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Research Article

Protection and shelf-life extension of mango by a volatile compound, 3-methylpentanoic acid from endophytic bacterium, *Bacillus safensis*

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ABSTRACT

Postharvest decay caused by *Colletotrichum gloeosporioides* (anthracnose) and *Lasiodiplodia theobromae* (stem-end rot) accounts for significant economic losses in global mango (*Mangifera indica* L.) value chains. While synthetic fungicides remain the primary control measure, escalating concerns regarding chemical residues and environmental toxicity necessitate the development of bio-based alternatives. This study identified and characterized the potent antifungal efficacy of 3-methylpentanoic acid (3-MP), a volatile organic compound (VOC) derived from the endophyte *Bacillus safensis*. *In vitro* assays demonstrated that 3-MP exerts dose-dependent inhibition on critical fungal life stages, including mycelial expansion, conidial germination, and appressorium formation. Complete inhibition was achieved at concentrations as low as 200 µM for *C. gloeosporioides* and 300 µM for *L. theobromae*. *In vivo* application significantly suppressed disease severity, while storage trials revealed that 3-MP treatments (specifically at 100 µM) simultaneously delayed fruit ripening and minimized physiological weight loss. As a "Generally Recognized as Safe" (GRAS) compound, 3-MP represents a dual-action breakthrough: it serves as both a high-efficacy bio-fumigant and a physiological preservative. These findings provide a sustainable framework for replacing synthetic agrochemicals with microbial-derived volatiles, offering a scalable solution for the long-term preservation of tropical fruits.

Introduction

Mango (*Mangifera indica* L.) is among the most economically and nutritionally significant tropical fruits globally, with extensive cultivation across Asia, Africa, and Latin America (Arauz, 2007). Its international prominence is driven by high consumer demand for its unique organoleptic profile and antioxidant density, making it a cornerstone of rural livelihoods and global trade (Singh et al., 2013). However, the mango value chain is severely constrained by its highly perishable nature. Postharvest losses, estimated at 20–40% in key producing regions, represent a critical bottleneck that threatens global food security and supply chain sustainability (Pawde et al., 2026).

The primary drivers of this deterioration are specialized fungal pathogens, most notably *C. gloeosporioides*, the causal agent of anthracnose, and *L. theobromae*, which induce stem-end rot

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(Dofuor et al., 2023; Gariba et al., 2025). Anthracnose presents as quiescent infections that manifest during ripening as necrotic, sunken lesions, often rendering the fruit unmarketable after significant investment in transport (Nelson, 2008). Similarly, stem-end rot initiates rapid internal tissue breakdown from the pedicel, causing catastrophic browning and pulp softening (Gariba et al., 2025).

Current mitigation strategies rely almost exclusively on synthetic fungicides. However, the modern regulatory landscape and consumer preferences are shifting away from these traditional inputs due to escalating concerns regarding toxicological residues, the emergence of multi-drug-resistant pathogen strains, and long-term environmental degradation (Karim et al., 2024; Yang et al., 2021). Consequently, there is an urgent research imperative to identify "green" bio-preservatives that offer high efficacy without compromising consumer safety.

Microbial antagonists, particularly within the genera *Bacillus*, *Pseudomonas*, *Lysobacter*, and *Trichoderma*, have demonstrated profound potential in suppressing pre- and postharvest pathogens through multifaceted mechanisms, including nutrient competition, antibiosis, and the induction of systemic resistance (Islam et al. 2005; Tahir et al., 2017; Cao et al., 2019; Chakraborty et al. 2020; Surovy et al. 2024). A particularly compelling frontier in biological control is the use of Volatile Organic Compounds (VOCs) (Surovy et al. 2023). These metabolites comprising alcohols, aldehydes, and organic acids that can diffuse through storage atmospheres to inhibit fungal morphogenesis without requiring direct contact with the fruit surface, thereby preserving the natural microbiome and reducing application costs (Liu et al., 2018; Massawe et al., 2018; Surovy et al., 2023).

Within this context, 3-methylpentanoic acid (3-MP), a volatile metabolite synthesized by *Bacillus safensis*, has emerged as a high-potential candidate for postharvest application (Paul et al., 2025; Sujon et al., 2026). As a compound naturally occurring in various edible plants and classified as "Generally Recognized as Safe" (GRAS) by the U.S. Food and Drug Administration, 3-MP aligns with stringent food safety standards (Cohen et al. 2020). Preliminary evidence suggests that 3-MP may disrupt fungal cell wall integrity and interfere with essential signaling pathways (Gupta et al., 2025; Paul et al., 2025; Sujon et al., 2026). However, a significant knowledge gap exists regarding the specific efficacy thresholds of 3-MP against the mango pathogen complex and its secondary effects on fruit ripening physiology. We hypothesize that 3-MP acts as a potent bio-fumigant that can simultaneously suppress the mycelial and conidial development of *C. gloeosporioides* and *L. theobromae* while slowing the metabolic respiration associated with fruit senescence. To test this hypothesis, the current study aimed to (i) quantify the in vitro and in vivo antifungal potency of 3-MP against major mango pathogens; (ii) evaluate its impact on physiological weight loss and shelf-life extension; and (iii) assess its potential as a scalable, sustainable alternative to synthetic fungicides. By bridging the gap between microbial secondary metabolism and practical postharvest management, this work seeks to provide a robust framework for enhancing the global mango value chain through eco-friendly innovation.

Materials and Methods

Fungal Strain and Culture Conditions

The fungal pathogens *C. gloeosporioides* and *L. theobromae* were collected from the Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI) (Table 1). Each isolate was maintained on dried filter paper at 4 °C until experimentation. The isolates were separately cultured on potato dextrose agar (PDA; 42 g/L) and incubated at 25 °C for 7 to 8 days for reactivation. A small block of each fungal culture was then transferred to fresh PDA medium and incubated under the same conditions (Paul et al., 2022). Sporulation was induced as follows: *C. gloeosporioides* formed acervuli on PDA cultures, from which conidia were harvested, and *L. theobromae* developed pycnidia that released conidia in gelatinous masses. Conidia from these pathogens were suspended in sterile distilled water for

microscopic observation. Germination was evaluated by monitoring spore development under a compound microscope, and germinated spores were manually counted and standardized to 1×10^5 spores/mL using a hemocytometer (Neubauer, 0.0025 mm²) (Kumar et al., 2011). For the *in vivo* disease suppression assay, the susceptible mango cultivar BARI Aam-3 was selected as the host.

Table 1. List of fungal isolates with host origin and disease association.

Isolate name	Strain Name	Disease Name	Source of isolation
Colletotrichum gloeosporioides	MHPR2	Anthracnose	BARI Aam-3
Lasiodiplodia theobromae	MAHR5	Stem-end rot	BARI Aam-3

Chemicals and preparation of working solution

3-MP (Sigma-Aldrich, product no. W343706) was employed in this study (Fig. 1). A concentrated stock solution was prepared in dimethyl sulfoxide (DMSO), using the minimal volume required to achieve solubility. Working solutions of 50 µM, 100 µM, 200 µM, and 300 µM were subsequently formulated, with the final DMSO concentration maintained below 1% (v/v). This DMSO concentration has previously been demonstrated to exert no measurable effect on hyphal growth or sporulation of the fungal pathogen (Paul et al., 2022).

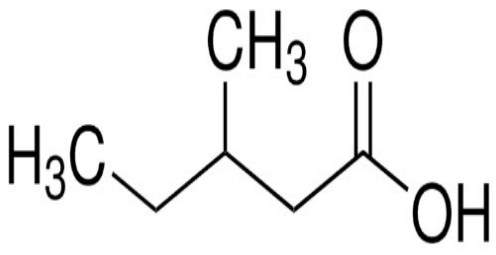

Fig. 1. Chemical structure of 3-Methylpentanoic Acid.

Bioassay with 3-MP on mycelial growth

3-MP was evaluated against *C. gloeosporioides* at 0 (Control), 100, and 200 µM for 7 days and *L. theobromae* at 0 (Control), 100 and 300 µM for 5 days. For each assay, an autoclaved filter paper was affixed to the lid of a Petri dish, and the respective 3-MP concentration was applied to it. A 2 mm mycelial block of the test isolate was placed in the center of a PDA plate, and the lids were sealed tightly to prevent the loss of 3-MP. The radial mycelial growth (cm) of each pathogen was measured at days 7 and 5 of post-incubation of *C. gloeosporioides* and *L. theobromae*, respectively. Measurements were taken for inhibition zones and colony diameters influenced by the test compound. Radial growth inhibition percentage (RGIP) (± standard error) (Riungu et al., 2008) was calculated from mean values as:

$$\text{RGIP (\%)} = \frac{\text{Radial growth in control plate-Radial growth in treated plate}}{\text{Radial growth in control plate}} \times 100$$

Inhibition of conidial germination and morphological alteration in *C. gloeosporioides*, and *L. theobromae*

The inhibitory effect of 3-MP on conidial germination was assessed on *C. gloeosporioides*, and *L. theobromae* at different concentrations (50 µM, 100 µM, and 200 µM) over varying time intervals. A sterile filter paper moistened with sterile water was placed at the base of each Petri dish to maintain humidity, along with a microscope slide. A 20 µL droplet of conidial suspension (1×10^6 conidia/mL) from each pathogen was placed on the slide. Filter paper strips containing the specified 3-MP concentrations were attached to the inner surface of the Petri dish lid, and the dish was securely sealed. Incubation was carried out at 25 °C in darkness, with conidial germination of *C. gloeosporioides* and *L. theobromae* monitored at 12 and 24 hours. For each treatment, 100 conidia from three replicates were analyzed using a Zeiss Primo Star microscope (40× magnification), and images were recorded with a Zeiss Axiocam ERc 5s camera. The experiment included three replications and was repeated three times.

Suppressive activities of 3-MP on postharvest pathogens of mango

To evaluate the suppressive effect of 3-MP on postharvest diseases of mango, surface-disinfected fruit (treated with 1.0% sodium hypochlorite) were subjected to wound inoculation assays. Two wounds were made on each mango fruit using a sterile cork-borer, and each wound was inoculated with a 20 µL droplet of conidial suspension (1×10^5 conidia/mL) of *C. gloeosporioides* and *L. theobromae* (Konsue et al., 2020). The fruits were placed in sealed plastic boxes containing sterile filter papers treated with 3-MP. For *C. gloeosporioides*, concentrations of 120 and 200 µM were applied, while for *L. theobromae*, concentrations of 160 and 320 µM were used. The boxes were tightly sealed to ensure continuous exposure of the fruits to 3-MP throughout the incubation period. Inoculated fruits were incubated at 28 ± 1 °C with relative humidity ≥ 90%. Disease severity was assessed at 5 days post-inoculation for *C. gloeosporioides* and 3 days post-inoculation for *L. theobromae*.

Effect of 3-MP on mango shelf life

Freshly harvested mango fruits were air-dried at room temperature and subsequently placed in plastic boxes. The fruits were stored in a climatic cabinet maintained at 20 °C and 85% relative humidity. Two experimental groups were established: untreated mangoes (control) and mangoes treated with 3-MP at 50 µM and 100 µM concentrations. Color change was monitored visually after 10 days of storage. Initially, the peel exhibited a green coloration, which gradually transitioned to yellow as ripening progressed. Weight loss was determined 10 days after storage by recording fruit mass relative to its initial harvest weight. The percentage of weight loss was calculated using the following equation (Tuffour et al., 2026):

$$\text{Weight loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100\%$$

Statistical analysis

Experiments were arranged in a completely randomized design to evaluate the biological activity of 3-MP. Data were analyzed using one-way analysis of variance (ANOVA), and treatment means were compared using Tukey's honest significant difference (HSD) post hoc test at a significance level of $p \leq 0.01$. All statistical analyses were performed using R software (version 4.5.1, accessed 5 January, 2026) integrated into RStudio (version 2025.09.1+401, accessed 5 January, 2026). Results are presented as mean ± standard error (SE) from three biological replicates in Tables and Figures.

Results

3-MP inhibited the fungal mycelial growth

A dose-dependent inhibition of mycelial growth was observed in both postharvest pathogens of mango. *C. gloeosporioides* displayed progressive sensitivity to 3-MP, with 41.11 ± 0.61% inhibition at 100 µM after 7 days and complete suppression achieved at 200 µM (Figure 2, A and B). *L. theobromae* also exhibited marked responsiveness, showing 78.84 ± 0.64% inhibition at 100 µM and complete inhibition at 300 µM by 5 days post-incubation (Fig. 2, A and B).

Inhibition of Conidial Germination and Appressorium Formation by 3-MP

Conidial germination assays revealed a concentration-dependent inhibitory effect of 3-MP on both *C. gloeosporioides* and *L. theobromae*. For *C. gloeosporioides*, germination at 12 h in the control was 43.0 ± 1.52%, whereas treatment with 3-MP reduced germination to 17 ± 1.0% at 50 µM, 12.67 ± 0.67% at 100 µM, and near complete inhibition (0.67 ± 0.33%) at 200 µM. By 24 h, control conidia reached 88.66 ± 1.76% germination, while inhibition persisted with 51.33 ± 2.40% at 50 µM, 32.66 ± 1.45% at 100 µM, and 4 ± 1.15% at 200 µM (Fig. 3, A and B). Germ tubes were malformed, and appressorium formation was severely impaired at higher concentrations (Table 3). For *L. theobromae*, control conidia showed 98.66 ± 1.33% germination at 12 h. In contrast, 3-MP reduced germination to 83.33 ± 0.88% at 50 µM, 42.33 ± 1.33% at 100 µM, and 3 ± 0.57% at 200 µM. At 24 h, control conidia reached 99.67 ± 0.33%, while inhibition remained evident with 95.33 ± 0.88% at 50 µM, 70.0 ± 1.25% at 100 µM, and 5.67 ± 0.66% at 200 µM (Fig. 3, C and D). Conidia exposed to higher concentrations displayed collapsed morphology and failed germ tube elongation (Table 3).

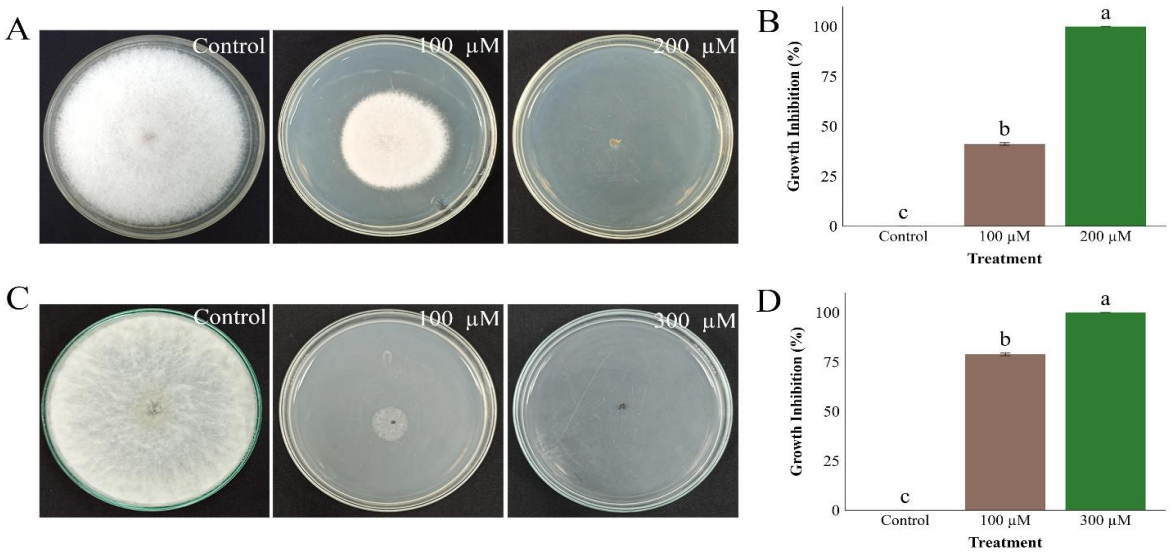

Fig. 2. Inhibitory effects of 3-MP on fungal mycelial growth. (A) Micrograph representing the suppression of mycelial growth of *C. gloeosporioides* (7 days) in PDA; (B) Data represents the growth inhibition percentage of *C. gloeosporioides* by 3-MP across all concentrations. (C) Micrograph representing the suppression of mycelial growth of *L. theobromae* (5 days) (D) Data represents the growth inhibition percentage of *L. theobromae* by 3-MP across all concentrations. Error bars represent the standard error calculated from three biological replicates. Different letters above the bars indicate statistically significant differences between treatments (P ≤ 0.01), as determined by one-way ANOVA followed by the Tukey–HSD post hoc test.

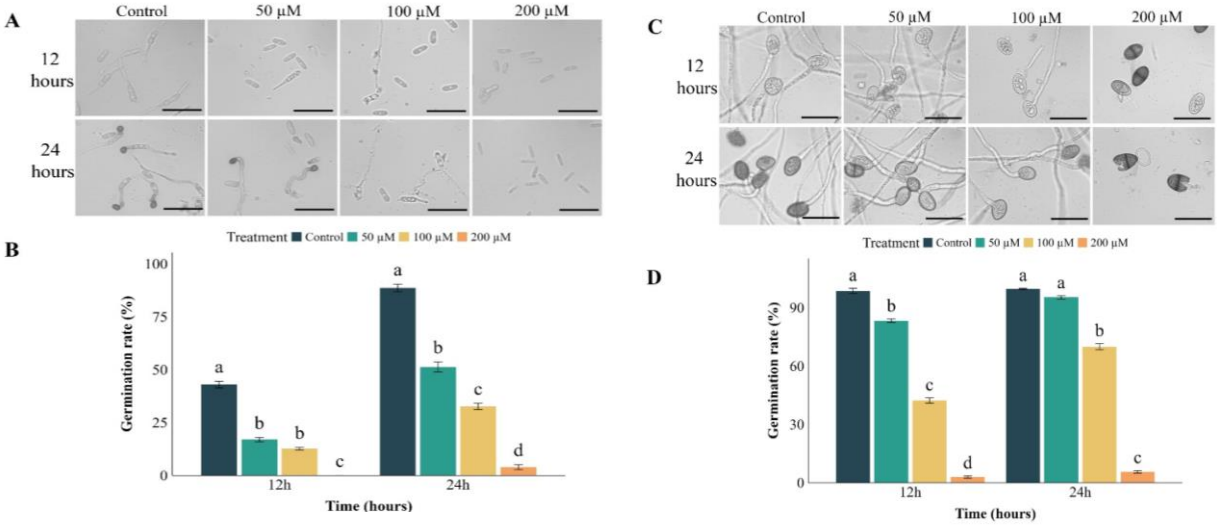

Fig. 3. Effect of 3-MP on spore germination and morphology alteration. (A) Time-course microscopic observations of *C. gloeosporioides* conidia exposed to 3-MP, Images were captured at 12 and 24hours post-incubation. (B) Quantitative analysis of *C. gloeosporioides* conidial germination rate (%) under different treatments over time. (C) Time-course microscopic observations of *L. theobromae* conidia exposed to 3-MP, Images were captured at 12 and 24 hours post-incubation. (D) Quantitative analysis of *L. theobromae* conidial germination rate (%) under different treatments over time. Each data point represents the mean ± SE from three independent replicates, with 100 conidia counted per replicate. Different letters above bars indicate statistically significant differences among treatments at each time point (Tukey's HSD, p < 0.01). Bar = 25 µm.

Table 2. Effects of 3-MP on germination of conidia and morphology of germ tubes and appressoria of *C. gloeosporioides* at 50 µM, 100 µM, and 200 µM *in vitro*.

Treatment	Time (h)	Germinated (% ± SE)	Major morphological changes occurred in the treated conidia
Control	12	43.01 ± 1.52[a]	Significant mycelial elongation
	24	88.66 ± 1.76[a]	Fully developed hyphal networks
50 µM	12	17 ± 1.0[b]	Hyphal growth was reduced with fewer branches
	24	51.33 ± 2.40[b]	Developed hyphal networks but appressoria formed
100 µM	12	12.67 ± 0.67[b]	Conidia with limited growth and abnormal germ tube elongation
	24	32.66 ± 1.45[c]	Deformed germ tube and no appressoria formed
	12	0.67 ± 0.33[c]	Near no conidia germination observed
200 µM	24	4 ± 1.15[d]	Conidia appeared shrunken, no germination

Table 3. Effects of 3-MP on germination of conidia and morphology of germ tubes and appressoria of *L. theobromae* at 50 µM, 100 µM, and 200 µM *in vitro*.

Treatment	Time (h)	Germinated (% ± SE)	Major morphological changes occurred in the treated conidia
Control	12	98.66 ± 1.33[a]	Significant conidial germination
	24	99.67 ± 0.33[a]	Fully developed hyphal networks
50 µM	12	83.33 ± 0.88[b]	Significant conidial germination
	24	95.33 ± 0.88[a]	Developed hyphal networks
100 µM	12	42.33 ± 1.33[c]	Conidia with limited growth,
	24	70.0 ± 1.25[b]	Significant conidial germination but fewer branches
200 µM	12	3 ± 0.57[d]	Near no conidia germination observed
	24	5.67 ± 0.66[c]	Conidial cell wall ruptured, showing cell wall leakage.

3-MP suppressed the anthracnose and stem-end-rot disease development in mango

Application of 3-MP significantly reduced postharvest disease development in mango fruit inoculated with *C. gloeosporioides* and *L. theobromae*. The suppressive effect was dose-dependent and consistent across both pathogens, as reflected in reductions in disease severity compared to untreated controls. In the case of *C. gloeosporioides*, control fruit exhibited high anthracnose severity (62.22 ± 2.22%), whereas treatment with 120 µM 3-MP reduced severity to 37.77 ± 2.22%. A more pronounced suppression was observed at 200 µM, where disease severity decreased to 4.44 ± 2.22% (Fig. 4, A and B). Similarly, for *L. theobromae*, control fruit showed severe stem-end rot symptoms (82.22 ± 2.22%), which were reduced to 40.46 ± 3.45% and 8.89 ± 2.22% following treatment with 160 µM and 320 µM 3-MP, respectively (Fig. 4, C and D). Statistical analysis confirmed significant differences among treatments.

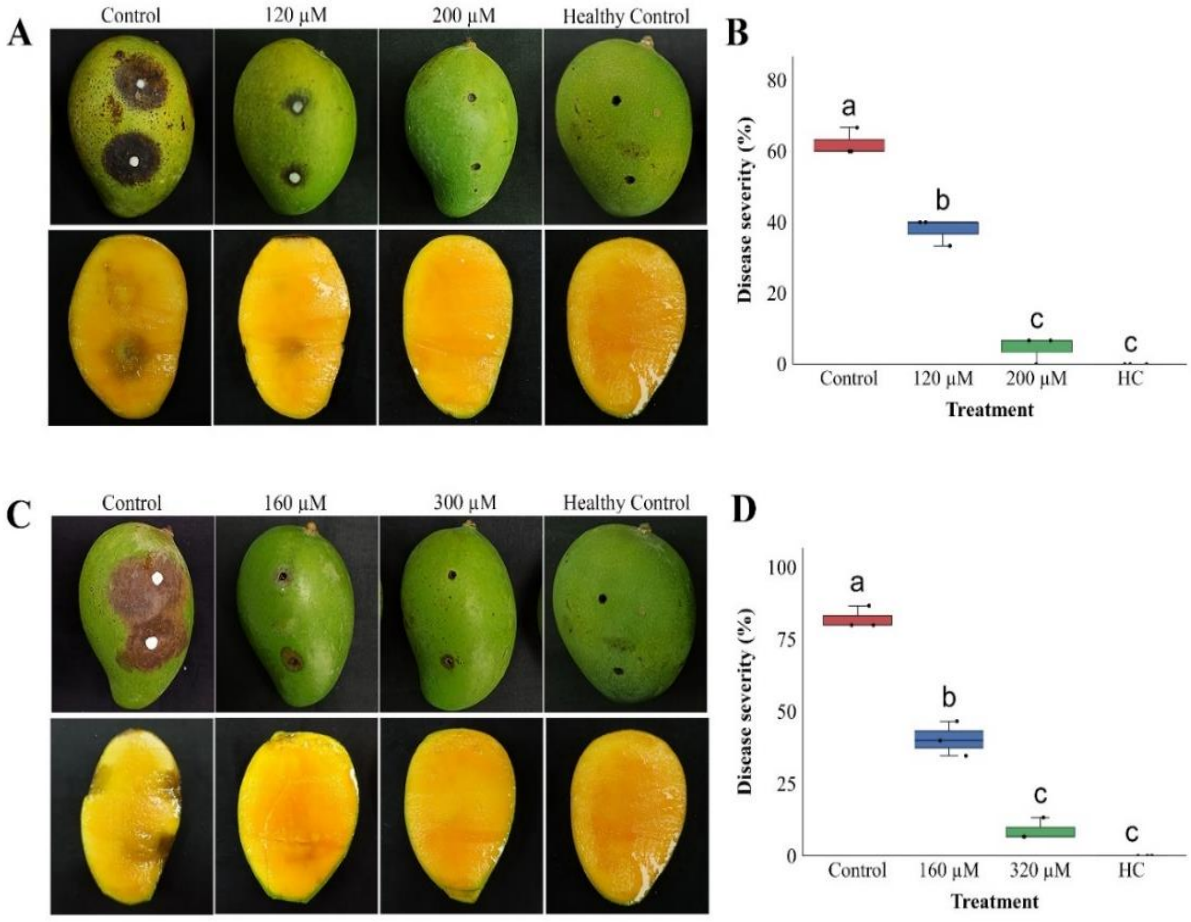

Fig. 4. Effect of 3-MP on disease suppression of postharvest *C. gloeosporioides* and *L. theobromae* pathogen (A) Effect of 3-MP on controlling mango anthracnose caused by *C. gloeosporioides* (B) Anthracnose disease severity (%) in mango treated with 3-MP, relative to the untreated control (C) Effect of 3-MP on controlling mango stem-end rot caused by *L. theobromae*. (D) Stem end rot disease severity (%) in mango treated with 3-MP, relative to the untreated control. Different letters above bars indicate statistically significant differences among treatments at each time point (Tukey's HSD, $p \leq 0.01$).

3-MP delayed ripening and reduced weight loss of mango

Application of 3-MP had a marked impact on the postharvest performance of mango fruits stored at 20 °C and 85% relative humidity. After 10 days of storage, the control fruits displayed rapid peel color transition from green to deep yellow accompanied by visible signs of overripening and clear signs of decay. In contrast, fruits treated with 3-MP retained a more gradual and uniform color change. The 100 µM treatment was most effective in slowing ripening and maintaining greener hues (Fig. 5A). Weight loss measurements supported these visual observations (Fig. 5B). Control fruits exhibited the highest percentage (7.84±0.96) of mass reduction after 10 days, whereas 3-MP treatments reduced this loss in a concentration-dependent manner. The 100 µM treatment consistently minimized weight loss (3.63±0.91), suggesting that 3-MP helps preserve fruit integrity by slowing metabolic activity and water loss during storage.

Discussion

In this study, 3-MP derived from *B. safensis*, demonstrated potent antifungal efficacy against two devastating mango pathogens. Beyond its biocontrol potential, 3-MP is a naturally occurring constituent of edible flora like snake fruit (Wijaya et al., 2005) and holds GRAS (Generally Recognized as Safe) status from the U.S. FDA. This safety profile is a critical distinction, as it addresses increasing consumer demand for residue-free produce and underscores the suitability of the compound for direct agricultural application (Cohen et al., 2018, 2020).

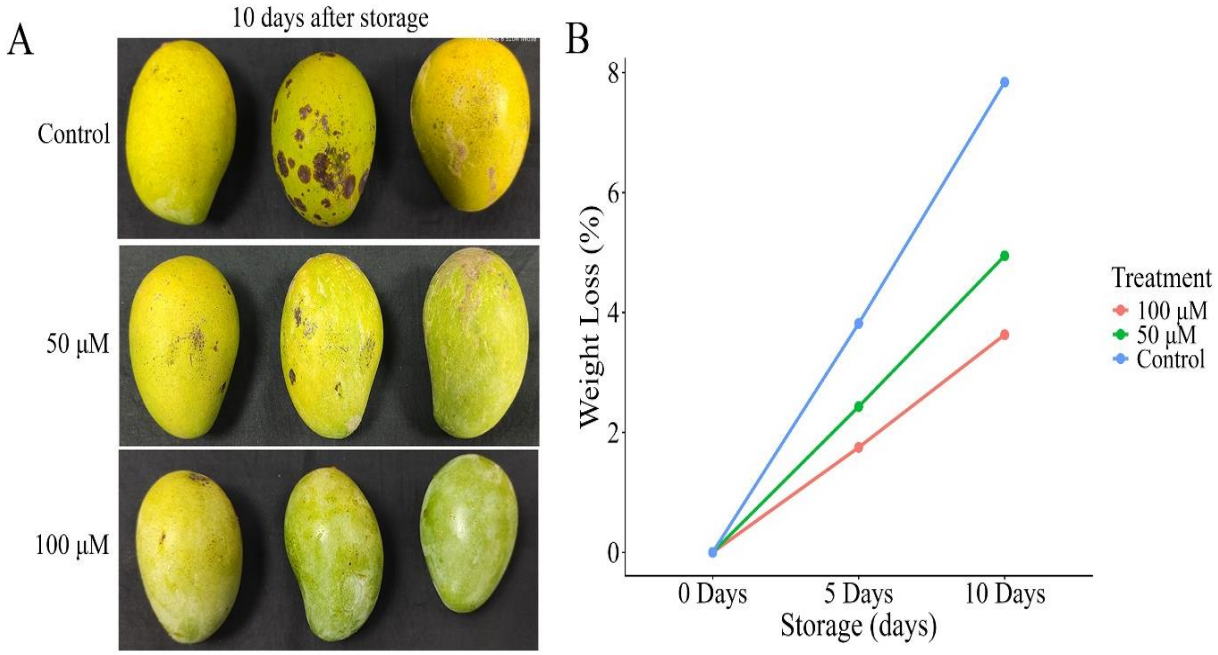

Fig. 5. Effect of 3-MP on mango ripening and weight loss. (A) Visual appearance of mango fruits after 10 days of storage at 20 °C and 85% relative humidity. (B) Percentage of fruit weight loss at 0, 5, and 10 days.

The broad-spectrum inhibitory activity of 3-MP against *C. gloeosporioides* and *L. theobromae* was characterized by a multi-stage assault on fungal development. By suppressing mycelial growth, conidial germination, and appressorium formation (Sujon et al., 2026), 3-MP effectively disrupts the pathogen's life cycle before host penetration can occur. This dose-dependent inhibition aligns with the established mechanisms of other *Bacillus*-derived VOCs, which typically compromise cell wall integrity and interfere with essential signaling pathways (Gupta et al., 2025). While Massawe et al. (2018) highlighted the inhibition of *Sclerotinia sclerotiorum* by endophytic VOCs, our findings specifically position 3-MP as a high-potency agent, achieving complete inhibition of *C. gloeosporioides* and *L. theobromae* at concentrations as low as 200–300 µM. This reinforces previous suggestions by Paul et al. (2025) that 3-MP targets the Cell-Wall Integrity (CWI) and MAPK pathways, which are vital for fungal survival under stress.

A key novelty of this study lies in the observed morphological disruption induced by 3-MP. The transition from healthy conidia to collapsed, deformed germ tubes represents a significant loss of virulence. Similar "morphological collapse" has been documented in *Rahnella aquatilis* (Kong et al., 2020) and *Streptomyces corchorusii* (Li et al., 2024), but the efficacy of 3-MP in preventing appressorium formation is particularly noteworthy. Since the appressorium is the "mechanical drill" used by *C. gloeosporioides* to breach the mango cuticle, its inhibition explains the dramatic reduction in disease severity observed in our *in vivo* assays.

Furthermore, the application of 3-MP provided a dual-benefit rarely seen with synthetic fungicides: simultaneous disease suppression and physiological preservation. By reducing weight loss (3.63% vs. 7.84% in controls) and delaying peel yellowing, 3-MP appears to modulate the ripening rate of mangoes stored at 20 °C. This mirrors the effects of 2,5-dimethylpyrazine (Janamatti et al., 2022) and suggests that 3-MP may reduce the metabolic "burst" associated with both ripening and pathogen-induced tissue breakdown. This synergy between antifungal activity and shelf-life extension provides a significant commercial advantage for postharvest management of mango (Archana et al. 2021).

While 3-MP presents a promising bio-based alternative to synthetic fungicides, its successful transition to commercial application necessitates further exploration. Future research should prioritize molecular docking studies to elucidate the precise protein targets within the Cell-Wall Integrity (CWI) and MAPK pathways, thereby confirming its specific mode of action at a molecular level. Additionally, advancements in formulation science are required to develop slow-release sachets or specialized bio-coatings capable of maintaining effective VOC concentrations throughout the duration of large-scale commercial cold storage. Finally, synergy studies should assess the compatibility of 3-MP with other "green" technologies, such as UV-C treatment or modified atmosphere packaging (MAP), to establish a holistic and robust postharvest defense system for global mango supply chains.

Conclusion

This study identifies the volatile metabolite 3-methylpentanoic acid (3-MP), derived from *Bacillus safensis*, as a potent and multifaceted antifungal agent against the primary postharvest mango pathogens *C. gloeosporioides* and *L. theobromae*. Our results demonstrate that 3-MP effectively disrupts the fungal life cycle by inhibiting mycelial proliferation, suppressing conidial germination, and compromising critical infection structures—specifically germ tubes and appressoria—culminating in near-complete disease suppression at concentrations of 200 µM and 300 µM, respectively. Distinguished by its "Generally Recognized as Safe" (GRAS) status and natural occurrence, 3-MP offers a high-efficacy, residue-free alternative to conventional synthetic fungicides, aligning with global shifts toward sustainable agriculture. By simultaneously extending shelf life and reducing

pathological decay, this compound addresses key vulnerabilities in the postharvest supply chain and reinforces the viability of microbial volatiles as eco-friendly biocontrol agents. This study lays the groundwork for using 3-MP in pest management, but future research must identify its molecular targets and optimize delivery for commercial storage to support the global mango industry.

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Authors contribution

Md. Shahrear Parvaj Sujon: Writing–original draft, Methodology, Validation, Investigation, Visualization, Software, Formal analysis, Data curation; Avi Kumar Badhon: Methodology, Investigation, Writing – review and editing; Abdullah Al Kafi, Methodology, Investigation, Writing – original draft; Abdullah Al Nayeem: Investigation, Writing– review and editing; Md. Mynul Islam: Validation, Writing – review and editing; Dipali Rani Gupta: Supervision, Resources, Validation, Writing– review and editing; Tofazzal Islam: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing– review and Editing.

Conflict of interest

The authors declare no conflict of interest.

Declaration of Generative AI Use

During the preparation of this work, the author used Gemini to improve readability and precision of the writing. After using this tool, the authors reviewed and edited the content as needed and takes full responsibility for this content of the published article.

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