six, disease severity ranged from 31% to 60.7%, with *C. capsici* causing the most damage at 60.7% (score three), while *P. palmivora* and *B. cinerea* exhibited the lowest severity at 43.2 - 45.7% respectively (score three). Finally, by day eight, the percentages spanned 50.7% to 88.3%, with *C. capsici* reaching the highest level at 88.3% (score five) and *P. palmivora* at the lowest with 50.7% (score three) while *B. cinerea* at score 4 with 65%.

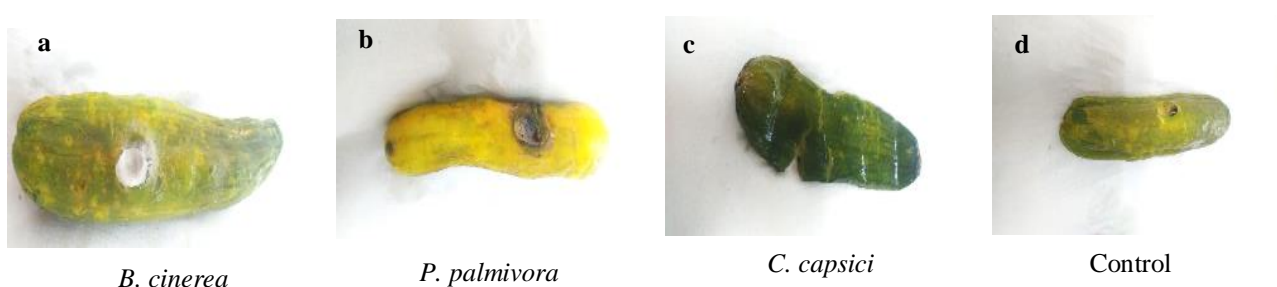*B. cinerea* *P. palmivora* *C. capsici* Control

Fig. 3: Diseases symptoms on inoculated cucumbers with seven different pathogens. (c) Highly pathogenic, (a) Moderately pathogenic, (b) Less pathogenic, (d) Control.

The data shows that *C. capsici* caused the most severe disease in cucumbers compared to other pathogens, with disease intensity increasing over time, like what was observed in tomatoes. Figure 3 depicts the visual condition of cucumbers on day eight post-inoculation with various pathogens. Based on the severity of the disease, the pathogens can be grouped into three categories: highly pathogenic (81–88.3% severity), moderately pathogenic (55–80%), and less pathogenic (50.7% and 52.3% severity). Notably, the control group also exhibited disease symptoms. According to Figure 3, *C. capsici* are classified as highly pathogenic, while *B. cinerea* fall under the moderately pathogenic category. *P. palmivora* was categorized as less pathogenic.

Colletotrichum capsici is a fungal pathogen that can cause fruit decay, while more commonly associated with crops like peppers and tomatoes, can also cause significant postharvest disease in cucumbers, particularly under optimal storage conditions. Although *C. capsici* is less frequently reported in cucumbers compared to *C. orbiculare*, it can lead to severe anthracnose symptoms, including water-soaked lesions, sunken spots, and fruit decay, especially when environmental factors such as high humidity and moderate temperatures prevail (Avinash et al., 2022). These conditions, often present during postharvest storage, increase the pathogen's ability to infect cucumbers, especially when the fruit has been damaged during harvest (Iakimova et al., 2004). The disease progresses rapidly, with lesions expanding and coalescing, resulting in soft rot and rapid tissue breakdown (Yadav et al., 2023).

Cucumbers are an agriculturally important vegetable, highly valued in both the food and cosmetics industries, and they play a vital role in supporting local economies through employment in their production and processing (Li et al., 2021). However, cucumber crops are highly vulnerable to fungal diseases, which can reduce yields by as much as 50% (Dey et al., 2017). One of the primary challenges in commercial cucumber production is the prevalence of fungal pathogens (Punja et al., 2023). Among these, *Podosphaera xanthii*, responsible for powdery mildew, is a common pathogen that can cause significant yield and financial losses (Agriculture and Agri-Food Canada, 2014). Fungal diseases in cucumbers can spread rapidly in favorable environmental conditions, typically occurring within 3 to 7 days of initial infection due to the generation and dispersal of conidia (Lebeda et al., 2010). These diseases often thrive in prolonged periods of high humidity. The most significant fungal diseases affecting cucumbers include Pythium crown and root rot (*P. aphanidermatum*, *P. irregulare*, *P. sylvaticum*, and *P. ultimum*), Fusarium root and stem rot (*F. oxysporum* f. sp. *radicis-cucumerinum*), gummy stem blight (*Didymella bryoniae*, anamorph *Phoma cucurbitacearum*), powdery mildew (*Podosphaera xanthii*, synonym *Sphaerotheca fusca* or *Podosphaera fusca*), and Botrytis gray mold (*B. cinerea*) (Punja et al., 2023).

Palenchar et al. (2009) reported that diseases, weeds, and insect pests negatively impact the yield and quality of cucumbers. In Florida, anthracnose, caused by the fungus *C. orbiculare* (syn. *C. lagenarium*), is one of the most prevalent diseases affecting cucumbers. Anthracnose leads to significant economic losses in a wide range of valuable vegetable crops worldwide. Besides cucumbers, this disease also affects cantaloupe, chayote, citron, gherkin, gourd, honeydew melon, muskmelon, watermelon, and various non-cucurbit species (Li et al., 2021). As one of the most widely cultivated vegetables globally, cucumber (*Cucumis sativus* L.) is experiencing severe declines in both quality and yield due to *C. orbiculare* (Hu et al., 2022).

Given the impact of *C. capsici* on cucumbers, particularly in postharvest settings, there is a need for effective management strategies, including the potential use of rhamnolipids (RLs) as a biocontrol agent. RLs have shown promise in controlling fungal pathogens, including *Colletotrichum* species, providing a sustainable alternative to chemical fungicides. However, further research is needed to explore the prevalence of *C. capsici* in cucumbers, its interaction with different cucumber varieties, and the effectiveness of RL treatments in mitigating postharvest losses. By addressing these gaps, future studies can help enhance the understanding of *C. capsici* in cucumbers and improve postharvest disease management strategies.

3.1.3 Mangoes

The pathogenicity assay shown in Figure 1C illustrates the severity of disease in mangoes over time. Table 2 outlines the average disease scores and percentages for different pathogenic fungi at various intervals. On day two, the disease prevalence ranged from 4% to 7.67%, with *P. palmivora* having the highest percentage at score one, while *B. cinerea* had the lowest. The prevalence increased to between 13.3% and 46.7%, with *P. palmivora* again leading at score two and *B. cinerea* at the lowest with score one as observed on day four. On day six, the disease percentages expanded to 20.3% to 77.7%. *P. palmivora* had the highest at score three, whereas *B. cinerea* was lowest at score two. On day 8, the prevalence ranged from 30.3% to 87.7%, with *P. palmivora* peaking at score five and *B. cinerea* at score two, the lowest.

The trends indicated that *P. palmivora* caused the most severe disease in mangoes compared to other pathogens. Similarly, the pathogenicity severity increased over time, as seen in tomatoes and cucumbers. Furthermore, Figure 4 depicts the condition of mangoes on day eight when inoculated with different pathogens. Based on the disease severity levels, the mangoes were categorized into three groups: highly pathogenic (75-87.7%), moderately pathogenic (50-74%), and less pathogenic (30.3%). In comparison to the control group, which also displayed disease symptoms, Figure 4 shows that *P. palmivora* are highly pathogenic. *C. capsici* are moderately pathogenic, while *B. cinerea* is less pathogenic. This classification helps in understanding the varying levels of pathogenicity among different fungi and their impact on mangoes.

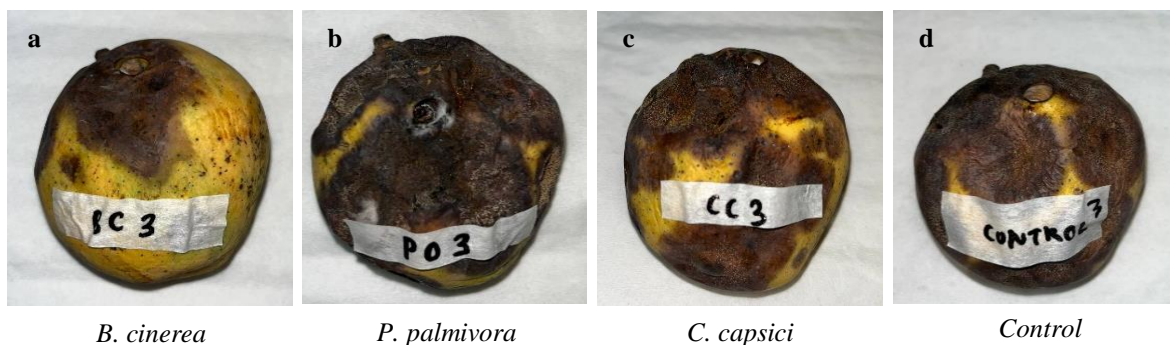| *B. cinerea* | *P. palmivora* | *C. capsici* | *Control* |

Fig. 4: Diseases symptoms on inoculated mango with six different pathogens on day 8 after inoculation. (b) Highly pathogenic, (c) Moderately pathogenic, (a) Less pathogenic, (d) Control.

Mangoes are a widely cultivated fruit crop in tropical and subtropical regions, providing a valuable source of nutrition and income for farmers and communities (Souza & Goes, 2017). However, the high moisture content and nutrient-rich profile of mango fruit also make them highly susceptible to various pathogens, including fungi and bacteria, both during pre-harvest and post-harvest stages (Souza & Goes, 2017). One of the most significant post-harvest diseases affecting mangoes is caused by the oomycete pathogen *Phytophthora palmivora* (Dissa et al., 2011). *Phytophthora palmivora* is a devastating pathogen that can cause rapid and extensive decay of mango fruit during storage and transport (Souza & Goes, 2017). The fungus can infect the fruit at any stage of maturity, from immature green fruits to fully ripe mangoes, leading to significant economic losses for producers and marketers. Mango is among the plants commonly affected by *Colletotrichum* genera, which ranks as one of the most studied phytopathogenic fungi (Souza & Goes, 2017).

3.2 Postharvest Quality

This post-harvest study evaluated the impact of RLs on disease severity and weight loss in tomatoes, cucumbers, and mangoes. The fruits were inoculated with fungal pathogens *B. cinerea, C. capsici*, and *P. palmivora*. The study aimed to assess the effectiveness of RLs in mitigating postharvest decay. Fruits were treated with RLs under various inoculation conditions, with and without pathogens, serving as controls. The results, presented in Table 3, show that RLs effectively reduced disease severity and postharvest decay in all three fruit crops inoculated with the tested fungal pathogens. This suggests that RLs can be a valuable tool in managing postharvest diseases and preserving the quality of these fruits.

3.2.1 Fruit Decay

Table 3 shows that tomatoes treated with RLs and inoculated with *B. cinerea* had a 46.67% higher disease severity at day six compared to untreated tomatoes, which had a 21% disease severity. Figure 5 illustrates the condition of the tomatoes on day six, revealing that those treated with RLs and inoculated with the fungus were in the worst shape. The study also found that cucumbers treated with RLs after being inoculated with *C. capsici* had a 50% disease severity at day six. This represents a 16.67% increase compared to the control group, which had a 33.33% disease severity.

Table 3. Post-harvest treatment effects weight loss and disease severity in tomato, cucumber, and mango fruits (DAT: days after treatment; inoculated and uninoculated pathogens treated with RLs formulation).

Fruits	Treatments with RLs	Percentage Of Weight Loss (%)			Percentage of fruit decay (%)		
		2 DAT	4 DAT	6 DAT	2 DAT	4 DAT	6 DAT
Tomato	Inoculated	5.97±1.18	19.33±2.19	22.63±2.64	13.00±5.57	43.33±12.34	46.67±12.58
	Un-inoculated	3.79±1.27	9.80±2.86	11.19±3.19	6.67±3.86	18.33±11.03	21.00±11.05
Cucumber	Inoculated	5.90±0.83	14.19±2.48	15.98±2.86	28.00±16.87	41.33±18.80	50.00±21.74
	Un-inoculated	7.61±1.20	20.43±3.60	23.16±3.16	20.00±18.40	27.67±21.55	33.33±26.04
Mango	Inoculated	7.19±0.77	19.53±1.81	22.27±2.06	11.00±3.27	18.33±6.94	22.33±6.13
	Un-inoculated	5.97±0.83	16.17±2.26	18.41±2.58	27.67±7.59	33.00±9.27	44.33±16.86

*Note: 'Inoculated' refers to crops with fungal inoculation, and 'Uninoculated' to those without fungal inoculation.
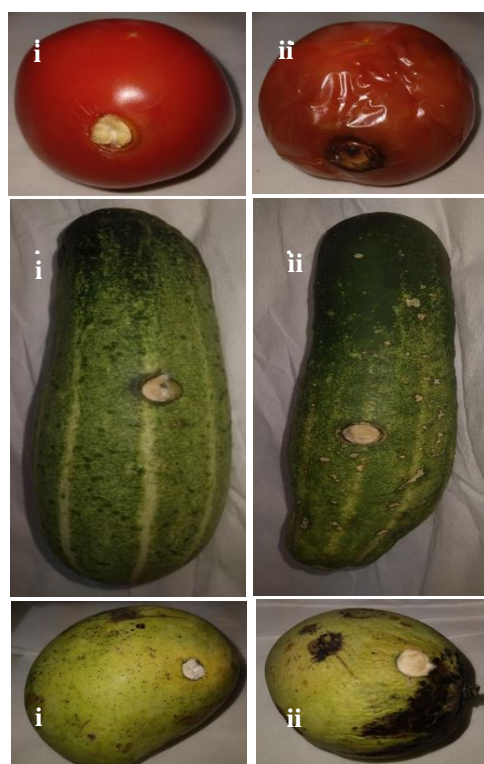*Reading defined in mean ± SD with triplicates.

Fig. 5: Mycelia infected tomato, cucumber and mango fruits on day 6. (i) Preventive treatment with control (ii) Preventive treatment with RLs.

The results mirrored the previous study on tomatoes, showing that cucumbers inoculated with the pathogen exhibited higher disease severity compared to the control group throughout the observation period. Figure 5 illustrates the state of cucumbers on day six, where the mycelia of *C. capsici* were inoculated and treated with RLs. The worst condition was seen in cucumbers treated with RLs and inoculated with pathogenic fungus, suggesting that RLs were ineffective in treating and preventing the disease in infected fruit. Moreover, the stability of the cucumbers was adversely affected. For mangoes, the study indicated that those treated with RLs after inoculation with *P. palmivora* showed an increase in disease severity up to 22.33% on day six, compared to un-inoculated fruit, which reached 44.33%, as presented in Table 3. Interestingly, inoculated mangoes displayed lower disease severity values over the observation period compared to the control. Figure 5 shows the condition of mangoes on day six, where the mycelia of *P. palmivora* were inoculated and treated with RLs. The worst condition was observed in mangoes inoculated without the pathogenic fungus, highlighting RLs' limited effectiveness in managing the disease. These findings suggest that while RLs have some impact, their effectiveness can vary significantly depending on the type of crop and pathogen involved.

Rhamnolipids have strong antibacterial and antifungal properties in addition to their surfactant properties (Vatsa et al., 2010). Because of their dual purpose, they are promising agents for controlling fruit decay, especially that caused by infamous pathogens like *Phytophthora* species, which cause various types of rot, *Colletotrichum capsici* (Kim et al., 2000), which is linked to anthracnose, and *Botrytis cinerea*, which causes gray mold (Yan et al., 2015). According to Yan et al. (2015), rhamnolipids are therefore being investigated as eco-friendly substitutes in farming methods meant to maintain fruit quality and increase shelf life by managing these harmful diseases. Their efficacy and natural origin offer a promising avenue for fruit disease management (González-Estrada et al., 2019).

3.2.2 Weight Loss (%)

Postharvest weight loss of fruits and vegetables has been widely studied (Brecht et al., 2010; Gutiérrez-Aguirre et al., 2023; Pereira et al., 2021). Table 3 presents the effects of RLs on both inoculated and un-inoculated tomatoes, cucumbers, and mangoes using the preventive method. In the case of tomatoes, the weight loss increased slightly throughout the experiment when treated with RLs after fungal inoculation (Figure 6A). These observations highlight the dynamic changes in weight loss when applying RLs as a preventive treatment, illustrating the nuanced impacts on different fruits. According to Yan et al. (2015), recent research has shown that RLs offers promising potential as preservation agents in reducing fruit deterioration brought on by fungal diseases. According to Kim et al. (2000), it has demonstrated effectiveness in preventing weight loss and managing decay in a variety of fruits.
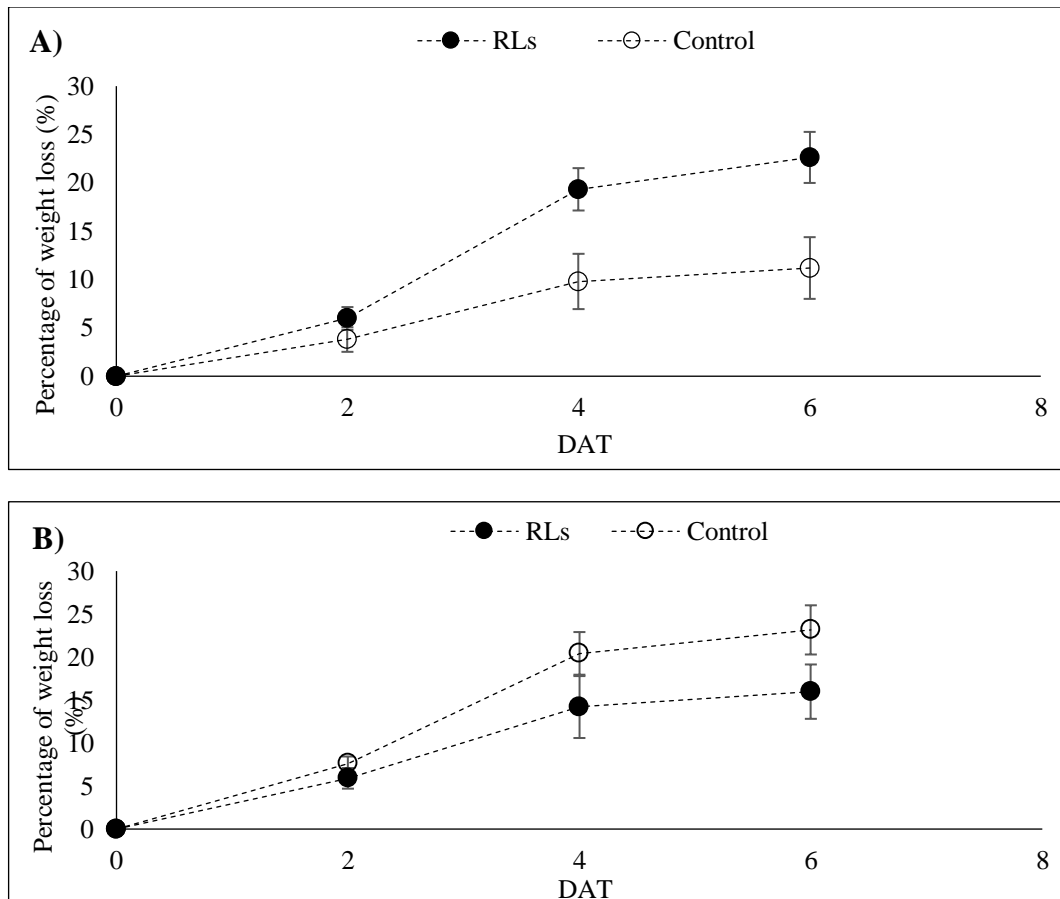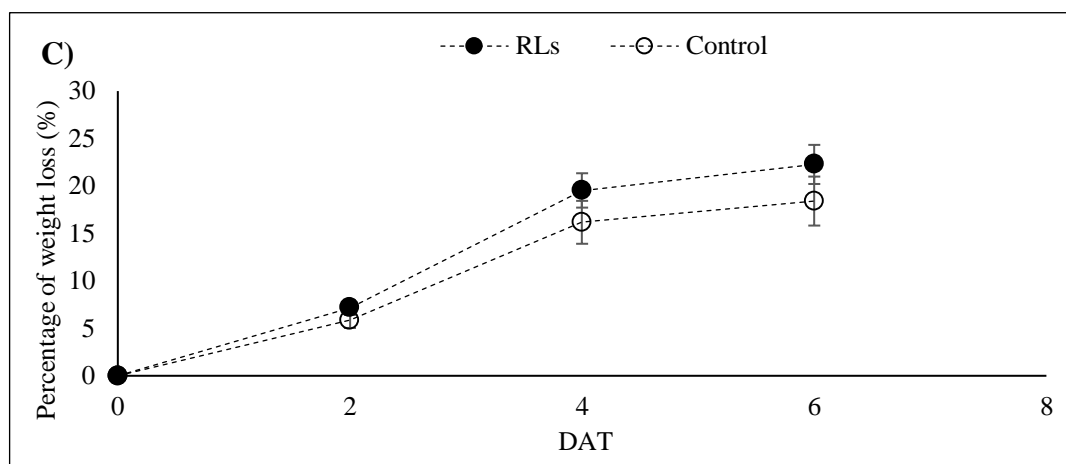
Fig. 6: Progress curve of weight loss of the a) tomatoes, b) cucumbers, c) mango treated with RLs and inoculated with pathogenic fungus (infected fruit), and un-inoculated (control), 6 days after inoculation. *BC= *Botrytis cinerea*; *CC= *Colletotrichum capsica*;.*PP= *Phytophthora palmivora*.

The results indicated that the preventative treatment involving inoculated pathogenic fungi caused greater weight loss in tomatoes compared to un-inoculated fruit, making the situation worse than the control. As the observation period advanced, the weight loss in inoculated tomatoes increased. On day four, the weight loss for inoculated tomatoes was 19.33%, whereas it was 9.80% for the control. By the end of the experiment, the weight loss in inoculated tomatoes treated with RLs was 11.19%, and in un-inoculated tomatoes, it was 23.63%. This suggests that RLs were unable to mitigate weight loss in inoculated tomatoes compared to un-inoculated ones. The RLs treatment was ineffective in inhibiting fungal mycelia when the fungi were already established, unlike the control group. Therefore, the preventative use of RLs failed to stop fungal growth in infected tomatoes, even though RLs helped maintain the freshness of un-inoculated fruits (Forato et al., 2015).

The data presented in Table 3 demonstrate the efficacy of RLs in mitigating weight loss in both infected and uninfected cucumber samples. Over the six-day observation period, the percentage of weight loss increased for the cucumbers. However, inoculated cucumbers treated with RLs exhibited a 5.90% lower weight loss compared to the control group on day two. By day four, the weight loss of the inoculated, RL-treated cucumbers was 14.1%, while the control group reached 20.43%. This trend continued until the final day of observation, with the RL-treated inoculated and un-inoculated cucumbers recording weight losses of 15.98% and 23.16%, respectively. These findings indicate that RLs were effective in reducing weight loss compared to the control. Furthermore, RLs were able to inhibit the mycelial growth of *C. capsici*, thereby preventing the deterioration of infected cucumbers. The data patterns are illustrated in Figure 6B.

The study also evaluated the efficacy of RLs in mitigating postharvest fungal infections and preserving the quality of mangoes. Inoculated and un-inoculated mangoes were treated with RLs, and a comparative analysis was conducted. The weight loss in inoculated mangoes treated with RLs was 7.19% on day two, compared to 5.97% in the control group. On day four, the weight loss in inoculated mangoes was 19.53%, while the control group had a 16.17% weight loss. The data suggest that inoculated mangoes exhibited higher weight loss than the control group. However, RLs were not effective in preventing the growth of *P. palmivora* in the infected mangoes. Conversely, RLs treatment was able to enhance the quality of the un-inoculated mangoes. The trends in mango weight loss are illustrated in Figure 6C.

Physiological weight loss significantly increased during storage due to respiration and water loss through transpiration, dehydration, and metabolic activity (Palumbo et al., 2022). It was reported that RLs can prolong the life of oranges with a chitosan-RL coating (Palumbo et al., 2022). This observation corroborates previous research utilizing chitosan independently or blended with other coating substances. Chitosan's ability to create a selectively permeable barrier against oxygen, carbon dioxide, and humidity during storage notably decreases weight loss whenever applied as an edible coating. Consequently, the coated produce is safeguarded from water loss, dehydration, and shriveling (Arnon et al., 2014; Gol et al., 2013; Valero et al., 2013).

In comparison to other biofungicides, RLs treatment as a protective agent exhibits varying effects on weight loss and fruit decay. While *Trichoderma* and *Bacillus* species have demonstrated effectiveness in reducing postharvest weight loss and fruit decay (Zeriouh et al., 2011), RLs show potential in delaying weight loss and preserving fruit freshness, although this varies depending on the specific pathogens and fruit types involved. Unlike biofungicides incorporating plant extracts, such as pomegranate peel extract studied by Cruz-Valenzuela et al. (2022), RLs offer a microbial-derived alternative. Additionally, previous studies have shown that coatings can help prevent moisture loss in fresh-cut produce

(Cofelice et al., 2019; Forato et al., 2015). For instance, a study on cashew gum-carboxymethylcellulose edible coatings on whole and minimally processed mangoes revealed decreased mass loss and maintained firmness.

More broadly, the efficacy of biofungicides, including RLs, is influenced by factors such as crop variety, pathogen type, and environmental conditions. This underscores the necessity for further comparative research to identify the optimal biofungicidal agents for specific crops and disease scenarios, enabling evidence-based decision-making for sustainable agricultural disease management. These investigations demonstrate RLs' antifungal qualities and their capacity to control the deterioration brought on by these harmful pathogens, which eventually helps to increase shelf life and lower financial losses for the farming sector (Crouzet et al., 2020).

3.2.3   Total Soluble Solid (TSS)

Total soluble solids (TSS) content, reflecting the combined concentrations of sugars and dissolved minerals in fruits and vegetables, was assessed on the final day of the study. Table 4 presents the TSS percentages for tomatoes, cucumbers, and mangoes. The study analyzed the total soluble solids (TSS) content, measured by Brix readings, in inoculated and non-inoculated fruit and vegetable samples treated with RLs. For tomatoes, the TSS reading in inoculated samples was 2.5, while non-inoculated samples reached a Brix value of 4.1. This suggests that pathogen infection reduced the sugar content despite RLs application (Juárez-López et al., 2014; Okanume et al., 2017). In cucumbers, RL -treated inoculated samples showed a TSS of 3.4, higher than the 2.9 observed in uninoculated samples. This indicates that RLs helped maintain sugar levels even when cucumbers were exposed to pathogens (Auerswald et al., 1999).

Mangoes displayed higher TSS values compared to tomatoes and cucumbers, with Brix values of 8.93 and 9.27 for inoculated and uninoculated samples, respectively. This minor difference suggests that natural variations in sugar content play a more significant role in mangoes than RLs treatment effects. Mandal et al. (2018) reported that using salicylic acid as a protective agent on mangoes enhanced shelf life and maintained quality. Fruits treated with 2.0 mM salicylic acid showed minimal weight loss and low TSS after 15 days of storage. Conversely, Islam et al. (2013) found that gibberellic acid increased various physicochemical metrics, including TSS, extending shelf life and delaying property changes.

Table 4. Total soluble solid (TSS) of tomatoes, cucumbers, and mango at day 6 of treatment.

Fruits	Treatment with RLs	Total Soluble Solid (ºBrix)
Tomato	Inoculated	2.5 ± 0.51
	Un-inoculated	4.1 ± 0.25
Cucumber	Inoculated	3.4 ± 0.51
	Un-inoculated	2.9 ± 0.29
Mango	Inoculated	8.93 ± 1.75
	Un-inoculated	9.27 ± 1.56

*TSS defined in mean ± SD with triplicates.

The increased TSS in inoculated samples, as shown in Table 4, can be attributed to various factors. TSS is crucial for assessing fruit and vegetable quality, quantifying dissolved sugars and minerals (Alemán-Ramirez et al., 2020). The impact of RLs treatment on TSS varies depending on fruit type, pathogen, and protective agent employed, emphasizing the complexity of these interactions. Further research is needed to understand the full potential of RLs, particularly in mangoes.

4.   Conclusion

As conclusion, this study demonstrateds the potential of RLs as a biofungicide in postharvest management of fungal diseases in tomatoes, cucumbers, and mangoes. The efficacy of RLs was shown to vary across crops and pathogens. Rhamnolipids have limited efficiency against *B. cinerea* in tomatoes, *C. capsici* in cucumbers and *P. palmivora* in mangoes demonstrating the pathogen and crops-specificity of their activity. Additionally, RLs performed better as preventive treatment rather than as curative treatment. While RLs suppressed fungal infections in non-infected fruits, they were less effective in infected fruits, with some treatments significantly increasing disease severity relative to untreated controls. This emphasizes the need for proper timing in RLs application for optimal disease management. The *in-vivo* study of postharvest quality, RLs treatment led to significant reduction in weight loss, and fruit decay particularly in cucumbers, but showed less pronounced effect on other fruits. Moreover, RLs effectively slowed weight loss, fruit decay and total soluble solids (TSS) content was also improved, although the extent of this improvement varied by fruit variation. While RLs were effective as preventive agents, they had limited efficacy in treating already infected crops.

This suggests that RLs have potential as alternatives to chemical pesticides and preservative agents in postharvest management, particularly in preventing the onset of infections.

Future research recommendations, the study should focus on optimizing RL formulations for more effective and consistent applications, as well as investigating the potential of combining RLs with other biofungicides or protective coating to enhance efficacy against a broader range of pathogens. In other hand, it is essential to include positive and negative control for each tested pathogen to provide a baseline comparison and accurately assess the effectiveness of RL treatments in mitigating postharvest deterioration.

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