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Harnessing Rhamnolipids from Waste Glycerol for Effective Biocontrol of Fungal Pathogens in Cucumber and Melon Plants

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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

Abstract: The aim of the present study was to evaluates the efficacy of rhamnolipids (RLs) as biopesticides for controlling common fungal diseases, includingpowdery mildew and stem rot, in cucumber and melon plants.The minimum inhibitory concentration (MIC) tests were conducted against *Botrytis cinerea, Fusarium oxysporum cubenses spp.* TR4*, Ganoderma boninense, Colletotrichum capsici, Rigidoporus microporus,* and *Pyricularia oryzae* in a 96-well plateassay. RLs inhibited >50% growth of all tested pathogens (except F. oxysporum TR4) at 0.1-1 g/L within 24-96 hours.Field trials,conducted in a randomized complete block design (RCBD), compared RLs (0.3, 0.5, and 0.7 g/L) to chemical fungicides, and untreated controls. RL treatments significantly enhanced cucumberfruit weight compared to controls, though results for melons were less consistent. Plant survival rates were highest with RL concentrations of 0.5 and 0.7 g/L, > 90% survival, comparable to chemical fungicides. These findings highlight RLs as an eco-friendly alternative to chemical pesticides, offering solutions for crop protection.

Keywords: Pest management, Biofungicide, Rhamnolipid, Biosurfactants, Antifungal*, Pseudomonas aeruginosa*

1. Introduction

Plant diseases and pests account for up to 40% of global crop production losses, resulting in economic damages over US$ 220 billion annually (FAO, 2024). The reliance on synthetic fungicides for disease management remains the primary strategy; however, their indiscriminate use has led to ecological imbalances, the emergence of resistant pathogens, and adverse effects on non-target organisms (Duke et al., 2023; Pathak et al., 2022). These challenges underscore the urgent need for eco-friendly and sustainable alternatives, such as biocontrol agents, to enhance agricultural productivity and environmental health.

Cucumbers (*Cucumis sativus*) and melons (*Cucumis melo*), crucial crops for global food security are highly susceptible to soil- and airborne diseases fungal diseases, resulting in significant yield reductions and hampering plant development (Seo & Kim, 2017). Notably, powdery mildew, caused by *Podosphaera xanthii, can lead to* losses of up to 50% in cucurbit crops, especially in semi-arid regions with high humidity and low temperatures (El-Naggar et al., 2012; Tanaka et al.,2017). The overuse of chemical pesticides to combat these diseases exacerbates environmental degradation, emphasizing the need for natural and sustainable alternatives.

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Rhamnolipids (RLs), biosurfactants derived from *Pseudomonas aeruginosa* RS6, have emerged as promising biocontrol agents due to their low toxicity, biodegradability, and broad-spectrum antimicrobial properties (Baskaran et al., 2021; Monnier et al., 2020). RLs inhibit fungal growth by altering microbial cell structures and distrupting critical biological processes (Botcazon et al., 2022). Previous studies have shown RLs' potential in reducing disease incidence and promoting plant recovery in various crops, including cucumber and tomato (Kim et al., 2000; Yoo et al., 2005). However, most research has focused on laboratory-scale evaluations, with limited studies exploring RLs; efficiency field conditions.

This study aims to adress these gaps by evaluating RLs produced from waste glycerol by *P. aeruginosa* RS6 as a sustainable biopesticide. Unlike prior research, this work uniquely investigates RLs' antifungal efficacy against a broad spectrum of economically significant fungal pathogens, including *Botrytis cinerea, Fusarium oxysporum f. sp. cubenses* TR4*, Ganoderma boninense, Colletotrichum capsici, Rigidoporus microporus,* and *Pyricularia oryzae*. The study combines minimum inhibitory concentration (MIC) test assay with field trials using a randomized complete block design (RCBD) in cucumber and melon crops to assess RLs' potential as an eco-friendly alternative for disease management in commercial agriculture.

2. Materials and methods

2.1. Production and purification of RLs

The preparation of growth media, inocula and the production of RLs by *P. aeruginosa* RS6 were performed as described by Baskaran et al. (2021). Fermentation for the RLs production was conducted in a 7 L fermenter (InforsHT, Switzerland) with a working volume of 5 L under batch cultivation at 30°C for 72 h. The cell-free supernatant containing RLs were subjected to solvent extraction procedures described by Smyth et al. (2010). The collected RLs were then concentrated using a rotary evaporator (Model, BUCHI, Switzerland) to achieve a viscous brown extract. The RLs produced were further characterized by its mono- and di-RLs compositions by HPLC following methods described earlier (Baskaran et al., 2021). The SPE-purified RLs and cell free fermentation broth were used in MIC tests and field planting trials, respectively using cucumber and melon Manis Terengganu as test plants.

2.2 Analyses of RLs

2.2.1 Determination of RLs concentration

The concentration of produced RLs was determined using the HPLC-UV analytical method as described by Smyth et al. (2010) and Baskaran et al. (2021).

2.3. Preparation of pathogenic fungi

Botrytis cinerea, Fusarium oxysporum cubense TR4, Ganoderma boninense, Colletotrichum capsici, Rigidoporus microporus, and Pyricularia oryzae were obtained from the Microbial Culture Collections at the Institute of Bioscience (IBS), Universiti Putra Malaysia. Pure cultures of these pathogens were sub-cultured onto Potato Dextrose Agar (PDA) plates and incubated at 28 ± 2°C for 5-7 days. These pathogens were later used in the minimum inhibitory concentration (MIC) analysis to determine the antifungal activity of RLs.

2.4. Minimum inhibitory concentration (MIC)

The antifungal activity of RLs against six different pathogenic fungal strains, including *B. cinerea, P. oryzae, C. capsici, R. microporus, G. boninense,* and *F. oxysporum cubense* TR4, was assessed using the microdilution method in 96-well flat-bottom plates, as described by Luna et al. (2011), with minor modifications to the medium type and temperature used for fungal growth. Potato dextrose broth (PDB, DifcoTM, Becton, USA) was prepared at a concentration of 24 g/L, autoclaved at 121°C for 15 min, and used to determine MICs. The spores (1 x 10^{-6} spore suspension mL^{-1}) were harvested from mature cultures after 5-7 days and added to each well (40 µL), except for the 11th and 12th columns. The microplates were then incubated at 30°C for 2-5 days. The growth development of pathogens was measured daily at 620 nm using a spectrophotometric micro-titre plate reader (MultiSkan Go, Model, Switzerland).

Growth inhibition was calculated using the following equation:

$$PGI\,(\%) = (\Delta C - \Delta T) / \Delta C \times 100 \qquad \text{Equation 1 (Broeckaert et al., 1990)}$$

Where: ΔC: Absorbance of the control micro-culture at 620nm

ΔT: Absorbance of the treatment micro-culture at 620nm

2.5. Microscopic observations of fungal morphology

To determine the effects of RLs at various concentrations on the mycelial structure of pathogens, comparisons were made with a control group (Kim et al., 2000). The mycelium structures were observed under a light microscope (BX40, Olympus, Singapore). For more detailed analysis, scanning electron microscopy (SEM) (JSM 5610LV, JOEL, Japan) and transmission electron microscopy (TEM) (MODEL JEM 2100, Japan Electron Optics Laboratory Co., Tokyo, Japan) were employed. The fungal preparations for SEM and TEM analyses followed the protocols reported by Cruz et al. (2021) and Lopez et al. (2012) respectively.

2.6. Field planting experiment

2.6.1. Plant material and growing conditions

Field trials were conducted in Kuala terengganu, Malaysia, from March to August 2021. Cucumber (*Cucumis sativus L.*) and melon manis Terengganu (*Cucumis melo L.*) sourced from Green World Genetics Sdn. Bhd., Malaysia and Advance Seed Co. Ltd., Thailand. The seeds were treated with RLs at 0.3 g/L, 0.5 g/L, and 0.7 g/L, chemical pesticides, and a water (control). Treated seeds were soaked for one hour. The seeds were germinated and transplanted into polybags containing cocopeat medium. RLs and fungicides were applied weekly starting 10 days post-sowing. Fertilizers were supplied automatically three times daily using a drip system. The fertilizers used for the melon plantings were sourced from Yara International Sdn. Bhd., Malaysia, while HIBOR and sodium molybdate were obtained from Fertitrade Sdn. Bhd., Malaysia. Fertilizers A and B, commonly used by farmers and purchased from Yara International Sdn. Bhd., Malaysia, were employed for the cucumber crop.

2.6.2. Experimental area and design

The study utilized a 1,271-square-feet (ft) greenhouse, in which plant spacing within each polybag at half a ft and spacing greenhouses 3.5 ft apart. The study employed a randomized complete block design (RCBD) with three replications (Hafez et al., 2020). Each replication consisted of 10 experimental plants. The plants underwent treatments following RCBD design involving three RL concentrations (0.3 g/L, 0.5 g/L, and 0.7 g/L), chemical treatment (Antracol at 0.5 g/L and Pegasus at 1 g/L), and a control group (water). Treatments were applied weekly with two spray methods, using 25–40 mL of solution per plant (Hafez et al., 2020). The RLs concentrations (0.3, 0.5, 0.7 g/L) were prepared by diluting the 15 g/L of RLs with tap water. Two fertilizers labeled A (green) and B (yellow) were supplied. Antracol 70 WP fungicide was applied every three days at 0.5 g/L. Weeding was applied every three days. Fertilizers A and B were supplied via an automated dropper system thrice daily for five minutes each (morning, afternoon, evening).

2.6.3. Plant growth and yield parameters

The efficacy of RLs as a biofungicide was determined through field planting trials of melon and cucumber by observing and measuring plant growth and yield. Plant height, leaf width, and fruit weight were recorded weeekly (Hafez et al., 2020). Fruit weight was assessed after harvest to evaluate marketable yield.

2.7. Statistical analysis

Data were analyzed using one-way ANOVA and Tukey HSD post-hoc tests (IBM SPSS version 27.0). A significant level of $P \leq 0.05$ was applied.

3. Results and discussion

3.1. Minimum inhibitory concentration (MIC)

A 96-well microtiter plate experiment was conducted to assess the antifungal activities of RLs against spores of *B. cinerea, G. boninense, P. oryzae, R. microporus, F. oxysporum cubense spp.* TR4, and *C. capsici*. Benomyl 50 WP, a chemical fungicide, was used as a control. Percentage inhibition was recorded every 24 hours up to 96 hours of spore incubation. The results, summarized in Table 1, indicate that RLs were highly effective against most pathogens, often at concentrations lower than those required by chemical control. For instance, RLs inhibited *G. boninense* growth i at 0.078 mg/mL whereas Benomyl 50 WP required higher concentrations (2.5 mg/mL). Similarly, RLs demonstrated superior inhibition of *C. capsici* (0.156 mg/mL) compared toBenomyl, which was ineffective at suppressing this pathogen. RLs delayed germination of *B. cinerea* conidia at 48 hours. At the lowest concentration (0.078 mg/mL), RLs achieved >50% growth inhibition. Benomyl 50 WP showed 52% and 78% inhibition at higher concentrations of 0.313 mg/mL. RL-treated cultures of *P. oryzae* showed >80% spore inhibition at the lowest concentration (0.020 mg/mL). Benomyl 50 WP required higher concentrations (1.25 mg/mL) to inhibit spore growth by >80% at 96 hours. RLs treatment on *F. oxysporum f. sp. cubense* TR4 was ineffective, with growth inhibition below 35% despite prolonged incubation. On the other hand, Benomyl 50 WP inhibited >50% of conidia growth. RLs inhibited spore germination of *R. microporus* at 0.039 mg/mL

with >80% growth inhibition. Similarly, Benomyl 50 WP achieved >50% inhibition at the lowest concentration (0.02 mg/mL) after 24 hours of incubation.

Ganoderma boninense can cause basal stem rot, which negatively impacts oil palm production by reducing palm oil fruit yield and potentially leading to plant death (Irma et al., 2018; Naher et al., 2012). The inhibitory effect of RLs derived from *P. aeruginosa* on the growth of *G. boninense* was reported and RLs can suppress the growth of *G. boninense* by 70% and underscores the potential of these biosurfactants as biocontrol agents against this notorious oil palm pathogen (Irma et al., 2018; Ramli, 2016). *C. capsici* is one of the most studied fungal pathogens worldwide and this pathogen is responsible for anthracnose or fruit-rot diseases in chillies and red rot diseases in sugarcane ((Goswami et al., 2015; Than et al., 2008). The application of RLs at concentrations 0.025-0.5 mg/mL and 0.1 mg/mL, inhibited the growth and spore germination of *C. capsici* and *Colletotrichum* spp. by 60-90% and >80%, respectively (Goswami et al., 2015; Than et al., 2008). Robineau et al. (2020) reported a similar effect of RLs against *B. cinerea* through inhibition of conidial germination, where a distinct effect was seen after 24 h, with a maximum 54 2.7% growth inhibition compared to the control. The study by Shalini et al. (2017) underscores the efficacy of biosurfactants in mitigating the specific growth of *P. oryzae* and the findings revealed that even at a concentration as low as 1%, biosurfactants can significantly reduce the growth of *P. oryzae* by 38.4%.

Fusarium oxysporum spp. is an important pathogen that causes wilt and wilt disease in tomato plants (Deepika et al., 2015). *Fusarium oxysporum* (*Fusarium* wilt) showed inhibition at 0.27 mg/mL when RLs were loaded with carvacrol (Paramalingam et al., 2021). Hadi et al. (2022) reported that 10 ppm of RL effectively inhibited the growth of *R. microporus* by 34.36%. In the present study, the inhibition levels differed for each pathogen tested with selective preference for RLs and Benomyl 50WP. Benomyl 50WP worked best at 0.5 g/L, and the effectiveness of the treatment decreased when the concentration was below 0.5 g/L. On the other hand, the lowest concentration of RLs inhibited pathogen growth in the range of 0.3-1.0 g/L, and it worked for certain pathogens; however, the best minimum concentration for RLs was 0.5 g/L. Consequently, MIC tests carried out in the present work have demonstrated that the RL successfully suppresses the proliferation of the most pathogens.

Table 1: Minimum inhibitory concentration (MIC) of fungal pathogens when treated with RLs and chemical using Benomyl 50WP compared with positive (PDB + fungus) and negative controls (PDB only) at different concentrations and incubation times.

Pathogen	Incubation time (h)	RLs (mg/mL)	% Growth Inhibition	Benomyl (mg/mL)	% Growth Inhibition
G. boninense	96	0.078	>50	2.5	>50
C. capsici	24	0.156	>50	-	<10
B. cinerea	48	0.078	>50	0.313	52
P. oryzae	96	0.020	>80	1.25	>80
F. oxysporum TR4	24	0.3	<35	0.5	>50
R. microporus	24	0.039	>80	0.02	>80

*Positive and negative controls value were not shown in the table

3.4. Microscopic observation of fungal pathogens

3.4.1. Light microscope observations

As discussed earlier, RLs successfully inhibited the growth of *B. cinerea, F. oxysporum cubense spp.* TR4, *G. boninense, C. capsici, R. microporus*, and *P. oryzae* in MIC studies. To further examine the impact of RL concentration on the mycelium structure of these pathogens, light microscopy was used, comparing treated and untreated cultures (Fig. 1).

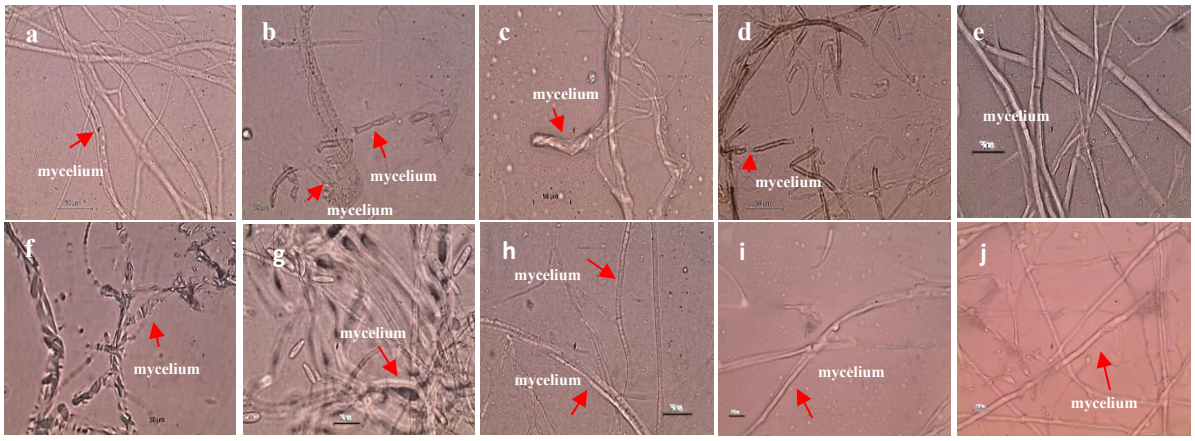


Fig. 1: The morphology of *P. oryzae was* observed under the light microscope at 1000X. Mycelia and spore structure of *P. oryzae*, (a) control, with the treatment of RLs at concentrations of (b) 5 mg/mL. Mycelia structure of *C. capsici*, (c) control, with the treatment of RLs at concentrations of (d) 1.25 mg/mL. Mycelia structure of *R. microporus*, (e) control, when treatment with RLs at concentrations of (f) 5 mg/mL. The mycelia structure of *F. oxysporum cubenses TR4*, (g) control, when treated with RLs at concentrations of (h) 5 mg/mL. Mycelia structure of *G. boninense*, (i) control, with the treatment of RLs at concentration of (j) 5 mg/mL.

3.4.2. SEM and TEM observations

To further observe the impact of RLs on the mycelium structure of pathogens, SEM and TEM were used. Fig. 2 shows SEM images of *C. capsici* at various RL concentrations. Abnormal growth, lysis, shrinking, and mycelium disruption were noted (Fig. 2b-d). The control group exhibited normal growth and smooth surfaces (Fig. 2a). Under TEM, untreated (control) *C. capsici* displayed typical ultrastructural elements, including normal cell walls with a two-layer structure, plasmalemma, cytosol with nucleus and nucleolus, lipids, mitochondria, and vacuoles (Fig. 3). The cell wall and plasmalemma were firmly attached, with a spherical nucleus and evenly dispersed cytoplasm. Mitochondria were ovoid shaped with average electron density, and lipids and glycogen were the main intracellular nutrients. In contrast, treated *C. capsici* micrographs demonstrated that RLs at a minimum concentration of 0.7 g/L caused irreversible ultrastructural changes to the mycelium (Fig. 3b). Organelle disintegration and cytoplasmic content precipitation were visible. The cell wall appeared shrunken, porous, and uneven, with a largely damaged or non-adherent plasmalemma and a more electron-dense outer cell wall layer.

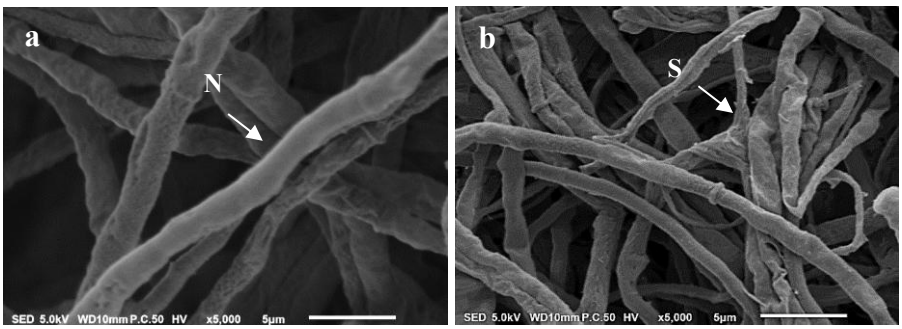


Fig. 2: Effect of RLs on *C. capsici* mycelium. (a) control SEM micrographs and (b) treated SEM micrographs. *N= normal growth and smooth surface; S= shrinkage; L= lysis

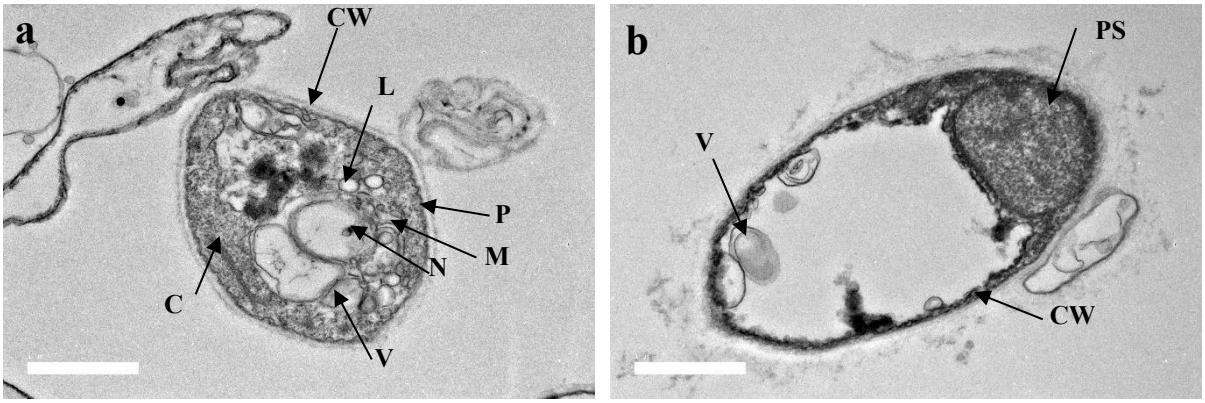


Fig. 3: Transmission electron micrograph of *C. capsici*: (a) control: showing a cross-section of a *C. capsici* mycelium with the normal ultra-structural components; (b) showing cross-section of a *C. capsici* mycelium treated with RLs with minimum concentration (0.7g/L). *Cell wall (CW); periplasmic space (PS); plasmalemma (P); cytoplasm (C); mitochondrion (M); vacuole (V); nucleus (N); lipids (L)

SEM micrographs of *R. microporus* treated with RLs (Fig. 4b) demonstrated several adverse effects on the mycelium's morphology compared to the controls (Fig. 4a). The treated mycelium exhibited abnormal growth, lysis, shrinkage, disruption, aggregation, and pore formation. In contrast, the control group showed normal growth, smooth surfaces, and healthy spores attached to the mycelium. Under TEM observation, untreated (control) *R. microporus* displayed typical ultrastructural components, including a two-layer cell wall structure, plasmalemma, cytosol with nucleus and nucleolus, lipids, mitochondria, and vacuoles (Fig. 5a). The plasmalemma was firmly attached to the cell wall, the nucleus was spherical, and the cytoplasm was uniformly dispersed. Mitochondria were ovoid-shaped, abundant, and exhibited typical electron density. Lipids and glycogen served as the main intracellular nutrient stores. In contrast, *R. microporus* treated with RLs at a concentration of 0.7 g/L exhibited significant ultrastructural changes (Fig. 5b). Organelle disintegration and cytoplasmic content precipitation were evident. The cell wall appeared shrunken, porous, and abnormally shaped. The plasmalemma was largely destroyed or detached from the cell wall, with the outer cell wall layer showing increased electron density.

SEM and TEM images observed supported the findings that the RLs have made changes in *C. capsici* and *R. microporous* mycelium. The functional integrity of the fungal cell`s components is necessary to maintain the viability and germination quality (Gow et al., 2017). The loss of viability and germination quality of the RL-treated mycelium was caused by the precipitation of the cytoplasm and the death of the nucleus and cytoplasmic organelles. In addition, RLs caused irreversible modifications that undermined the cell wall's ability to function as a barrier and eliminated the potential of activating enzymes linked to the cell wall (Gow et al., 2017).

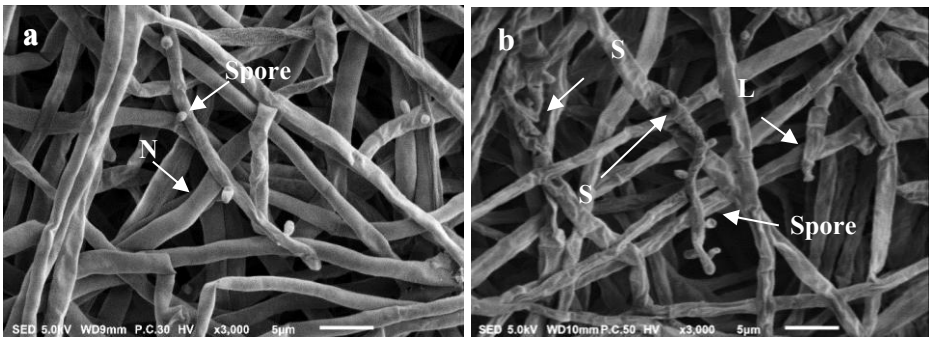


Fig. 4: Effect of RLs on *R. microporus* mycelium. (a) control SEM micrographs and (b) treated SEM micrographs. N= normal growth and smooth surface; S= shrinkage; L= lysis

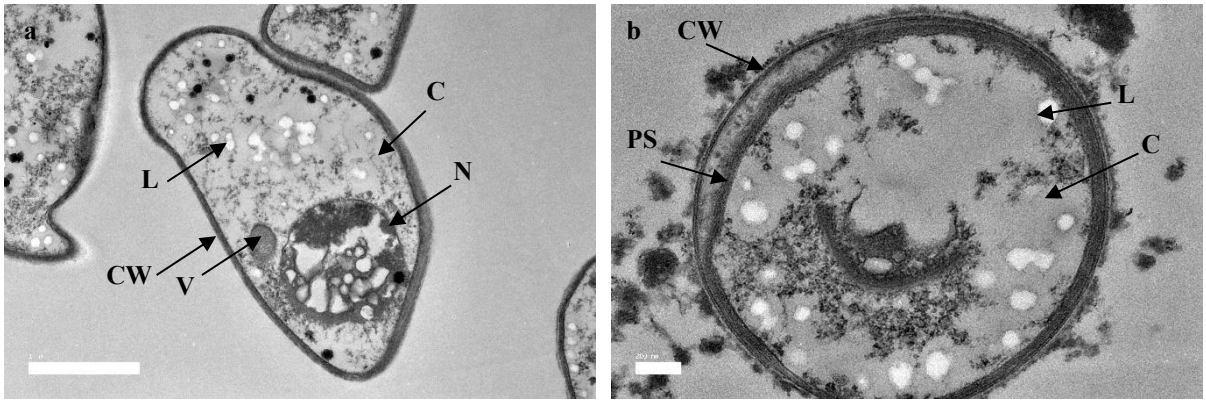


Fig. 5: Transmission electron micrograph of *R. microporus*: (a) control: showing cross section of a *R. microporus* mycelium with the normal ultra-structural components; (b) showing cross section of a *R. microporus* mycelium treated with RLs. Cell wall (CW); periplasmic space (PS); cytoplasm (C); vacuole (V); nucleus (N); lipids (L).

3.4.3. Effectiveness of RLs by comparing with chemical fungicides

The effectiveness of RLs at specific concentrations appears to be pathogen dependent, potentially linked to the molecular characteristics of the pathogens. RLs likely act by disrupting fungal cell membranes and inhibiting germination, as observed through light microscopy and SEM/TEM analyses. The ability of RLs to cause lysis, shrinkage, and distruption of the fungal mycelium at low concentrations (0.039-0.078 mg/mL) suggested that they exerted strong surfactant properties, destabilizing the cell wall and plasma membrane integrity. Moreover, comparing RL treatments with chemical fungicides like Benomyl 50 WP reveals significant advantages for RLs, particularly in terms of cost and environmental sustainability. Although Benomyl was more effective at higher concentrations such as 2.5 mg/mL for *G. boninense*, RLs performed better at much lower concentrations (0.078 mg/mL), suggesting a more efficient mode of action. Moreover, RLs are biodegradable and non-toxic to humans and animals, offering a safer alternative to chemical fungicides, which are associated with long-term environmental impacts and resistance issues (Goswami et al., 2015). Therefore, a detailed cost benefit analysis, including the production cost of RLs versus synthetic fungicides, would be valuable to further emphasize the practicality of RLs in commercial agriculture, and further mechanistic studies should focus on identifying specific interactions between RLs and fungal cell components to better understand why RLs are more effective against certain pathogens.

3.5. Field planting trials

3.5.1. Cucumber plant growth

Field planting trials for cucumber and melon plants under greenhouse conditions were conducted to demonstrate the ability of RLs produced from *P. aeruginosa* RS6 to control fungal diseases. Unlike previous studies, this work did not first infect the plants with fungal pathogens. Instead, the trials were carried out alongside commercial planting plots, allowing researchers to collect data that more accurately represents the real-world application of RLs as biofungicides. This study aimed to provide a realistic assessment of RLs' efficacy in controlling fungal diseases in cucumber and melon plants. Such an approach is valuable for understanding how RLs perform under conditions that mimic commercial agricultural practices, which is crucial for evaluating their practicality and potential adoption by farmers. Cucumber plants were treated with chemical fungicide (0.5 g/L), a fermentation broth containing RLs at different concentrations (0.3-0.7 g/L), and water (as a control group). The application of RLs at all concentrations reduced the disease incidence by 96%.

3.5.2. Percentage of cucumber plant survival

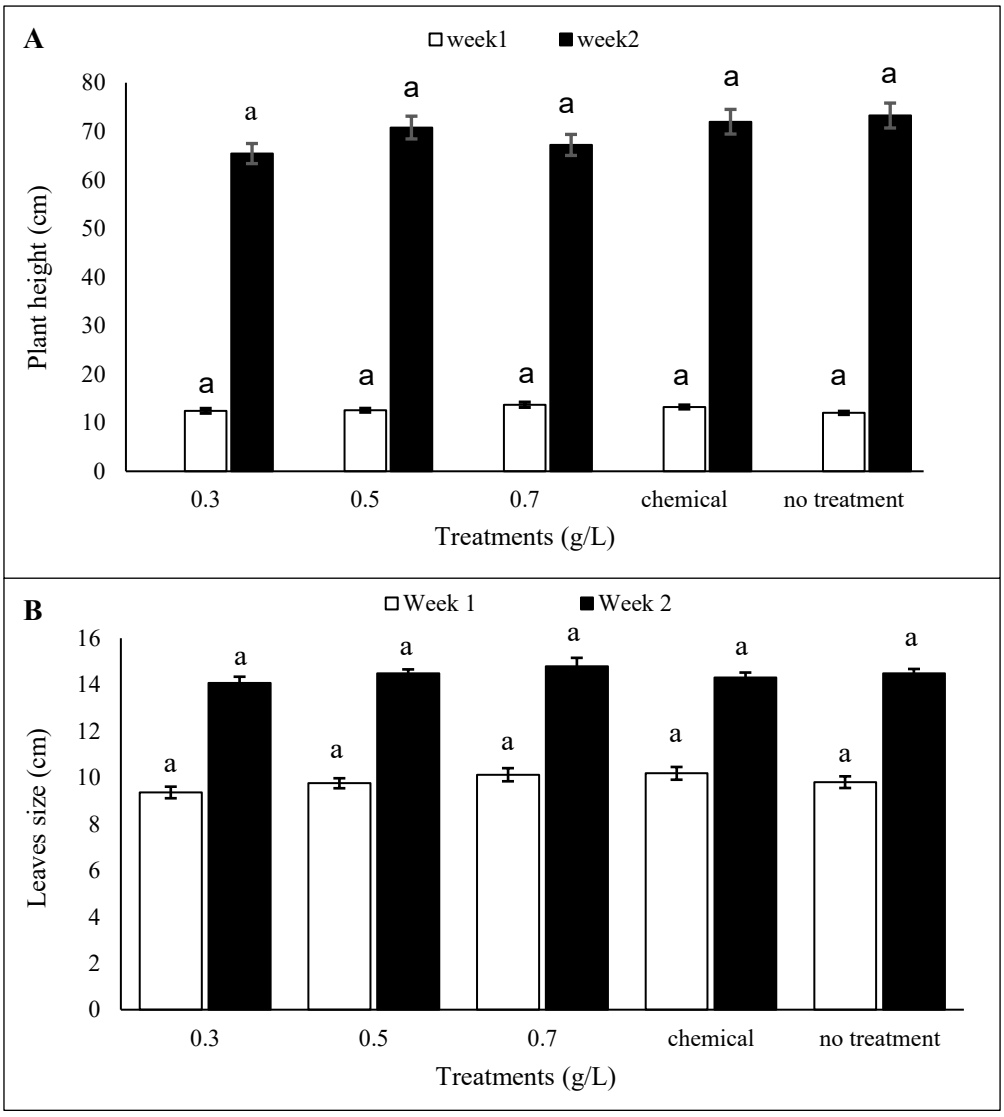
Table 2 illustrates the impact of different RL concentrations on the protection of cucumber plants against infection. In the control group (water), 76% of the plants survived, indicating a moderate level of infection protection. On the other hand, the application of chemical fungicides (Antracol at 0.5 g/L) has resulted in a higher survival rate, with 90% of cucumber plants resisting infection, demonstrating the effectiveness of chemical fungicide. Notably, the use of 0.3 g/L of RLs increased the survival rate to 88%, indicating substantial protection against the disease. When the RLs concentration was increased to 0.5 g/L, the survival rate improved to 92%, indicating a higher level of protection compared with the lower RLs concentration. The most significant increase in survival (96%) was observed at RL concentration of 0.7 g/L, highlighting the excellent protection provided by higher RL concentrations, leading to a substantial reduction in disease incidence. As RL concentration increases, the percentage of survival plants also increases, indicating a dose-dependent response. In addition, the efficacy of RLs as a protective agent was compared with that of the control group (water).

Table 2: The effect of RLs concentration on protection against infection of cucumber plants

	Treatments				
	Water (control)	**Antracol (0.5 g/L)**	**RLs (0.3 g/L)**	**RLs (0.5 g/L)**	**RLs (0.7 g/L)**
Percentage of the survival plant (%)	76%	90%	88%	92%	96%

3.5.3. Cucumber plant growth

In terms of average plant height (Fig. 6a), no significant impact was observed from any of the treatments, despite the highest mean height observed in the control group. The efficacy of treatment was more than 50% compared to the control. Plant height slightly increased after the third week after sowing. For average leaf width (Fig. 6b) on days 9 and 18 after sowing, RLs at a concentration of 0.7 g/L resulted in slightly wider leaves compared with those of the chemical pesticide treatment and control, but with no significant difference. Leaf widths ranged from 14.1 cm to 14.8 cm, with the highest width observed for plants treated with 0.7 g/L RLs, followed by plants treated with 0.5 g/L RLs, chemical pesticides, control, and 0.3 g/L RLs. There was a significant difference in cucumber weight (Fig. 6c). Cucumber weights ranged from 1.2 kg to 2.9 kg, with a mean of 2.12 kg for the control, 2.05 kg for 0.3 g/L RLs, 2.12 kg for 0.5 g/L RLs, 1.92 kg for 0.7 g/L RLs, and 1.96 kg for the chemical treatment. In conclusion, RLs produced by *P. aeruginosa* RS6 as a biopesticide demonstrated satisfactory efficacy against plant pathogenic fungi on cucumber plants. There were significant effects of the type of pesticide used on cucumber weight, with RL treatment at 0.5 g/L producing the highest mean yield.



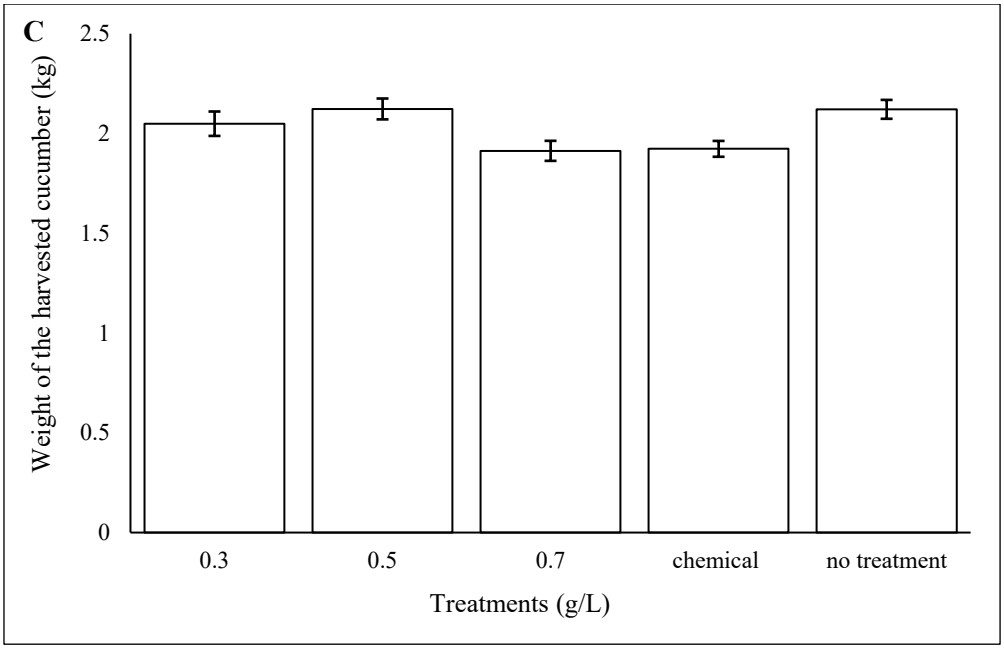


Fig. 6: a) The average height of the cucumber plant taken in Week 1 and Week 2 since seeds were sown. b) The average size of the cucumber leaves taken on Week 1 and Week 2 since seeds were sown. c) The average weight of the harvested cucumbers. The error bars indicated the triplicate reading of standard error. The subscripts on the top of the bar indicate the homogenous subset groups based on HSD Tukey-b.

3.5.4. Melon plant survival

Table 3 illustrates the effect of RL concentrations on melon plant protection against infection. The survival rate of melon plants in the control group (water) was 70%, indicating a notable incidence of infection. Application of chemical fungicides (Antracol at 0.5 g/L) resulted in a higher survival rate of 92%, suggesting efficient disease control took place. Remarkably, the use of RLs at 0.3 g/L resulted in a survival rate of 87%, signifying a moderate level of protection against infection. When the RL concentration was increased to 0.5 g/L, the survival rate further improved to 90%, indicating a more pronounced protective effect. Notably, the application of RLs at a concentration of 0.7 g/L resulted in a significant increase in the survival rate of melon plants, thus offering effective protection against infections and diseases caused by pathogenic fungi.

The results obtained from the melon planting trials align with those from the cucumber, affirming the effectiveness of RLs produced from *P. aeruginosa* RS6 as a biofungicide. Application of fermentation broth containing RLs at concentrations ranging from 0.3 to 0.7 g/L resulted in a significant reduction in disease incidence, achieving a remarkable 96% reduction in melon plants. RLs have demonstrated significant potential in reducing disease incidence compared to the control group, offering a promising eco-friendly alternative to chemical pesticides in agriculture.

3.5.5. Melon plant growth

The growth of melon plants was significantly affected by each treatment. Melon plants that were treated with 0.3 g/L RLs exhibited the highest average height (Fig. 7a). Significant effects of pesticide type on melon plant height were observed ($p > 0.05$). The melons treated with 0.5 g/L RLs showed the highest average leaf width per plant (Fig. 7b). Leaf widths ranged from 20.8 cm to 26.8 cm, whereby the widest average melon leaves were observed in the 0.3 g/L RL treatment group, and the narrowest leaves were observed in the control group. A significant effect of RL concentration on melon leaf width was observed ($p > 0.05$). Melon weights ranged from 0.00 kg to 1.75 kg, and no significant effect of pesticide type on melon weight ($p > 0.05$) observed (Fig. 7c). In conclusion, plant height and leaf width were significantly influenced by the type and concentration of RLs used, whereas melon fruit weight did not show significant differences between treatments.

Table 3: The effect of RLs concentration on protection against infection of melon plants.

	Treatments				
	Water (control)	Antracol (0.5g/L)	RLs (0.3 g/L)	RLs (0.5 g/L)	RLs (0.7 g/L)
Percentage of the survival plant (%)	70%	92%	87%	90%	96%

These results obtained in the present studies were consistent with those of other research, where the use of RLs in cucumber plants prevented the development of infections caused by various pathogens (Kim et al., 2000; Yoo et al., 2005). Sha et al. (2012) demonstrated that *P. aeruginosa* ZJU211-derived RLs exhibited strong activity against *Oomycetes* on tomato plants. The absence of RLs resulted in 100% plant damage, whereas the application of RLs resulted in significant recovery rates. Application of RLs at 0.25 mg/mL, and at 0.5 mg/mL has resulted in 70% of plant growth recovery, and full recovery, respectively after the plants were first infected with pathogens. Importantly, RLs at 1 mg/mL are safe for cucumber, tomatoes, and grass, indicating their potential as environmentally friendly alternatives to chemical pesticides. Pot studies on eggplants infected with *F. oxysporum f. sp. melongenae* showed that RL treatments at a concentration of 0.25 mg/mL were more effective than synthetic fungicides in controlling the infection. This finding further highlights the potential of RLs as a sustainable and efficient solution for plant disease management (Nalini & Parthasarathi, 2018). Deepika et al. (2015) investigated the antifungal activity of *P. aeruginosa* KVD-HM52 and its RLs against *F. oxysporum* wilt disease in tomato plants. Purified RLs at a concentration of 0.2 mg/mL completely inhibited disease severity in tomato plants after 14 d of pot studies. This demonstrates the strong inhibitory effect of RLs against fungal pathogens and their potential as a biopesticide.

Collectively, the results from the previous studies, along with the findings in the current study, indicated that RL has the potential as biopesticide for controlling plant diseases. The field trials for cucumber and melon plants confirmed the high efficacy of RLs as biofungicide. RLs at 0.7 g/L provided the best disease control, resulting in a survival rate of 96% for cucumber plants and significant disease reduction (96%) in melon plants. While RLs did not significantly affect plant height or leaf width, they did increase survival rates and reduce disease incidence compared to the control. Notably, RLs at 0.5 g/L yielded the highest cucumber weight (2.12 kg), suggesting that RLs not only protect plants but may also have positive effects on yield. The use of different concentrations of RLs has shown dose-dependent effects on disease protection, and the efficacy of RLs in reducing disease incidence and severity suggests its promising application in sustainable agricultural production.

Moreover, these field trials demonstrated the potential for RLs as a viable biofungicide in real-world agricultural settings. Application of RLs (0.3-0.7 g/L) resulted in up to 96% disease reduction in cucumber and melon plants, with survival rates reaching 96% at 0.7 g/L RLs. These results suggest that RLs could be incorporated into integrated pest management systems, offering an eco-friendly alternative to chemical pesticides. In terms of scaling, RLs could be integrated into commercial farming practices by utilizing fermentation broths, which are cost-effective and relatively easy to apply. However, challenges related to the large-scale production of RLs need to be addressed, including optimizing fermentation conditions and ensuring consistent RL yields. Additionally, RLs should be tested in field trials across different regions and conditions to confirm their effectiveness under varied environmental factors.

Comparing RLs to chemical fungicides, RLs provided comparable protection, with advantages in sustainability and reduced chemical input. Scaling these results to larger commercial operations could offer farmers an effective, eco-friendly alternative to synthetic pesticides. However, further research is required to fully understand the mechanism of action and optimize the application methods to maximize their effectiveness in crop protection and explore the potential synergistic effects of RLs in combination with other biofungicides or natural protective coatings to enhance their effectiveness and broaden their applicability in commercial agriculture.

4. Conclusion

This study highlights the efficacy of rhamnolipids (RLs) as a promising biofungicide for managing postharvest fungal infections in tomatoes, cucumbers, and mangoes. The results demonstrated that 0.5 mg/mL was the optimal concentration, effectively reducing disease severity, minimizing weight loss, and preserving fruit quality. However, the performance of RLs varied across fruits and pathogens, underscoring their preventive rather than curative potential. For instance, RLs showed notable success in reducing fungal infections in cucumbers but were less effective against *B. cinerea* in tomatoes.

To advance the practical application of RLs, future research should include large-scale field trials under diverse environmental conditions to validate these findings. Additionally, testing RLs on other high-value crops and exploring their potential synergy with other biofungicides or postharvest coatings would provide deeper insights and improve efficacy. These efforts could pave the way for RLs to become a sustainable and effective alternative for postharvest disease management.

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Conflict of Interest

The authors declare no conflicts of interest.

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