International Journal of Food Microbiology 430 (2025) 111037

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Effective control of snake fruit (*Salacca zalacca*) rot using *Trichoderma asperelloides* SKRU-01: A safe approach to preserving fruit quality

Sawai Boukaew [a,*], Julalak Chuprom [b], Jirayu Buatong [c], Sujirat Sornprasit [a], Sureeporn Wijitsopa [a], Karistsapol Nooprom [a], Rachasak Boonhok [d]

[a] Faculty of Agricultural Technology, Songkhla Rajabhat University, Songkhla 90000, Thailand
[b] School of Languages and General Education, Walailak University, Nakhon Si Thammarat 80160, Thailand
[c] International Center of Excellence in Seafood Science and Innovation, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand
[d] Department of Medical Technology, School of Allied Health Sciences, Walailak University, Nakhon Si Thammarat 80160, Thailand

ARTICLE INFO

Keywords:
Postharvest disease management
Fungal inhibition
Biocontrol agents
Food safety
Shelf-life extension

ABSTRACT

Rot disease, caused by the fungal pathogen *Peniophora salaccae* SKRU002, affects the quality of snake fruit production. In the pursuit of sustainable disease management, biocontrol using *Trichoderma asperelloides* SKRU-01 offers a promising solution. This study evaluated the antagonistic potential of *T. asperelloides* SKRU-01 against *P. salaccae* SKRU002 in both *in vitro* assays and snake fruit trials, while also assessing its impact on fruit quality. *In vitro* dual culture assays revealed that *T. asperelloides* SKRU-01 inhibited *P. salaccae* SKRU002 growth by 62.5 % over 10 days through efficient nutrient colonization. Microscopic analysis confirmed that *T. asperelloides* SKRU-01 hyphae penetrated and wrapped around *P. salaccae* SKRU002, causing cytoplasmic lysis. Additionally, *T. asperelloides* SKRU-01 culture filtrates (20 % *v/v*) completely inhibited *P. salaccae* SKRU002 growth in both solid and liquid media. LC-QTOF/MS analysis identified 31 secondary metabolites, including toyocamycin and antimycin A1. In snake fruit trials, *T. asperelloides* SKRU-01 culture filtrates provided 100 % protection against disease incidence (DI) and severity (DS), comparable to Mancozeb®. The application of *T. asperelloides* SKRU-01 spores (10^7 spores/mL) reduced DI and DS to 0 % within 1–3 days post-pathogen inoculation, demonstrating both protective and curative effects. Furthermore, while *P. salaccae* SKRU002 significantly affected fruit quality—causing weight loss, color changes, and reductions in total soluble solids, phenolic content, titratable acidity, and antioxidant activity—the simultaneous application of *T. asperelloides* SKRU-01 mitigated these effects without compromising fruit quality. These findings indicate the antagonistic activity of *T. asperelloides* SKRU-01 and its metabolites against *P. salaccae* SKRU002, suggesting their potential as biofungicidal agents for managing rot disease in snake fruit.

1. Introduction

Salacca zalacca (Gaertn.) Voss, commonly known as snake fruit, is a tropical fruit widely distributed throughout Southeast Asia, emerging as a vital economic commodity in Thailand and neighboring countries, with global exports to destinations such as Russia, the United Arab Emirates, and the Maldives (Supapvanich et al., 2011; Phothisuwan et al., 2021). Famed for its white, firm pulp, distinguished by a sweet, slightly acidic, and astringent flavor, the snake fruit is esteemed as a bountiful provider of vital nutrients, encompassing vitamins, minerals, dietary fiber, and bioactive compounds that enhance its antioxidant properties (Aralas et al., 2009; Suica-Bunghez et al., 2016). Despite its nutritional value, the cultivation and pre- and post-harvest stages of snake fruit present a significant challenge to consumer acceptance due to fungal infections. Addressing this challenge and preserving the fruit's desirable attributes necessitates alternative methods for controlling mold growth (Anoraga and Bintoro, 2020).

Between 2022 and 2023, symptoms of *P. salaccae* SKRU002 infection were detected in approximately 30 % of snake fruits in Thung Naree Subdistrict, Pabon District, Phatthalung Province, Southern Thailand. External symptoms included severely dried fruits covered with fungus, and exhibiting yellowish-white colored fungal hyphae. Cross-sectioning of infected snake fruit samples revealed shrunken skin and pulp with a black-brown color, fruit rot, and the presence of white fungal hyphae

* Corresponding author.
E-mail address: sawai.bo@skru.ac.th (S. Boukaew).

https://doi.org/10.1016/j.ijfoodmicro.2024.111037
Received 27 September 2024; Received in revised form 12 December 2024; Accepted 19 December 2024
Available online 21 December 2024

(Boukaew et al., 2024a). These concerns have spurred research endeavors to uncover efficient and eco-friendly approaches for controlling plant pathogen diseases, with the goal of reducing economic losses.

Biological control emerges as a pivotal strategy in managing fungal diseases, employing live microorganisms to regulate phytopathogen populations below economically damaging levels. The investigation of antagonistic microorganisms, such as bacteria, yeast, and fungi, isolated from crops or soils showcasing antagonistic activity against plant pathogenic fungi, has emerged as a rapidly growing area of research (Ayaz et al., 2023; Fenta et al., 2023).

Microbial biocontrol agents are poised as promising and safe alternatives to synthetic fungicides, holding significant potential for effective disease management. Different types of antagonistic microorganisms, such as *Trichoderma* spp. (Díaz-Gutiérrez et al., 2021; Erazo et al., 2021; Boukaew et al., 2023a; Zhan et al., 2023; Boukaew et al., 2024b, 2024c), *Bacillus* spp. (Calvo et al., 2020; You et al., 2021; Xie et al., 2021; Liu et al., 2023), *Streptomyces* spp. (Boukaew et al., 2023b, 2023c, 2023d; Nazari et al., 2023; Li et al., 2024), and *Pseudomonas* spp. (Elsharkawy et al., 2023; Mehmood et al., 2023), have garnered attention in minimizing phytopathogenic impacts. Within this realm, the genus *Trichoderma* stands out as a powerful reservoir of efficient biocontrol agents combating plant pathogenic fungi (Amira et al., 2017; Brito et al., 2020; da Costa et al., 2021; Díaz-Gutiérrez et al., 2021; Erazo et al., 2021; Boukaew et al., 2023a; Zhan et al., 2023; Boukaew et al., 2024b, 2024c). Numerous species, such as *T. harzianum* (Youssef et al., 2016; Amira et al., 2017), *T. asperelloides* (Boukaew et al., 2023a, 2024a, 2024b), *T. viride* (Verma et al., 2007; Bae et al., 2016; Awad et al., 2018), *T. atroviride* (Savazzini and Longa, 2009; Chilosi et al., 2020), *T. asperellum* (Shang et al., 2020), and *T. saturnisporum* (Martínez et al., 2016), have demonstrated exceptional biological control effects. Successful cases of employing biocontrol *Trichoderma* against fungal phytopathogens have been well-documented. These strains deploy multiple mechanisms to combat pathogens, including (i) rapid reproduction on simple nutrients, enabling effective colonization of surfaces (Chet and Inbar, 1994; Belanger et al., 1995; Verma et al., 2007; Savazzini and Longa, 2009), (ii) production of antimicrobial compounds including cell wall-degrading enzymes, antibiotics, cytotoxic agents, and volatile organic compounds (Sid-Ahmed et al., 2003; Verma et al., 2007; Savazzini and Longa, 2009), and (iii) possession of biostimulating capabilities (Harman et al., 2004; Shoresh et al., 2010).

In recent years, researchers worldwide have shown increasing interest in identifying effective *Trichoderma* strains to manage plant pathogens across diverse crops. Examples include the control of *Fusarium solani* in peanut (Erazo et al., 2021) and olive (Amira et al., 2017) cultivation, *F. oxysporum* in stevia (Díaz-Gutiérrez et al., 2021), *Botrytis cinerea* in tomato (Geng et al., 2022), *Ceratocystis fimbriata* in mango (Brito et al., 2020), *Colletotrichum musae* in banana (da Costa et al., 2021), and *Lasiodiplodia theobromae* in mango fruits (Zhan et al., 2023). In our previous studies, *T. asperelloides* SKRU-01 demonstrated strong inhibitory effects against *Aspergillus flavus* and *A. parasiticus* in peanuts, effectively preventing spoilage in food and feed (Boukaew et al., 2023a, 2024b, 2024c). These effects were attributed to its production of antimicrobial secondary metabolites and volatile organic compounds (Boukaew et al., 2023a, 2024b, 2024c). While *T. asperelloides* SKRU-01 is recognized for its antifungal activity, this study expands its application by evaluating its efficacy specifically against *P. salaccae* SKRU002.

The objectives are threefold: (i) to evaluate the *in vitro* efficacy of *T. asperelloides* SKRU-01 against *P. salaccae* SKRU002, (ii) to assess the strain's protective and curative potential on snake fruit while evaluating its effectiveness in preserving fruit quality, and (iii) to identify the antifungal agents produced, using liquid chromatography–quadrupole time-of-flight mass spectrometry (LC-QTOF/MS). Our goal is to enhance understanding of the effectiveness of these antifungal agents against *P. salaccae* SKRU002 and to propose practical applications in agriculture.

2. Material and methods

2.1. Sources of materials

2.1.1. Microorganisms and inoculum preparation

The antagonistic *T. asperelloides* SKRU-01 was isolated from loam soil samples collected from cultivation plots at the Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Thailand, and identified through molecular characterization based on the internal transcribed spacers (ITS), *rpb*-2, and *tef*-1 regions (Boukaew et al., 2023a). The strain was cultured on potato dextrose agar (PDA) at 30 °C for 7 days. Spore inoculum was prepared by suspending it in sterile 0.1 % Tween 80. Spore counts were then performed using a hemacytometer before dilution to attain concentrations ranging from 10^4 to 10^8 spores/mL with sterile distilled water.

The pathogen *P. salaccae* SKRU002 was isolated from snake fruits exhibiting rot symptoms, collected from cultivation fields in Phatthalung Province, Thailand (coordinates: 7°20′63″N, 100°12′47″E). This isolate was identified through morphological examination and molecular phylogenetic analysis, targeting the ITS and the large subunit (nrLSU) regions of nuclear ribosomal DNA (rDNA) (Boukaew et al., 2024a). The strain was then cultured and maintained on PDA plates at 30 °C for seven days.

2.1.2. Mancozeb®

The chemical fungicide Mancozeb® (80 % WP) was prepared according to the manufacturer's recommendations, with a final concentration of 1.2 g/L (1,200 ppm).

2.1.3. Snake fruit preparation

Snake fruits (cv. 'Sumalee') were harvested from a farm in Thung Naree Subdistrict, Pabon District, Phatthalung Province, Southern Thailand. The fruits, averaging 20 per kilogram, were collected 38 weeks after pollination. They were carefully selected to ensure uniform size and the absence of any visible diseases or wounds. Each fruit was then scrubbed with a brush, rinsed with sterile distilled water, and air-dried for 30 min in a laminar flow cabinet. The prepared fruits were immediately used for laboratory experiments, which were conducted at 30 °C.

2.2. In vitro detection of antagonistic activity of T. asperelloides SKRU-01 against P. salaccae SKRU002

2.2.1. Dual culture effect and microscopic analysis of the interaction

The antagonistic interaction between *T. asperelloides* SKRU-01 and *P. salaccae* SKRU002 was evaluated using a dual culture assay as described by Boukaew et al. (2023a). Colony diameters were measured daily, and the percentage of inhibition (%) was calculated using the following formula (Eq. (1)):

$$\text{Inhibition of hyphal growth (\%)} = [(\text{Colony diameter of SKRU002 in control} - \text{Colony diameter of SKRU002 with SKRU} - 01) / \text{Colony diameter of SKRU002 in control}] \times 100 \quad (1)$$

The experiment was conducted with three replications and was repeated twice.

Microscopic analysis of the mycoparasitic interaction between *T. asperelloides* SKRU-01 and *P. salaccae* SKRU002 was performed using confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) on a 7-day-old dual culture plate. For CLSM, fungal mycelia from the interaction zone were stained with lactophenol cotton blue and observed under a light microscope (Zeiss LSM 800). For SEM, agar plugs from the interaction zone were fixed in 2.5 % glutaraldehyde for 2 h, rinsed with phosphate buffer (0.1 M, pH 7.2), and dehydrated in graded ethanol series (50 %–99.99 %). Critical point drying and gold coating were performed, and images were captured using an FEI Quanta 400 SEM.

2.2.2. Effect of *T. asperelloides* SKRU-01 culture filtrates against *P. salaccae* SKRU002

To prepare the culture filtrates, *T. asperelloides* SKRU-01 was cultured in potato dextrose broth (PDB) at 30 °C for 10 days (Boukaew et al., 2023a). Mycelial mats were subsequently filtered out using Whatman No. 1 filter paper to obtain a clear culture filtrate. This filtrate was then sterilized through a 0.22 μm Millipore membrane (Sartorius®), resulting in the production of culture filtrates (referred to as SKRU-01 culture filtrates). The effects of culture filtrates from *T. asperelloides* SKRU-01 were evaluated against *P. salaccae* SKRU002 in both solid and liquid media, as well as *in vivo* on snake fruit.

On solid media, varying concentrations of SKRU-01 culture filtrates (5 %–20 % *v/v*) were incorporated into PDA medium. Sterile water at the same concentrations as the SKRU-01 culture filtrates was used as a control. A 5 mm plug of *P. salaccae* SKRU002 was inoculated onto each plate and incubated at 30 °C for seven days. After the incubation period, colony diameters were measured, and inhibition percentages were calculated. The experiment included three replicates and was repeated twice.

For liquid culture, the same concentrations of SKRU-01 culture filtrates (5 %–20 % *v/v*) were added to PDB medium in conical flasks. Sterile water at the same concentrations as the SKRU-01 culture filtrates was used as a control. After inoculation with *P. salaccae* SKRU002 and incubation at 30 °C with shaking at 150 rpm, the resulting mycelial mats were filtered, dried, and weighed to calculate inhibition percentages (Li et al., 2011). This experiment also included three replications and was repeated twice.

Pearson's correlation coefficient (r) was used to evaluate the relationship between the concentration of *T. asperelloides* SKRU-01 culture filtrates (5 % to 20 %, *v/v*) and the inhibition of *P. salaccae* SKRU002. Fungal inhibition was assessed in both solid and liquid culture systems.

2.3. Identification of antifungal compounds in *T. asperelloides* SKRU-01 culture filtrates using liquid chromatograph-quadrupole time-of-flight mass spectrometer (LC-QTOF/MS)

The characterization of antifungal compounds in SKRU-01 culture filtrates was carried out using LC-QTOF/MS to analyze its antimicrobial agents, following the protocol outlined by Alara et al. (2018). Experimental procedures for the ultra-high-performance liquid chromatography (UHPLC) column, mobile phase, and the LC-QTOF/MS instrument were conducted in accordance with methods previously described by Boukaew et al. (2023d). LC-MS chromatograms in $^{+}$ESI mode were compared using Mass Hunter METLIN Metabolite PCD and PCDL version 8 databases and libraries for compound identification.

2.4. In vivo biocontrol activity of *T. asperelloides* SKRU-01

2.4.1. Efficacy of *T. asperelloides* SKRU-01 culture filtrates against *P. salaccae* SKRU002 on snake fruit

An investigation was conducted to evaluate the potential protective effect of SKRU-01 culture filtrates against *P. salaccae* SKRU002 on snake fruit. Sterilized fruits were immersed in either SKRU-01 culture filtrates or Mancozeb® for 10 min at 30 °C. Fruits not inoculated with *P. salaccae* SKRU002 served as the negative control, while those inoculated with *P. salaccae* SKRU002 only served as the positive control. Subsequently, the fruits were air-dried for 30 min within a laminar airflow chamber before being individually placed into separate sterile plastic containers measuring 19.2 × 28 × 10.9 cm^3. These containers were maintained under conditions of 85–90 % relative humidity. The snake fruits were gently wounded at the center of the side surface using sterile fine needles, and a 5 mm-diameter mycelial plug of *P. salaccae* SKRU002 was inoculated directly into each wound. They were subsequently stored at 30 °C for 12 days. After the designated storage period, disease incidence (DI) was assessed by calculating the percentage of rotten snake fruit using the following formula (Eq. (2)) (Parafati et al., 2015):

$$\text{Disease incidence}\ (\%) = (\text{Number of rotten snake fruit}/\text{Total number of snake fruit}) \times 100 \quad (2)$$

Disease severity (DS) was also evaluated after the storage period using a 0-to-4 scale: 0 = no rot; 1 = 1–25 % of snake fruit with rot; 2 = 26–50 %; 3 = 51–75 %; 4 = 76–100 %. The DS index was determined using the following formula (Eq. (3)) (Parafati et al., 2015):

$$\text{Disease severity}\ (\%) = [\Sigma\ (\text{number of snake fruit in each category} \times \text{corresponding disease scale}) / (\text{total number of snake fruit examined} \times \text{highest disease scale})\,] \times 100 \quad (3)$$

Each treatment was replicated three times, with 10 fruits used per replicate, for a total of 30 fruits per treatment.

2.4.2. Efficacy of spore concentrations of *T. asperelloides* SKRU-01 against *P. salaccae* SKRU002 on snake fruit

The impact of spore concentrations ranging from 10^4 to 10^8 spores/mL of *T. asperelloides* SKRU-01 against *P. salaccae* SKRU002 on snake fruit was investigated. Sterilized snake fruits were gently wounded at the

center of the side surface using sterile fine needles, and 0.5 cm agar plugs were excised from 3-day-old PDA culture plates of *P. salaccae* SKRU002. Agar plugs were then placed directly onto wounded areas of the snake fruit. Subsequently, 10 μL of a spore concentration (10^4 to 10^8 spores/mL) of *T. asperelloides* SKRU-01 was applied on top of the mycelial agar plugs. Fruits that were not inoculated with any treatment served as the negative control, while those inoculated with *P. salaccae* SKRU002 served as the positive control. Afterward, they were placed individually inside separate sterile plastic containers measuring 19.2 × 28 × 10.9 cm^3. These containers were maintained under conditions of 85–90 % relative humidity. The fruits were then stored at 30 °C for 12 days, and disease incidence and disease severity were calculated as described above. Each treatment was replicated three times, with 10 fruits used per replicate, for a total of 30 fruits per treatment.

2.5. Assessing the biocontrol capacity of T. asperelloides SKRU-01 in halting the development of P. salaccae SKRU002 on snake fruit and preserving fruit quality

2.5.1. Efficacy of T. asperelloides SKRU-01 in halting the development of P. salaccae SKRU002 on snake fruit

The efficacy of *T. asperelloides* SKRU-01 in inhibiting the mycelial growth of *P. salaccae* SKRU002 on snake fruit was examined. Sterilized snake fruits were gently wounded at the center of the side surface using sterile fine needles, and agar plugs (0.5 cm) were excised from 3-day-old PDA culture plates of *P. salaccae* SKRU002. The agar plugs were then placed directly onto wounded areas of the snake fruit. Subsequently, 50 μL of a spore concentration at 10^7 spores/mL of *T. asperelloides* SKRU-01 was applied on top of the mycelial agar plugs at different time points: simultaneously, 1 day after pathogen development, 2 days after pathogen development, and 3 days after pathogen development. Fruits that were not inoculated with any treatment served as the negative control, while those inoculated with *P. salaccae* SKRU002 served as the positive control. Afterward, they were individually placed inside separate sterile plastic containers measuring 19.2 × 28 × 10.9 cm^3 and maintained under conditions of 85–90 % relative humidity. The fruits were then stored at 30 °C for 12 days, and disease incidence and disease severity were calculated as described above. Each treatment was replicated three times, with 10 fruits used per replicate, for a total of 30 fruits per treatment.

2.5.2. Monitoring the quality of snake fruit after inoculation with P. salaccae SKRU002 and application of T. asperelloides SKRU-01

Monitoring the quality of snake fruit post-inoculation with *P. salaccae* SKRU002 and application of *T. asperelloides* SKRU-01 involved the assessment of various parameters including pH, color, weight, total soluble solids (TSS), titratable acidity (TA), total phenolic content (TPC), and antioxidant activity (AA) over a 12-day storage period. Each treatment was replicated three times, with 10 fruits used per replicate, for a total of 30 fruits per treatment.

The snake fruit was sampled and weighed using an electronic balance. Weight loss was calculated using the following formula (Eq. (4)) (Shao et al., 2012):

$$\text{Weight loss (\%)} = [(\text{Fruit weight before storage} - \text{Fruit weight after storage}) / \text{Fruit weight before storage}] \times 100 \quad (4)$$

The color of the stored fruit was evaluated following treatment under different conditions. Using a colorimeter (HunterLab, ColorFlex EZ, USA), CIELAB color parameters (L^*, a^*, and b^*) were measured at three random points on the salacca pulp. In this context, L^* represented fruit brightness, a^* indicated the red/green spectrum (with negative values indicating green and positive values indicating redness), and b^* represented the yellow/blue value, with positive values corresponding to yellow and negative values to blue.

To analyze pH, TSS, TA, TPC, and AA, snake fruit samples were peeled, sliced into small pieces, and hand-pressed to extract juice into a beaker. Following this, the snake fruit juice was filtered and promptly analyzed for various parameters pertaining to fruit quality.

The pH of snake fruit juice was assessed using a pH meter (model FE20 - Mettler Toledo).

The TSS content was determined by applying a few drops of snake fruit juice onto a digital refractometer (Master Refractometer, Atago Co. Ltd., Japan) using a micropipette and recording the reading (Spinelli et al., 2024).

The TA of snake fruit juice was determined using the standard visual titration method (Ranganna, 1986). The measurement of TA (%) in the snake fruit juice was conducted using a modified method derived from Kalsi et al. (2023).

The TPC was determined by using the Folin–Ciocalteu method, with Gallic acid serving as the standard equivalent. The TPC content was assessed using a modified method adapted from Derakhshan et al. (2018). Briefly, 100 μL of 2.0 N Folin–Ciocalteu reagent (Sigma–Aldrich, St. Louis, USA) were mixed with 20 μL of diluted snake fruit juice sample. After 3 to 5 min, 80 μL of 7 % (*w/v*) sodium carbonate solution was added to the mixture, and the reaction proceeded for 30 min at room temperature in the dark. The absorbance was measured at 760 nm using a spectrophotometer (Parsaei et al., 2013).

To evaluate AA, the method proposed by Barreca et al. (2011) was adopted, employing 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH) with slight modifications. Initially, 1.18 mg of DPPH was dissolved in 25 mL of methanol to prepare the DPPH solution. Subsequently, 100 μL of DPPH solution was mixed with 100 μL of snake fruit juice and incubated for 30 min at room temperature in the dark. Additionally, a control was prepared using 100 μL of methanol solution instead of snake fruit juice. The absorbance was measured at 517 nm using a spectrophotometer, and the result was calculated using the following formula (Eq. (5)) (Barreca et al., 2011):

$$\text{DPPH free radical scavenging activity (\%)} = [(\text{Absorbance of control} - \text{Absorbance of the sample})/\text{Absorbance of control}] \times 100 \quad (5)$$

2.6. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 26 (IBM Corp., Armonk, NY, USA). Depending on the experimental design, data were analyzed using one- or two-way analysis of variance (ANOVA). ANOVA and Tukey's Honestly Significant Difference (HSD) test ($p < 0.05$) were used to assess normal distribution and to identify statistically significant differences between treated samples and the untreated control, respectively. For the two-way ANOVA conducted in the snake fruit tests, pathogen presence and *Trichoderma* application were included as independent factors, and their interaction effects were analyzed. Pearson's correlation coefficient (r) was calculated to evaluate the strength and significance of the relationship between culture filtrates concentration and inhibition percentage.

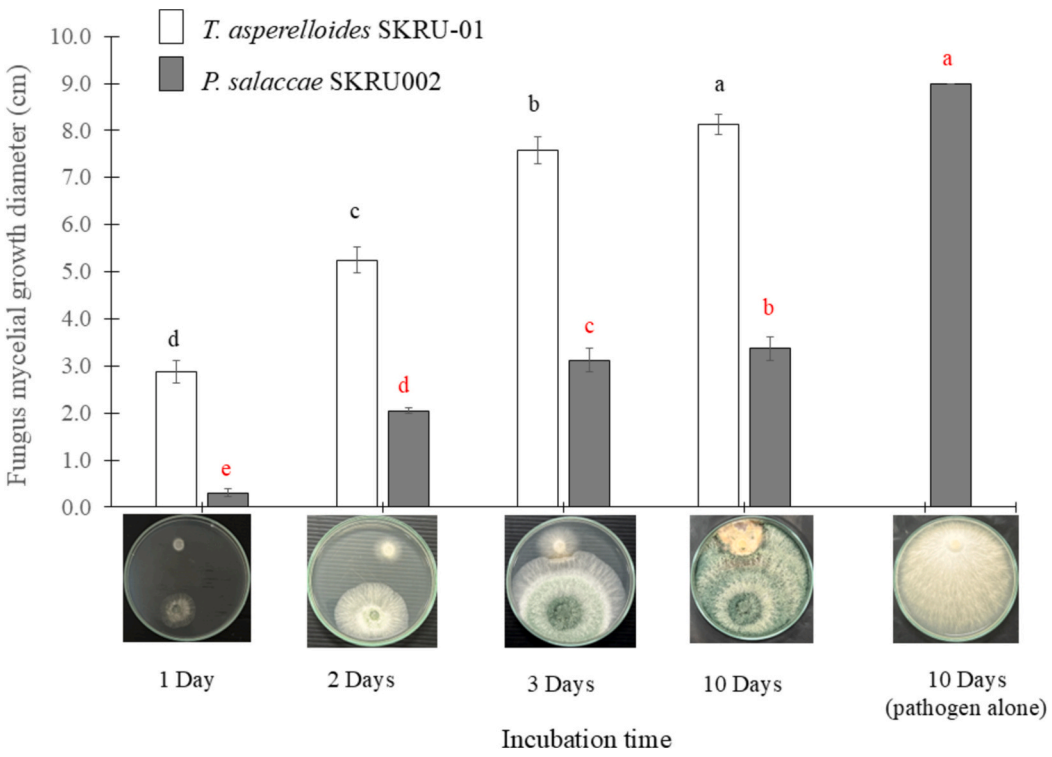

Fig. 1. The growth kinetics and mycoparasitic interactions of *T. asperelloides* SKRU-01 against *P. salaccae* SKRU002 on PDA were monitored over a 10-day incubation period at 30 °C. In the dual culture PDA plate, the upper section shows *P. salaccae* SKRU002, while the lower section indicates *T. asperelloides* SKRU-01. The data presented represent the mean of three replicates ± standard deviation (SD). Values with the same letter are not significantly different ($p > 0.05$) according to Tukey's multiple range test.

3. Results

3.1. In vitro detection of antagonistic activity of T. asperelloides SKRU-01 against P. salaccae SKRU002

To examine the competition and interaction between *T. asperelloides* SKRU-01 and *P. salaccae* SKRU002, both strains were cultured in the same Petri dishes for 10 days. Statistically significant differences ($p < 0.05$) in growth were observed between the antagonistic strain and the pathogen throughout the incubation period. The *in vitro* dual-culture experiments demonstrated that *T. asperelloides* SKRU-01 exhibited rapid growth compared to *P. salaccae* SKRU002, indicating a strong antagonistic effect (Fig. 1). From the third day onward, *P. salaccae* SKRU002 colonies began to collapse, revealing the inhibitory effects of *T. asperelloides* SKRU-01, which thickened at the junction and formed a prominent inhibition zone. After ten days, *P. salaccae* SKRU002 growth was limited to 3.38 cm, while the control reached 9.00 cm, resulting in an inhibition rate of 62.5 %.

The mycoparasitic interaction between *T. asperelloides* SKRU-01 and *P. salaccae* SKRU002 was observed using SEM (Fig. 2) and CLSM (Supplementary material: Fig. S1). SEM analysis of the fungal mycelium from the antagonism zone on the dual culture plate revealed that the mycelium of *T. asperelloides* SKRU-01 hooked, coiled, swirled, and penetrated the mycelium of *P. salaccae* SKRU002 (Fig. 2A, B, C). Pores in the mycelium of *P. salaccae* SKRU002 treated with *T. asperelloides* SKRU-01 were also detected (Fig. 2D). Additionally, profuse sporulation of *T. asperelloides* SKRU-01 on the mycelium of *P. salaccae* SKRU002 was observed (Fig. 2A, B, D). The effective mechanisms of *T. asperelloides* SKRU-01 on *P. salaccae* SKRU002 hyphae were further evident under CLSM (Fig. S1A, S1B). Moreover, cytoplasmic aggregation and vacuolization were observed in the mycelium of the fungal plant pathogen treated with *T. asperelloides* SKRU-01 (Fig. S1C), while no such effects were seen in the control (Fig. S1D). The hyphae of *P. salaccae* SKRU002 treated with *T. asperelloides* SKRU-01 (Fig. S1A, S1B, S1C) appeared larger compared to the untreated hyphae (control) (Fig. S1D).

The antifungal potential of *T. asperelloides* SKRU-01 culture filtrates at concentrations ranging from 5 % to 20 % (*v*/v) against *P. salaccae* SKRU002 in both PDA plates and PDB liquid medium is presented in Fig. 3. A significant positive correlation ($r = 0.96$, $p < 0.05$) was observed between the culture filtrates concentration and the inhibition percentage of *P. salaccae* SKRU002 in both media. Increasing concentrations of *T. asperelloides* SKRU-01 culture filtrates (5 % to 20 % (*v*/*v*)) resulted in progressively higher inhibition levels, with inhibition rates ranging from 54.29 % to 100 % in solid culture (PDA) and 56.47 % to 100 % in liquid culture (PDB). The minimum concentration required to achieve over 80 % inhibition was 15 % (*v*/*v*) for both media. At the highest concentration tested (20 % (*v*/*v*)), complete inhibition (100 %) was achieved.

3.2. Identification of bioactive compounds in T. asperelloides SKRU-01 culture filtrates by LC-QTOF/MS

The anti-*P. salaccae* SKRU002 activity in *T. asperelloides* SKRU-01 culture filtrates was analyzed using LC-QTOF/MS in electrospray ionization (ESI) positive ion mode. As summarized in Table 1, 31 bioactive compounds were identified based on retention time, molecular mass, and mass spectral fragmentation patterns. Notably, toyocamycin and antimycin A1, both recognized antibiotics (Supplementary material: Fig. S2), were identified among these compounds and have demonstrated antifungal activity (Chen et al., 2009; Yan et al., 2010; Battaglia et al., 2011; Shentu et al., 2012).

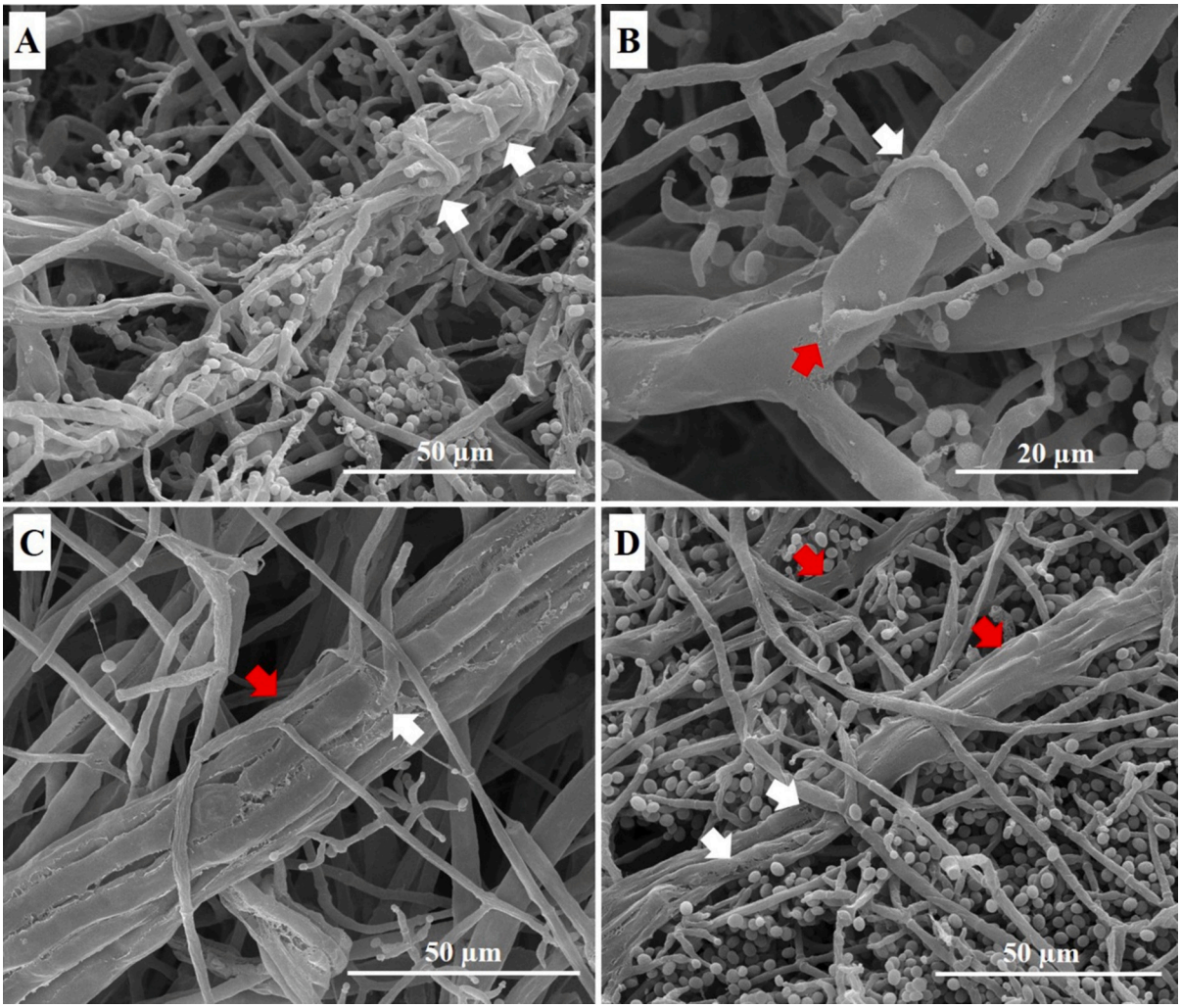

Fig. 2. SEM images showing mycoparasitism of *T. asperelloides* SKRU-01 on *P. salaccae* SKRU002. (A) Coiling and swirling structures; (B) hook structures (white arrow) and penetration points (red arrow); (C) attachment (red arrow) and penetration (white arrow); (D) hyphal pores (white arrow) and depressions on hyphae (red arrow).

3.3. In vivo biocontrol activity of T. asperelloides SKRU-01

3.3.1. Efficacy of T. asperelloides SKRU-01 culture filtrates against P. salaccae SKRU002 on snake fruit

The *in vivo* protective effect of *T. asperelloides* SKRU-01 culture filtrates against *P. salaccae* SKRU002 on snake fruit was assessed, with comparisons to Mancozeb® (Fig. 4). After 12 days, untreated control fruits inoculated with *P. salaccae* SKRU002 displayed high DI and DS, with values exceeding 100 % and 83.33 %, respectively (Fig. 4A). Treatment with *T. asperelloides* SKRU-01 culture filtrates significantly reduced DI and DS, achieving reductions comparable to those observed with Mancozeb® treatment (both DI and DS = 0 %; $p > 0.05$). Disease symptoms are illustrated in Fig. 4B, showing both external (Fig. 4B (a-d)) and cross-sectional (Fig. 4B (e-h)) views. In untreated control fruits, the surface was heavily colonized by yellowish-white fungal hyphae (Fig. 4B (b)), and cross-sections revealed black-brown rot affecting both the skin and pulp (Fig. 4B (f)). In contrast, fruits treated with either *T. asperelloides* SKRU-01 culture filtrates (Fig. 4B (g)) or Mancozeb® (Fig. 4B (h)) maintained normal tissue appearance similar to positive controls (Fig. 4B (e)).

3.3.2. Efficacy of spore concentrations of T. asperelloides SKRU-01 against P. salaccae SKRU002 on snake fruit

The efficacy of spore concentrations ranging from 10^4 to 10^8 spores/mL of *T. asperelloides* SKRU-01 against *P. salaccae* SKRU002 was investigated *in vivo* on snake fruit. The results, shown in Fig. 5, indicate that following treatment with various spore concentrations, a significant reduction in DI and DS caused by *P. salaccae* SKRU002 was observed ($p < 0.05$). Control fruits displayed DI and DS values of 100 % and 86.11 %, respectively—substantially higher than those in the spore treatments, which ranged from DI = 50.00 % to 0 % and DS = 33.33 % to 0 %. The application of 10^7 and 10^8 spores/mL resulted in a complete reduction of DI and DS to 0 % after 12 days. Based on these findings, 10^7 spores/mL was selected for further experiments.

3.4. Efficacy of T. asperelloides SKRU-01 in halting the development of P. salaccae SKRU002 on snake fruit

T. asperelloides SKRU-01, at a concentration of 10^7 spores/mL, was evaluated for its potential to inhibit the development of *P. salaccae* SKRU002 *in vivo* using snake fruit over a 12-day period. The mycelial growth of *P. salaccae* SKRU002 was assessed under different treatment conditions prior to the application of *T. asperelloides* SKRU-01 (Fig. 6A). When *P. salaccae* SKRU002 was allowed to establish on the fruit for 1, 2, and 3 days before applying *T. asperelloides* SKRU-01, mycelial growth reached 1.02 cm, 2.23 cm, and 3.42 cm, respectively. A two-way ANOVA revealed that both pathogen growth time and *T. asperelloides* SKRU-01 application significantly affected DI and DS (both $p < 0.0001$),

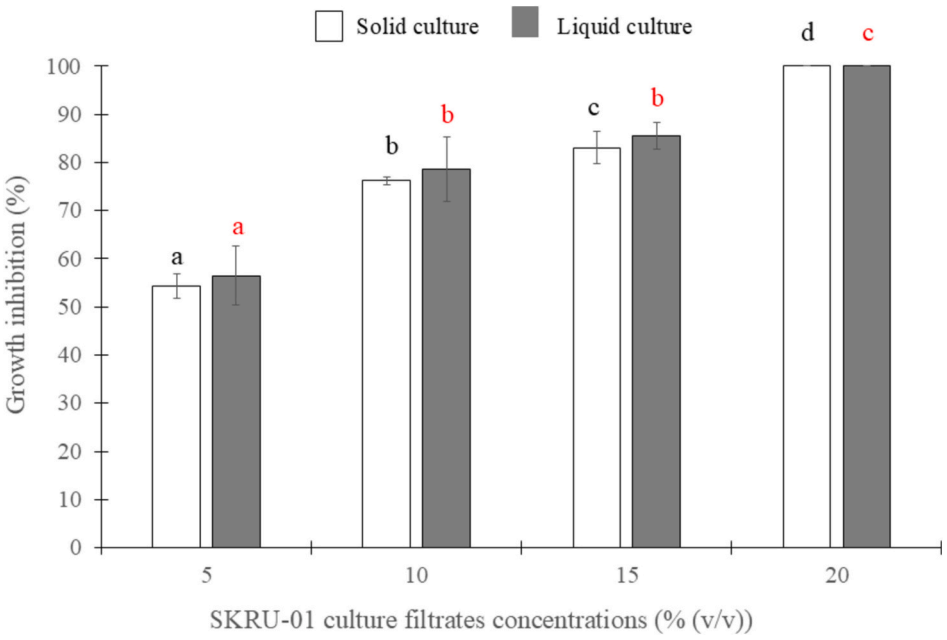

Fig. 3. *In vitro* antifungal activity of *T. asperelloides* SKRU-01 culture filtrates (SKRU-01 culture filtrates) at varying concentrations (5 % to 20 % *v/v*) against *P. salaccae* SKRU002 in PDA plates and PDB liquid medium. Data represent the mean of three replicates ± standard deviation (SD). Values with the same letter are not significantly different ($p > 0.05$) according to Tukey's multiple range test.

Table 1
Compounds emitted by *T. asperelloides* SKRU-01 produced in PDB medium detected by LC-QTOF/MS analysis.

No.	Predicted compounds	Retention Time (min)	Proportion (%)	Formula	*m/z*	Mass Error (mDa)	Matching Score (%)
1	O-Succinyl-L-homoserine	1.92	1.85	$C_8H_{13}NO_6$	220.0826	−4.5	94.86
2	Methyl nicotinate	1.94	2.31	$C_7H_7NO_2$	138.0553	−3.39	97.17
3	Epidermin	2.39	2.48	$C_{11}H_{19}NO_6$	262.129	−1.68	98.89
4	Toyocamycin	2.41	25.72	$C_{12}H_{13}N_5O_4$	314.0861	−0.44	95.22
5	Methyl 2,3-dihydro-3,5-dihydroxy-2-oxo-3-indoleacetic acid	2.54	1.49	$C_{11}H_{11}NO_5$	238.0719	−3.69	95.66
6	Piscidic Acid	2.62	0.56	$C_{11}H_{12}O_7$	274.0928	−2.02	93.95
7	N-Methylanthranilic Acid	2.69	3.62	$C_8H_9NO_2$	152.071	−2.65	97.55
8	1,1′-(Tetrahydro-6a-hydroxy-2,3a,5-trimethylfuro[2,3-d]-1,3-dioxole-2,5-diyl)bis-ethanone	3.29	1.81	$C_{12}H_{18}O_6$	276.1455	−4.55	92.82
9	3-Deazaneplanocin A	8.76	1.01	$C_{12}H_{14}N_4O_3$	263.1143	−2.84	90.85
10	Miraxanthin-III	9.46	0.32	$C_{17}H_{18}N_2O_5$	331.1289	−0.35	98.81
11	Pilocarpic acid	10.31	0.81	$C_{11}H_{18}N_2O_3$	227.1397	−2.88	95.46
12	3-O-(alpha-L-rhamnopyranosyl-(1–2)-alpha-L-rhamnopyranosyl)-3-hydroxydecanoic acid	10.91	0.32	$C_{22}H_{40}O_{11}$	481.2627	3.67	95.72
13	Ceanothine E	11.66	0.55	$C_{34}H_{40}N_4O_4$	569.3147	−4.29	98.42
14	7(14)-Bisabolene-2,3,10,11-tetrol	12.73	0.51	$C_{15}H_{28}O_4$	295.1887	−2.4	99.55
15	2,3-Dihydroabscisic alcohol	16.82	10.67	$C_{15}H_{24}O_3$	275.1625	−1.54	90.24
16	Ipomeatetrahydrofuran	18.03	1.07	$C_{15}H_{28}O_3$	279.1934	−1.22	97.25
17	Decyl isobutyrate	18.68	0.31	$C_{14}H_{28}O_2$	246.2426	0.39	95.78
18	Antimycin A1	20.63	17.90	$C_{28}H_{40}N_2O_9$	566.3066	−1.03	90.48
19	C16 Sphinganine	21.83	0.16	$C_{16}H_{35}NO_2$	274.2747	−2.46	91.33
20	Phytosphingosine	22.07	0.43	$C_{18}H_{39}NO_3$	318.3007	−1.42	98.19
21	Xestoaminol C	22.15	0.32	$C_{14}H_{31}NO$	230.2479	−0.25	99.13
22	10,20-Dihydroxyeicosanoic acid	22.44	0.27	$C_{20}H_{40}O_4$	362.327	−1.19	99.78
23	C17 Sphinganine	23.05	0.20	$C_{17}H_{37}NO_2$	288.2902	−1.7	98.59
24	Nummularine B	25.16	3.65	$C_{32}H_{41}N_5O_6$	614.2933	2.65	99.02
25	Asclepin	26.24	0.66	$C_{31}H_{42}O_{10}$	592.3117	−0.03	98.42
26	Mauritine A	29.68	3.77	$C_{32}H_{41}N_5O_5$	598.2976	3.87	94.17
27	Glycinoeclepin B	29.74	3.35	$C_{31}H_{42}O_9$	576.316	1.2	90.92
28	Cyclandelate	31.46	2.73	$C_{17}H_{24}O_3$	299.1618	−0.51	98.71
29	N-palmitoyl alanine	35.06	2.75	$C_{19}H_{37}NO_3$	350.2668	−0.68	99.57
30	N-stearoyl valine	41.30	1.67	$C_{23}H_{45}NO_3$	406.3288	0.94	99.42
31	6β-Hydroxy-3-oxo-5β-cholan-24-oic Acid	43.81	0.69	$C_{24}H_{38}O_4$	413.2658	0.94	99.34

with no significant interaction between these factors ($p = 0.431$), indicating that the effectiveness of *T. asperelloides* SKRU-01 remained consistent regardless of pathogen growth stage (Fig. 6B).

In the absence of a biocontrol agent, DI and DS (Fig. 6B) reached 100 % and 97.92 %, respectively. However, when *T. asperelloides* SKRU-01 was co-inoculated with the pathogen at different time points (simultaneously, 1, 2, or 3 days post-pathogen inoculation), it exhibited rapid growth and effectively antagonized the pathogen, reducing both DI and

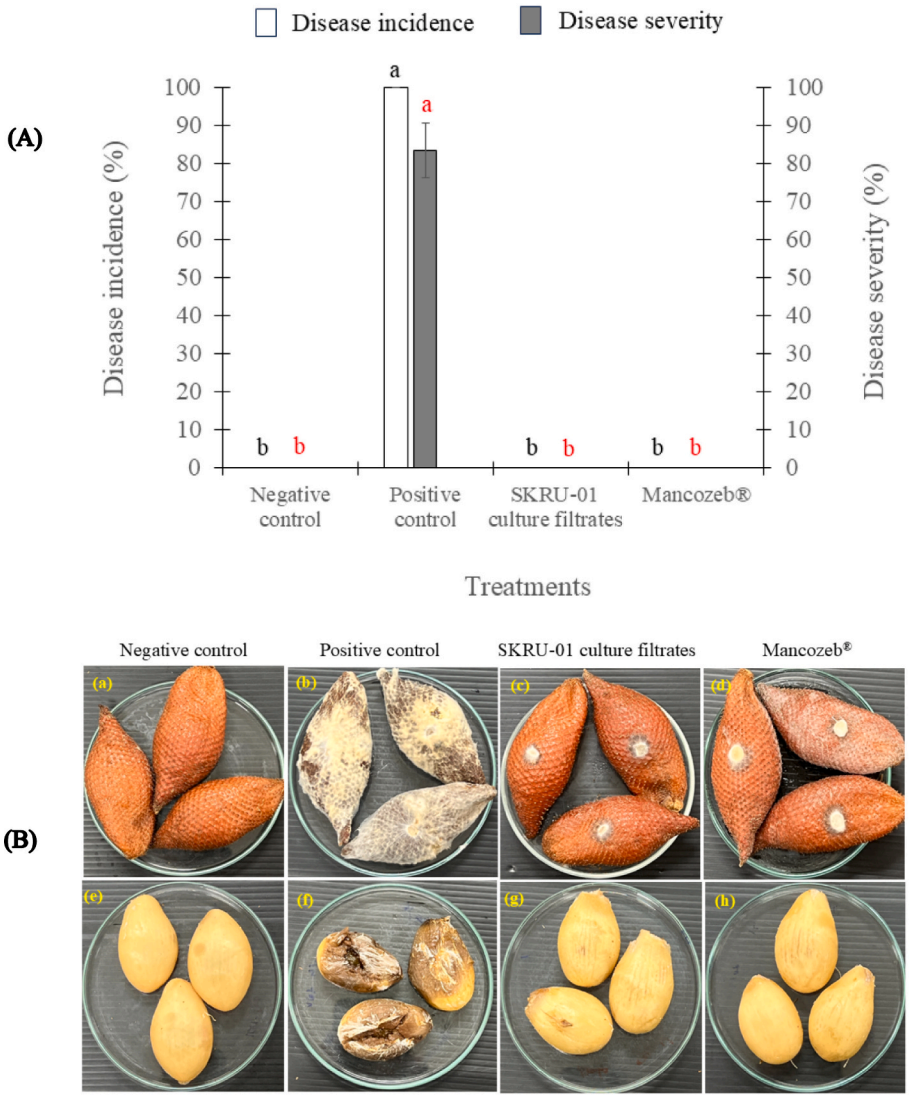

Fig. 4. Protective effects of *T. asperelloides* SKRU-01 culture filtrates and Mancozeb® on *P. salaccae* SKRU002 infection in snake fruit. Panel (A) illustrates the disease incidence and severity observed after 12 days of storage at 30 °C under high humidity. Panel (B) depicts symptoms on whole snake fruits (a-d) and cross-sectioned fruits (e-h). Data represent the mean of three replicates ± standard deviation (SD) ($n = 30$). Values with the same letter are not significantly different ($p > 0.05$) according to Tukey's multiple range test.
Note: The negative control received no treatment, and the pathogen was not inoculated, while the positive control involved inoculating the pathogen alone.

DS to 0 % (Fig. 6A). Fig. 6C presents external (Fig. 6C (a-f)) and cross-sectional (Fig. 6C (g-l)) symptoms of the snake fruit, comparing the negative control (inoculated with the pathogen) and samples treated with *T. asperelloides* SKRU-01. In the negative control (Fig. 6C (b)), the fruit surface was covered with yellowish-white fungal hyphae, and cross-sections revealed black-brown rotting flesh (Fig. 6C (h)). In contrast, snake fruit treated with *T. asperelloides* SKRU-01, whether applied simultaneously (Fig. 6C (i)) or 1, 2, or 3 days post-pathogen inoculation (Fig. 6C (j, k, and l)), exhibited normal tissue characteristics, similar to those of the positive control (Fig. 6C (g)). These results demonstrate that *T. asperelloides* SKRU-01 can rapidly colonize and inhibit the development of *P. salaccae* SKRU002, effectively preventing the rotting of snake fruit.

3.5. Monitoring the quality of snake fruit after inoculation with *P. salaccae* SKRU002 and application of *T. asperelloides* SKRU-01

The quality of snake fruit was monitored following inoculation with *P. salaccae* SKRU002 and the application of *T. asperelloides* SKRU-01. *T. asperelloides* SKRU-01 demonstrated notable efficacy in mitigating the effects of *P. salaccae* SKRU002 on snake fruit. Employing biological control, it was utilized to assess fruit quality preservation over a 12-day storage period under humid conditions (85–90 %), as detailed in Table 2. The findings unveiled significant improvements ($p < 0.05$) in various quality parameters such as color, weight, TSS, TA, TPC, and AA in snake fruit post-inoculation with *P. salaccae* SKRU002 and application of *T. asperelloides* SKRU-01.

In the positive control where *P. salaccae* SKRU002 was inoculated on snake fruit alone, alterations in color parameters (L^*, a^*, and b^*) of the snake fruit pulp were observed. However, co-inoculation of *T. asperelloides* SKRU-01 into the snake fruit simultaneously or at 1, 2, and 3 days after pathogen development did not significantly affect ($p > 0.05$) the snake fruit pulp color parameters compared to the negative control where no treatment was applied. Additionally, only the inoculation of *P. salaccae* SKRU002 on snake fruit alone resulted in a significant ($p < 0.05$) weight loss of 12.12 % (Table 2).

In the negative control (no treatment), TA and TSS of snake fruit juice were 11.73°Brix and 0.64 % citric acid, respectively. Inoculation

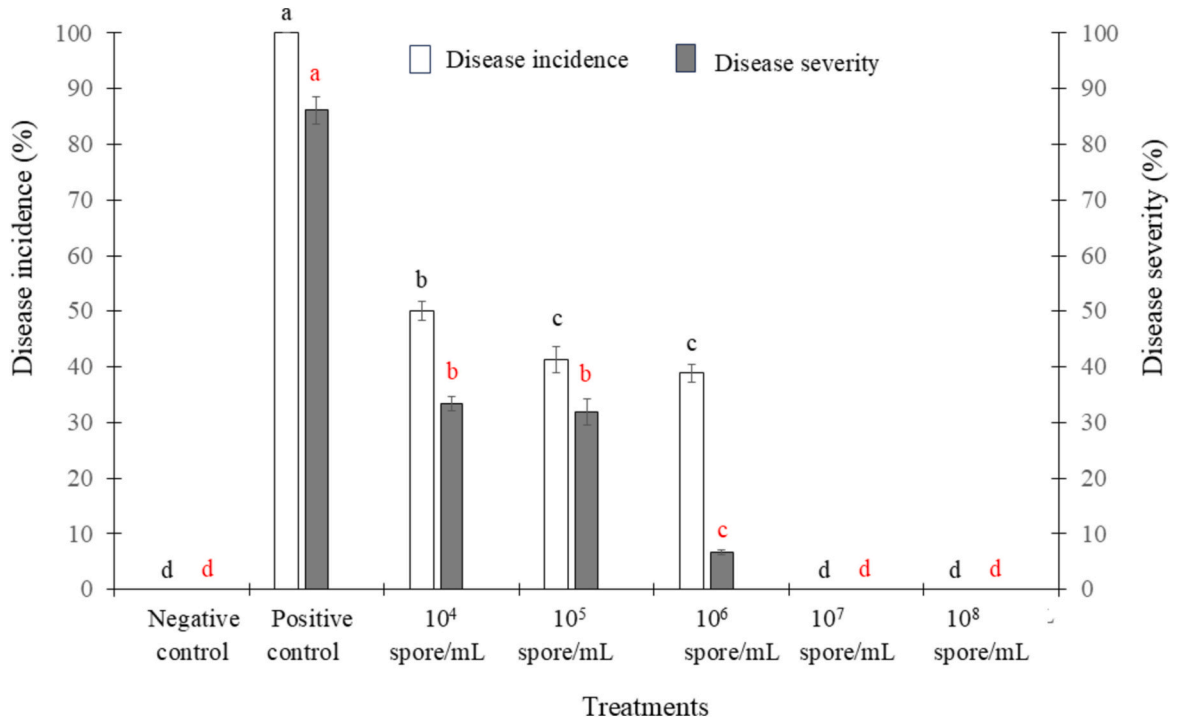

Fig. 5. Effect of *T. asperelloides* SKRU-01 spore concentrations (10^4 to 10^8 spores/mL) on disease incidence and severity of *P. salaccae* SKRU002 in snake fruit. Disease incidence and severity were assessed after a 12-day storage period at 30 °C under humid conditions. Data represent the mean of three replicates ± standard deviation (SD) (n = 30). Values with the same letter are not significantly different ($p > 0.05$) according to Tukey's multiple range test.
Note: The negative control received no treatment, and the pathogen was not inoculated, while the positive control involved inoculating the pathogen alone.

with *P. salaccae* SKRU002 reduced TA to 9.13°Brix and TSS to 0.30 % citric acid. However, co-inoculation with *T. asperelloides* SKRU-01 at various timings (simultaneously or 1 to 3 days post-inoculation) did not significantly affect ($p > 0.05$) TA and TSS, which ranged from 10.00 to 12.47°Brix and 0.34 % to 0.39 % citric acid, respectively (Table 2). The TPC of snake fruit juice after treatment with *T. asperelloides* SKRU-01 was significantly higher ($p < 0.05$) than the positive control, measuring 256.81 μg GAE/mL (simultaneously) to 87.11 μg GAE/mL (3 days post-inoculation), compared to 35.44 μg GAE/mL for the positive control and 223.59 μg GAE/mL for the negative control. Similarly, the AA of snake fruit juice following *T. asperelloides* SKRU-01 treatment ranged from 90.60 % to 94.68 % RSV, significantly higher than the 46.89 % RSV in the positive control, with the negative control at 95.81 % RSV. The AA from simultaneous application of *T. asperelloides* SKRU-01 was not significantly different ($p > 0.05$) from the negative control (Table 2).

4. Discussion

This study evaluated the efficacy of *T. asperelloides* SKRU-01 as a biocontrol agent for managing fruit rot in snake fruit caused by *P. salaccae* SKRU002. The findings demonstrate that *T. asperelloides* SKRU-01 effectively inhibits the growth of *P. salaccae* SKRU002 and helps maintain the quality of snake fruit during storage. The observed antagonistic activity can be attributed to the application of spore suspension and the production of bioactive compounds by *T. asperelloides* SKRU-01, both of which are known to suppress the growth of pathogenic fungi (Boukaew et al., 2023a, 2024b, 2024d).

In *in vitro* dual-culture experiments, *T. asperelloides* SKRU-01 effectively suppressed the mycelial growth of *P. salaccae* SKRU002, suggesting that it impedes colony expansion through the production of diffusible bioactive compounds and competitive nutrient acquisition. The observed antibiosis indicates the release of extracellular bioactive agents, while the rapid growth rate of *T. asperelloides* SKRU-01 provides a competitive advantage for space and nutrients (Benítez et al., 2004; Amira et al., 2017). After 10 days of co-incubation, *T. asperelloides* SKRU-01 outcompeted *P. salaccae* SKRU002, suggesting mycoparasitism as a potential mechanism of its antagonistic behavior. This finding is consistent with behaviors observed in other *Trichoderma* species, which utilize multiple mechanisms to inhibit fungal pathogens (Amira et al., 2017; Zhang et al., 2018; Ren et al., 2022; Madbouly et al., 2023).

This study investigates the effects of *T. asperelloides* SKRU-01 on the fungal pathogen *P. salaccae* SKRU002, showing morphological changes in the treated pathogens, including plasma membrane retraction, septae formation, and cell wall degradation leading to pore formation, consistent with previous research (Amira et al., 2017). Using CLSM and SEM, we demonstrated that *T. asperelloides* SKRU-01 effectively penetrates *P. salaccae* SKRU002, resulting in partial lysis and loss of cytoplasmic materials. The fungal cell walls, primarily composed of chitin, glucans, and proteins (Mukherjee et al., 2022), are susceptible to degradation by the hydrolytic enzymes produced by *Trichoderma* sp., such as chitinase, chitosanase, glucanase, and protease (Gruber and Seidl-Seiboth, 2012; Qualhato et al., 2013). This ability to produce hydrolytic enzymes is a key competitive mechanism for inhibiting fungal plant pathogens (Sánchez-Montesinos et al., 2021). The physical disruption of fungal structures by *T. asperelloides* SKRU-01, as evidenced by SEM images, indicates its capacity to leak cellular contents and inhibit the growth of *P. salaccae* SKRU002. Furthermore, the inhibitory effect of *T. asperelloides* SKRU-01 extends beyond direct contact; culture filtrates inhibit the mycelial growth of *P. salaccae* SKRU002, as demonstrated in both PDA plates and liquid culture. At a concentration of 20 % (*v/v*), the culture filtrates completely inhibited the growth of *P. salaccae* SKRU002. These findings suggest that the culture filtrates of *T. asperelloides* SKRU-01 contain bioactive compounds.

Species of *Trichoderma* are known for producing a variety of antibiotics, including trichogin, trichorzins, harzianins, trichotoxin, trichokindins (Tyskiewicz et al., 2022), gliovirin, gliotoxin, viridin, pyrones, and peptaibols (Vey et al., 2001), as well as trichocellins, tanshinone II

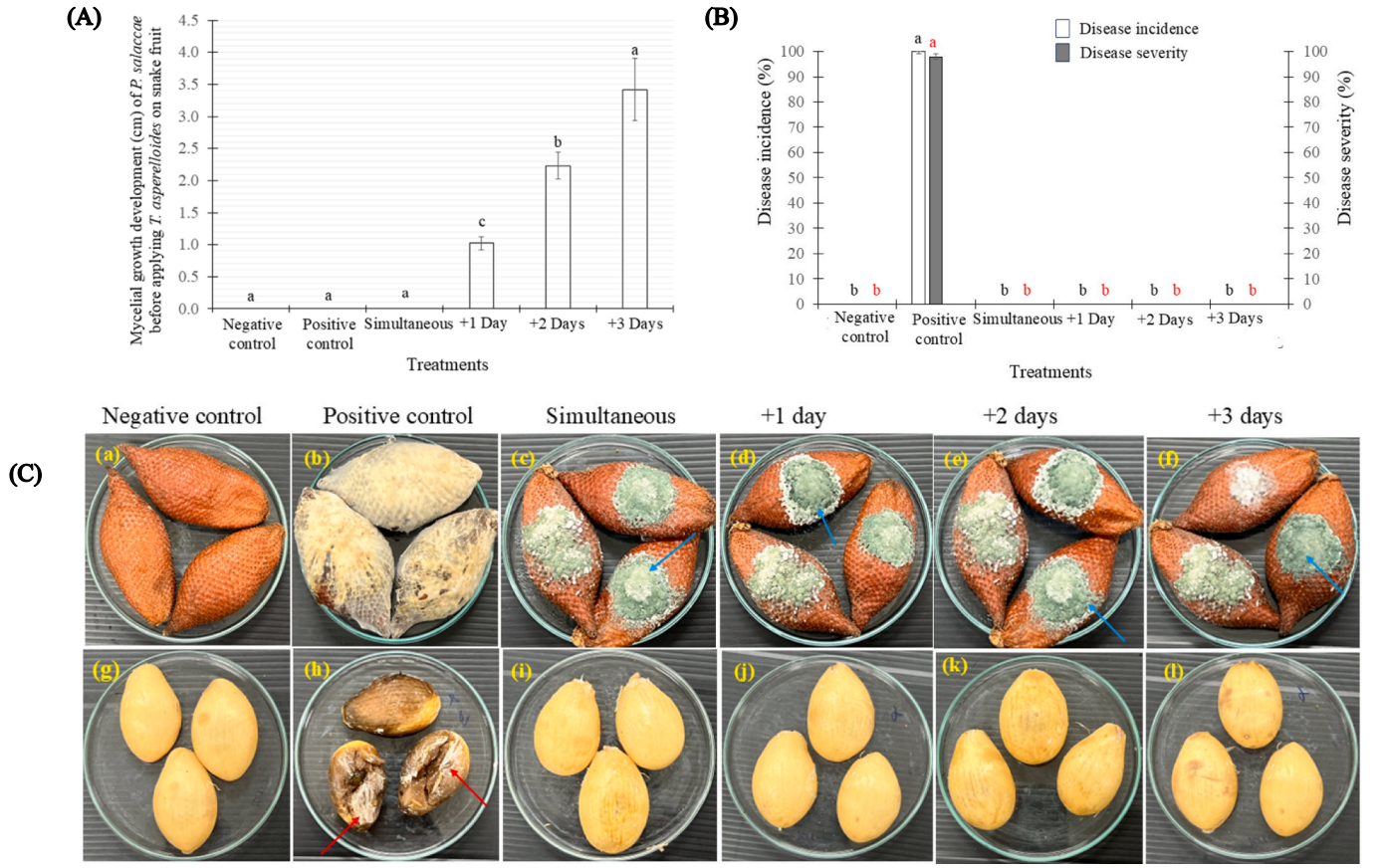

Fig. 6. Effect of *T. asperelloides* SKRU-01 application timing on *P. salaccae* SKRU002 development in snake fruit. Fruits were treated with 50 μL of *T. asperelloides* SKRU-01 (10^7 spores/mL) either simultaneously with or 1, 2, or 3 days after *P. salaccae* SKRU002 inoculation. After treatment, fruits were stored at 30 °C under high humidity for 12 days. (A) Mycelial growth of *P. salaccae* SKRU002 on snake fruit prior to *T. asperelloides* SKRU-01 application. (B) Disease incidence and severity after storage. (C) Symptoms on (a-f) whole and (g-l) cross-sectioned fruits. Blue arrows indicate areas where *T. asperelloides* SKRU-01 combats *P. salaccae* SKRU002 on the fruit surface, while red arrows highlight fungal growth within the fruit pulp. Data represent the mean of three replicates ± standard deviation (SD) (n = 30). Values with the same letter are not significantly different ($p > 0.05$) according to Tukey's multiple range test.
Note: The negative control received no treatment or pathogen inoculation. The positive control involved inoculating the pathogen alone. *T. asperelloides* SKRU-01 was applied 1, 2, or 3 days (+1, +2, +3, respectively) after *P. salaccae* SKRU002 or simultaneously.

Table 2
Displays the monitoring results for the quality of snake fruit after inoculation with the pathogen *P. salaccae* SKRU002 and application *T. asperelloides* SKRU-01 for fruit quality preservation after twelve days of incubation at 30 °C under humid conditions.

Parameters	Treatments					
	Negative control	Positive control	Simultaneous	+1 Day	+2 Day	+3 Day
pH	3.85	3.86	3.69	3.79	3.84	3.81
*L**	67.24^{a}	30.46^{b}	68.27^{a}	67.70^{a}	68.24^{a}	67.04^{a}
*a**	9.71^{c}	13.58^{a}	8.64^{cd}	11.01^{b}	9.80^{c}	8.91^{c}
*b**	34.53^{a}	22.65^{b}	33.18^{a}	33.92^{a}	31.59^{a}	33.24^{a}
Weight loss (%)	5.90^{b}	12.12^{a}	5.46^{b}	5.39^{b}	5.78^{b}	5.57^{b}
Total soluble solids (°Brix)	11.73^{a} ± 0.12	9.13^{c} ± 0.12	10.00^{b} ± 0.17	12.47^{a} ± 0.12	12.13^{a} ± 0.12	11.93^{ab} ± 0.12
Titratable acidity (% citric acid)	0.64^{a} ± 0.02	0.30^{d} ± 0.01	0.37^{bc} ± 0.00	0.39^{b} ± 0.01	0.34^{c} ± 0.01	0.35^{bc} ± 0.01
Total phenol content (μg GAE/mL)	223.59^{b} ± 9.34	35.44^{d} ± 8.83	256.81^{a} ± 5.93	91.98^{c} ± 5.00	86.22^{c} ± 3.01	87.11^{c} ± 3.97
% Radical scavenging activity	95.81^{a} ± 0.52	46.89^{c} ± 0.00	94.68^{a} ± 0.20	91.96^{b} ± 0.34	90.60^{b} ± 1.19	91.39^{b} ± 0.20

Note: The presented data represent the mean of three replicates ± standard deviation (SD) (n = 30). *L** indicated fruit brightness, *a** denoted the red/green spectrum (negative values indicating green, while positive values indicated redness), and *b** represented the yellow/blue value, where yellow corresponded to positive values and blue to negative values. Values with the same row do not differ significantly ($p > 0.05$) according to Tukey's multiple range test. The negative control received no treatment, and the pathogen was not inoculated. The positive control involved inoculating the pathogen alone. *T. asperelloides* SKRU-01 was applied 1, 2, or 3 days (+1, +2, +3, respectively) after *P. salaccae* SKRU002 or simultaneously.

A, and tanshinone I (Ming et al., 2012). These compounds have demonstrated antagonistic effects against several fungal pathogens (Ghisalberti and Sivasithamparam, 1991). In this study, we utilized LC-QTOF/MS analysis to identify 31 secondary metabolites in the culture filtrates of *T. asperelloides* SKRU-01, including toyocamycin and antimycin A1, both of which have been reported to suppress

phytopathogenic fungi (Chen et al., 2009; Yan et al., 2010; Battaglia et al., 2011; Shentu et al., 2012). Our findings indicate that *T. asperelloides* SKRU-01 possesses the ability to produce antibiotics that inhibit *P. salaccae* SKRU002 growth. Toyocamycin, a compound related to sangivamycin, exhibited antagonistic activity against *Rhizoctonia solani* and *C. gloeosporioides*, as well as inhibitory effects on the mycelial growth and conidial germination of *Magnaporthe oryzae* (Chen et al., 2009; Shentu et al., 2012). The diversity of secondary metabolites produced by *T. asperelloides* SKRU-01 suggests that they may have either individual or synergistic effects against *P. salaccae* SKRU002. Further research is needed to explore the efficacy and mechanisms of action of these metabolites.

In addition to evaluating the potential of *T. asperelloides* SKRU-01 to inhibit *P. salaccae* SKRU002 *in vitro*, its efficacy was also tested on salak fruits. The results demonstrated that snake fruits treated with *T. asperelloides* SKRU-01 culture filtrates and subsequently inoculated with *P. salaccae* SKRU002 mycelial plugs exhibited complete control, with both DI and DS recorded at 0 % after 12 days of storage. This level of control was comparable to the conventional fungicide Mancozeb®, which also maintained a DI and DS of 0 %. These findings indicate the efficacy of *T. asperelloides* SKRU-01 culture filtrates in inhibiting the development of *P. salaccae* SKRU002. In assessing the efficacy of varying spore concentrations of *T. asperelloides* SKRU-01, concentrations exceeding 10^7 spores/mL resulted in complete reductions in DI and DS. This effect was more pronounced than that observed with lower concentrations (10^4 to 10^6 spores/mL). The rapid colonization at higher spore concentrations suggests a competitive interaction for nutrients and space with the pathogen. However, even inoculation with 10^4 spores/mL exhibited protective effects against the fungal pathogen, indicating a complex relationship between spore concentration and antifungal activity.

Additionally, *T. asperelloides* SKRU-01 was co-inoculated into snake fruit at various time points—simultaneously, and 1, 2, and 3 days after pathogen inoculation. The results showed effective suppression of the pathogen, with both DI and DS reduced to 0 %, even when the pathogen was present. This suggests that *T. asperelloides* SKRU-01 can effectively counteract pathogen growth, potentially utilizing the pathogen as a nutrient source. The SEM results from dual culture plates likely revealed structural alterations or interactions between *T. asperelloides* SKRU-01 and the pathogen, enhancing nutrient competition and impeding infection initiation by *P. salaccae* SKRU002. These findings align with a substantial body of research demonstrating the efficacy of various *Trichoderma* species in managing plant pathogens across diverse crops, including *F. solani* in peanuts (Erazo et al., 2021), *F. oxysporum* in stevia (Díaz-Gutiérrez et al., 2021), and *Botrytis cinerea* in tomatoes (Geng et al., 2022). The versatility of *Trichoderma* as a biocontrol agent is further supported by studies showing its effectiveness against a range of pathogens. For example, *T. pinnatum* extracts have significantly reduced the severity of mango stem-end rot disease (Zhan et al., 2023).

The assessment of fruit and vegetable quality is vital for understanding their storage implications and economic value. Key quality parameters, such as weight loss rate, TSS, TA, TPC, and AA, serve as important indicators of fruit quality (Godana et al., 2020). In this study, *T. asperelloides* SKRU-01 demonstrated significant efficacy in controlling snake fruit rot caused by *P. salaccae* SKRU002. Additionally, the application of *T. asperelloides* SKRU-01 positively impacted fruit quality and delayed senescence. Notably, our results indicated that co-inoculation with *T. asperelloides* SKRU-01 resulted in reduced alterations in color parameters compared to the positive control, thereby helping to preserve levels of TA, TSS, TPC, and AA. While the effects of *T. asperelloides* SKRU-01 on plant growth have been extensively studied, its role in enhancing fruit storage quality remains underexplored. Further research is needed to clarify the specific mechanisms through which *T. asperelloides* SKRU-01 maintains fruit quality. These findings are consistent with previous research. For instance, Li et al. (2021) reported improved postharvest quality in strawberries treated with *Streptomyces* sp. H4 extracts, supporting the efficacy of biological control across various crops and pathogens. Similarly, Bu et al. (2021) demonstrated that *B. subtilis* L1–21 effectively managed gray mold in postharvest tomatoes caused by *B. cinerea*, highlighting the role of microbial agents in preserving fruit quality while controlling pathogens. Collectively, these studies highlight the effectiveness of biological control methods in managing postharvest diseases in various crops. Investigating microbial agents as sustainable alternatives to chemical fungicides is important for advancing agricultural practices.

5. Conclusion

Our *in vitro* dual-culture experiments demonstrated that *T. asperelloides* SKRU-01 effectively inhibits the growth of *P. salaccae* SKRU002 in PDA medium, achieving a 62.5 % inhibition by day 10. The CLSM and SEM analyses confirmed this inhibitory action, revealing that *T. asperelloides* SKRU-01 penetrates the hyphae of *P. salaccae* SKRU002 and induces partial lysis. Furthermore, culture filtrates of *T. asperelloides* SKRU-01 exhibited significant inhibitory effects, completely suppressing *P. salaccae* SKRU002 in both solid and liquid media, as supported by LC-QTOF/MS analysis. In trials conducted with snake fruit, the application of *T. asperelloides* SKRU-01 culture filtrates provided 100 % protection against DI and DS, comparable to the conventional fungicide Mancozeb®. Additionally, spores of *T. asperelloides* SKRU-01 effectively controlled pathogen growth, reducing both DI and DS to 0 %, even when the pathogen was present. Importantly, co-inoculation with *T. asperelloides* SKRU-01 did not adversely affect fruit quality, indicating its potential as a sustainable and effective solution for managing rot disease caused by *P. salaccae* SKRU002 in snake fruit.

CRediT authorship contribution statement

Sawai Boukaew: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Julalak Chuprom:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jirayu Buatong:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Sujirat Sornprasit:** Investigation. **Sureeporn Wijitsopa:** Investigation. **Karistsapol Nooprom:** Writing – review & editing. **Rachasak Boonhok:** Writing – review & editing.

Funding

This research was financially supported by the Faculty of Agricultural Technology, Songkhla Rajabhat University, the Thailand Research Fund (TRF) under the Research Team Promotion Grant (No. RTA 6080010), and the National Science, Research, and Innovation Fund (NSRF) of Prince of Songkla University (No. AGR6701189S).

Declaration of competing interest

The authors declare no competing interests regarding the publication of this study.

Acknowledgement

The authors would like to acknowledge the Medical Science Research and Innovation Institute, Research and Development Office, Prince of Songkla University, for providing the confocal laser scanning microscope. Special thanks to Assoc. Prof. Dr. Watcharapong Mitsuwan for providing the equipment used in the experimental study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2024.111037.

Data availability

The authors do not have permission to share data.

References

Alara, O.R., Abdurahman, N.H., Ukaegbu, C.I., Hassan, Z., Kabbashi, N.A., 2018. Dataset on LC-Q-TOF/MS tentative identification of phytochemicals in the extract of *Vernonia amygdalina* leaf through positive ionization. Data Br. 21, 1686–1689.

Amira, M.B., Lopez, D., Mohamed, A.T., Khouaja, A., Chaar, H., Fumanal, B., Gousset-Dupont, A., Bonhomme, L., Label, P., Goupil, P., Ribeiro, S., Pujade-Renaud, V., Julien, J.-L., Auguin, D., Venisse, J.-S., 2017. Beneficial effect of *Trichoderma harzianum* strain Ths97 in biocontrolling *Fusarium solani* causal agent of root rot disease in olive trees. Biol. Control 110, 70–78.

Anoraga, S.B., Bintoro, N., 2020. The effect of packaging and storage method on Snake fruit (*Salacca edulis* Rainw.) quality. In: Vol. 475. IOP Conference Series: Earth and Environmental Science. IOP Publishing. Article 012022. No. 1.

Aralas, S., Mohamed, M., Abu Bakar, M.F., 2009. Antioxidant properties of selected salak (*Salacca zalacca*) varieties in Sabah, Malaysia. Nutr. Food Sci. 39, 243–250.

Awad, N.E., Kassem, H.A., Hamed, M.A., El-Feky, A.M., Elnaggar, M.A.A., Mahmoud, K., Ali, M.A., 2018. Isolation and characterization of the bioactive metabolites from the soil derived fungus *Trichoderma viride*. Mycology 9, 70–80.

Ayaz, M., Li, C.-H., Ali, Q., Zhao, W., Chi, Y.-K., Shafiq, M., Ali, F., Yu, X.-Y., Yu, Q., Zhao, J.-T., Yu, J.-W., Qi, R.-D., Huang, W.-K., 2023. Bacterial and fungal biocontrol agents for plant disease protection: journey from lab to field, current status, challenges, and global perspectives. Molecules 28, 6735.

Bae, S.-J., Mohanta, T.K., Chung, J.Y., Ryu, M., Park, G., Shim, S., Hong, S.-B., Seo, H., Bae, D.-W., Bae, I., Kim, J.-J., 2016. *Trichoderma* metabolites as biological control agents against *Phytophthora* pathogens. Biol. Control 92, 128–138.

Barreca, D., Laganà, G., Ficarra, S., Tellone, E., Leuzzi, U., Galtieri, A., Bellocco, E., 2011. Evaluation of the antioxidant and cytoprotective properties of the exotic fruit *Annona cherimola* mill. (Annonaceae). Food Res. Int. 44, 2302–2310.

Battaglia, U., Long, J.E., Searle, M.S., Moody, C.J., 2011. 7-Deazapurine biosynthesis: NMR study of toyocamycin biosynthesis in *Streptomyces rimosus* using 2-13C-7-15N-adenine. Org. Biomol. Chem. 9, 2227–2232.

Belanger, R., Dufuor, N., Caron, J., Benhamou, N., 1995. Chronological events associated with the antagonistic properties of *Trichoderma harzianum* against *Botrytis cinerea*: indirect evidence for sequential role of antibiotics and parasitism. Biocontrol Sci. Tech. 5, 41–54.

Benítez, T., Ricon, A.M., Limon, M.C., Codon, A.C., 2004. Mecanismos de biocontrol de cepas de *Trichoderma*. Int. Microbiol. 7, 249–260.

Boukaew, S., Kumla, J., Prasertsan, P., Cheirsilp, B., Petlamul, W., Srinuanpan, S., 2023a. *In vitro* and *in situ* antifungal properties of a *Trichoderma asperelloides* SKRU-01 against aflatoxigenic aspergillus species. Food Control 154, 110025.

Boukaew, S., Zhang, Z., Prasertsan, P., Igarashi, Y., 2023b. Antifungal and antiaflatoxigenic mechanism of freeze-dried culture filtrate of *Streptomyces philanthi* RL-1-178 on the two aflatoxigenic fungi and identification of its active component. J. Appl. Microbiol. 134, 1–9.

Boukaew, S., Mahasawat, P., Petlamul, W., Chuprom, J., Sattayasamitsathit, S., Surinkaew, S., Chuprom, J., Prasertsan, P., 2023c. Application of antifungal metabolites from *Streptomyces philanthi* RL-1-178 for maize grain coating formulations and their efficacy as biofungicide during storage. World J. Microbiol. Biotechnol. 39, 157.

Boukaew, S., Prasertsan, P., Mahasawat, P., Sriyatep, T., Petlamul, W., 2023d. Efficacy of the antifungal metabolites of *Streptomyces philanthi* RL-1-178 on aflatoxin degradation with its application to prevent aflatoxigenic fungi in stored maize grains and identification of the bioactive compound. World J. Microbiol. Biotechnol. 39, 24.

Boukaew, S., Kumla, J., Suwannarach, N., 2024a. *Peniophora salaccae sp. nov.* (Russulales, Basidiomycota), a snake fruit (*Salacca zalacca*) pathogen from southern Thailand. Phytotaxa 662, 137–149.

Boukaew, S., Petlamul, W., Srinuanpan, S., Nooprom, K., Zhang, Z., 2024b. Heat stability of *Trichoderma asperelloides* SKRU-01 culture filtrates: potential applications for controlling fungal spoilage and AFB_1 production in peanuts. Int. J. Food Microbiol. 409, 110477.

Boukaew, S., Petlamul, W., Yossan, S., Srinuanpan, S., Nooprom, K., Zhang, Z., 2024c. Biofumigant potential and inhibition mechanism of *Trichoderma asperelloides* SKRU-01 volatile organic compounds for controlling aflatoxigenic *Aspergillus parasiticus* and *Aspergillus flavus* in stored peanuts. Food Control 157, 110194.

Boukaew, S., Chumkaew, K., Petlamul, W., Srinuanpan, S., Nooprom, K., Zhang, Z., 2024d. Biocontrol effectiveness of *Trichoderma asperelloides* SKRU-01 and *Trichoderma asperellum* NST-009 on postharvest anthracnose in chili pepper. Food Control 163, 110490.

Brito, R.A.S., Cavalcante, G.P., Stock, V.M., Colman, A.A., dos Santos, R.A., Sermarini, L. A., Maffia, D.P., 2020. *Trichoderma* species show biocontrol potential against *Ceratocystis* wilt in mango plants. Eur. J. Plant Pathol. 158, 781–788.

Bu, S., Munir, S., He, P., Li, Y., Wu, Y., Li, X., Kong, B., He, P., He, Y., 2021. *Bacillus subtilis* L1-21 as a biocontrol agent for postharvest gray mold of tomato caused by *Botrytis cinerea*. Biol. Control 157, 104568.

Calvo, H., Mendiara, I., Arias, E., Gracia, A.P., Blanco, D., Venturini, M.E., 2020. Antifungal activity of the volatile organic compounds produced by *Bacillus velezensis* strains against postharvest fungal pathogens. Postharvest Biol. Technol. 166, 111208.

Chen, Y., Li, Z., Wei, Y., Zhang, Y., Han, N., Gao, H., Sun, C., 2009. Study on a nucleotide antibiotic 3431 produced by a rhizosphere actinomycetes I06–03431. J. Biol. 26, 17–20.

Chet, I., Inbar, J., 1994. Biological control of fungal pathogens. Appl. Biochem. Biotechnol. 48, 37–43.

Chilosi, G., Aleandri, M.P., Luccioli, E., Stazi, S.R., Marabottini, R., Morales-Rodríguez, C., Vettraino, A.M., Vannini, A., 2020. Suppression of soil-borne plant pathogens in growing media amended with espresso spent coffee grounds as a carrier of *Trichoderma* spp. Sci. Hortic. 259, 108666.

da Costa, A.C., de Miranda, R.F., Costa, F.A., Ulhoa, C.J., 2021. Potential of *Trichoderma piluliferum* as a biocontrol agent of *Colletotrichum musae* in banana fruits. Biocatal. Agric. Biotechnol. 34, 102028.

Derakhshan, Z., Ferrante, M., Tadi, M., Ansari, F., Heydari, A., Hosseini, M.S., Conti, G. O., Sadarabad, E.K., 2018. Antioxidant activity and total phenolic content of ethanolic extract of pomegranate peels, juice and seeds. Food Chem. Toxicol. 114, 108–111.

Díaz-Gutiérrez, C., Arroyave, C., Llugany, M., Poschenrieder, C., Martos, S., Peláez, C., 2021. *Trichoderma asperellum* as a preventive and curative agent to control *Fusarium* wilt in *Stevia rebaudiana*. Biol. Control 155, 104537.

Elsharkawy, M.M., Khedr, A.A., Mehiar, F., El-Kady, E.M., Alwutayd, K.M., Behiry, S.I., 2023. Rhizobacterial colonization and management of bacterial speck pathogen in tomato by *Pseudomonas* spp. Microorganisms 11, 1103.

Erazo, J.G., Palacios, S.A., Pastor, N., Giordano, F.D., Rovera, M., Reynoso, M.M., Venisse, J.S., Torres, A.M., 2021. Biocontrol mechanisms of *Trichoderma harzianum* ITEM 3636 against peanut brown root rot caused by *Fusarium solani* RC 386. Biol. Control 164, 104774.

Fenta, L., Mekonnen, H., Kabtimer, N., 2023. The exploitation of microbial antagonists against postharvest plant pathogens. Microorganisms 11, 1044.

Geng, L., Fu, Y., Peng, Z., Yang, Z., Zhang, M., Song, Z., Guo, N., Chen, S., Chen, J., Bai, B., Liu, A., Ahammed, G.J., 2022. Biocontrol potential of *Trichoderma harzianum* against *Botrytis cinerea* in tomato plants. Biol. Control 174, 105019.

Ghisalberti, E.L., Sivasithamparam, K., 1991. Antifungal antibiotics produced by *Trichoderma* spp. Soil Biol. Biochem. 23, 1011–1020.

Godana, E.A., Yang, Q.Y., Wang, K.L., Zhang, H.Y., Zhang, X.Y., Zhao, L.N., Abdelhai, M. H., Legrand, N.N.G., 2020. Bio-control activity of *Pichia anomala* supplemented with chitosan against *Penicillium expansum* in postharvest grapes and its possible inhibition mechanism. LWT–Food Sci. Technol. 124, 109188.

Gruber, S., Seidl-Seiboth, V., 2012. Self versus non-self: fungal cell wall degradation in *Trichoderma*. Microbiol 158, 26–34.

Harman, G.E., Howell, C.R., Viterbo, A., Chet, I., Lorito, M., 2004. *Trichoderma* species, opportunistic avirulent plant symbionts. Nature 2, 43–56.

Kalsi, B.S., Singh, S., Alam, M.S., Bhatia, S., 2023. Application of thermosonication for guava juice processing: impacts on bioactive, microbial, enzymatic and quality attributes. Ultrason. Sonochem. 99, 106595.

Li, Q., Jiang, Y., Ning, P., Zheng, L., Huang, J., Li, G., Jiang, D., Hsiang, T., 2011. Suppression of *Magnaporthe oryzae* by culture filtrates of *Streptomyces globisporus* JK-1. Biol. Control 58, 139–148.

Li, X., Jing, T., Zhou, D., Zhang, M., Qi, D., Zang, X., Zhao, Y., Li, K., Tang, W., Chen, Y., Qi, C., Wei Wang, W., Xie, J., 2021. Biocontrol efficacy and possible mechanism of *Streptomyces* sp. H4 against postharvest anthracnose caused by *Colletotrichum fragariae* on strawberry fruit. Postharvest Biol. Technol. 175, 111401.

Li, X., Zhang, L., Zhao, Y., Feng, J., Chen, Y., Li, K., Zhang, M., Qi, D., Zhou, D., Wei, Y., Wang, W., Xie, J., 2024. Biocontrol potential of volatile organic compounds produced by *Streptomyces corchorusii* CG-G2 to strawberry anthracnose caused by *Colletotrichum gloeosporioides*. Food Chem. 437, 137938.

Liu, F., Gao, R., Zhang, F., Ren, Y., Li, W., He, B., 2023. Postharvest biocontrol of green mold (*Penicillium digitatum*) in citrus by *Bacillus velezensis* strain S161 and its mode of action. Biol. Control 187, 105392.

Madbouly, A.K., Rashad, Y.M., Ibrahim, M.I.M., Elazab, N.T., 2023. Biodegradation of Aflatoxin B_1 in maize grains and suppression of its biosynthesis-related genes using endophytic *Trichoderma harzianum* AYM3. J. Fungi 9, 209.

Martínez, F.D., Santos, M., Carretero, F., Marín, F., 2016. *Trichoderma saturnisporum,* a new biological control agent. J. Sci. Food Agric. 96, 1934–1944.

Mehmood, N., Saeed, M., Zafarullah, S., Hyder, S., Rizvi, Z.F., Gondal, A.S., Jamil, N., Iqbal, R., Ali, B., Ercisli, S., Kupe, M., 2023. Multifaceted impacts of plant-beneficial *Pseudomonas* spp. in managing various plant diseases and crop yield improvement. ACS Omega 8, 22296–22315.

Ming, Q., Han, T., Li, W., Zhang, Q., Zhang, H., Zheng, C., 2012. Tanshinone II A and tanshinone I production by *Trichoderma atroviride* D16, an endophytic fungus in *Salvia miltiorrhiza*. Phytomedicine 19, 330–333.

Mukherjee, P.K., Mendoza-Mendoza, A., Zeilinger, S., Horwitz, B.A., 2022. Mycoparasitism as a mechanism of *Trichoderma*-mediated suppression of plant diseases. Fungal Biol. Rev. 39, 15–33.

Nazari, M.T., Schommer, V.A., Braun, J.C.A., dos Santos, L.F., Lopes, S.T., Simon, V., Machado, B.S., Ferrari, V., Colla, L.M., Piccin, J.S., 2023. Using *Streptomyces* spp. as plant growth promoters and biocontrol agents. Rhizosphere 27, 100741.

Parafati, L., Vitale, A., Restuccia, C., Cirvilleri, G., 2015. Biocontrol ability and action mechanism of food-isolated yeast strains against *Botrytis cinerea* causing post-harvest bunch rot of table grape. Food Microbiol. 47, 85–92.

Parsaei, P., Karimi, M., Asadi, S.Y., Rafieian-Kopaei, M., 2013. Bioactive components and preventive effect of green tea (*Camellia sinensis*) extract on post-laparotomy intra-abdominal adhesion in rats. Int. J. Surgery 11, 811–815.
Phothisuwan, S., Matan, N., Matan, N., 2021. The influence of a closed system combining orange oil and mode of action on quality preservation of salacca fruit. Food Control 130, 108265.
Qualhato, T.F., Lopes, F.A.C., Steindorff, A.S., Brandao, R.S., Jesuino, R.S.A., Ulhoa, C.J., 2013. Mycoparasitism studies of *Trichoderma* species against three phytopathogenic fungi: evaluation of antagonism and hydrolytic enzyme production. Biotechnol. Lett. 35, 1461–1468.
Ranganna, S., 1986. Handbook of Analysis and Quality Control for Fruit and Vegetable Products. Tata McGraw-Hill Publishing Company, p. 1112.
Ren, X., Branà, M.T., Haidukowski, M., Gallo, A., Zhang, Q., Logrieco, A.F., Li, P., Zhao, S., Altomare, C., 2022. Potential of *Trichoderma* spp. for biocontrol of aflatoxin-producing *Aspergillus flavus*. Toxins 14, 86.
Sánchez-Montesinos, B., Santos, M., Moreno-Gavíra, A., Marín-Rodulfo, T., Gea, F.J., Diánez, F., 2021. Biological control of fungal diseases by *Trichoderma aggressivum* f. *europaeum* and its compatibility with fungicides. J. Fungi 7, 598.
Savazzini, F., Longa, C.M.O., 2009. Impact of the biocontrol agent *Trichoderma atroviride* SC1 on soil microbial communities of a vineyard in northern Italy. Soil Biol. Biochem. 41, 1457–1465.
Shang, J., Liu, B., Xu, Z., 2020. Efficacy of *Trichoderma asperellum* TC01 against anthracnose and growth promotion of *Camellia sinensis* seedlings. Biol. Control 143, 104205.
Shao, X.F., Tu, K., Tu, S., Tu, J., 2012. A combination of heat treatment and chitosan coating delays ripening and reduces decay in "gala" apple fruit. J. Food Qual. 35, 83–92.
Shentu, X., Yu, B., Bian, Y., Hu, J., Ma, Z., Tong, C., Yu, X., 2012. Biocontrol effect and taxonomy of antagonistic *Streptomyces* strain B28. Acta Phytopathologica Sinica 42, 105–109.
Shoresh, M., Harman, G.E., Mastouri, F., 2010. Induced systemic resistance and plant responses to fungal biocontrol agents. Annu. Rev. Phytopathol. 48, 21–43.
Sid-Ahmed, A., Ezziyyani, M., Pérez-Sánchez, C., Candela, M.E., 2003. Effect of chitin on biological control activity of *Bacillus* spp. and *Trichoderma harzianum* against root rot disease in pepper (*Capsicum annuum*) plants. Eur. J. Plant Pathol. 109, 418–426.
Spinelli, F.R., Andre, J.A., Celso, P.G., Vicenzi, M.S., Kinast, E.J., Lamb, C.R.C., Bertoldo, G., 2024. Soluble solids profile of Brazilian *Vitis labrusca* and hybrid grape musts, from the 2012–2022 harvest. J. Food Compos. Anal. 125, 105797.
Suica-Bunghez, I.R., Teodorescu, S., Dulama, I.D., Voinea, O.C., Simionescu, S., Ion, R. M., 2016. Antioxidant activity and phytochemical compounds of snake fruit (*Salacca zalacca*). IOP Conference Series: Materials Science and Engineering 133, 012051.
Supapvanich, S., Megia, R., Ding, P., 2011. Salak (*Salacca zalacca* (Gaertner) Voss). In: Yahia, E.M. (Ed.), Postharvest Biology and Technology of Tropical and Subtropical Fruits: Mangosteen to White Sapote, pp. 334–352e.
Tyskiewicz, R., Nowak, A., Ozimek, E., Jaroszuk-Scisel, J., 2022. *Trichoderma*: the current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. Int. J. Mol. Sci. 23, 2329.
Verma, M., Brar, S.K., Tyagi, R.D., Sahai, V., Prévost, D., Valéro, J.R., Surampalli, R.Y., 2007. Bench-scale fermentation of *Trichoderma viride* on wastewater sludge: rheology, lytic enzyme and biocontrol activity. Enzyme Microb. Technol. 41, 764–771.
Vey, A., Hoagland, R.E., Butt, T.M., 2001. In: Butt, T.M., Jackson, C., Magan, N. (Eds.), Toxic Metabolites of Fungal Biocontrol Agents. Fungi as Biocontrol Agents: Progress, Problems and Potential. CAB International, Bristol, pp. 311–346.
Xie, Z., Li, M., Wang, D., Wang, F., Shen, H., Sun, G., Feng, C., Wang, X., Chen, D., Sun, X., 2021. Biocontrol efficacy of *Bacillus siamensis* LZ88 against brown spot disease of tobacco caused by *Alternaria alternata*. Biol. Control 154, 104508.
Yan, L.-L., Han, N.-N., Zhang, Y.-Q., Yu, L.-Y., Chen, J., Wei, Y.-Z., Li, Q.-P., Tao, L., Zheng, Gu-H., Yang, S.-E., Jiang, C.-X., Zhang, X.-D., Huang, Q., Habdin, X., Hu, Q.-B., Li, Z., Liu, S.-W., Zhang, Z.-Z., He, Q.-Y., Si, S.-Y., Sun, C.-H., 2010. Antimycin A18 produced by an endophytic *Streptomyces albidoflavus* isolated from a mangrove plant. J. Antibiot. 63, 259–261.
You, W., Ge, C., Jiang, Z., Chen, M., Li, W., Shao, Y., 2021. Screening of a broad-spectrum antagonist-*Bacillus siamensis*, and its possible mechanisms to control postharvest disease in tropical fruits. Biol. Control 157, 104584.
Youssef, S.A., Tartoura, K.A., Abdelraouf, G.A., 2016. Evaluation of *Trichoderma harzianum* and *Serratia proteamaculans* effect on disease suppression, stimulation of ROS-scavenging enzymes and improving tomato growth infected by *Rhizoctonia solani*. Biol. Control 100, 79–86.
Zhan, X., Khan, R.A.A., Zhang, J., Chen, J., Yin, Y., Tang, Z., Wang, R., Lu, B., Liu, T., 2023. Control of postharvest stem-end rot on mango by antifungal metabolites of *Trichoderma pinnatum* LS029-3. Sci. Hortic. 310, 111696.
Zhang, F., Huo, Y., Cobb, A.B., Luo, G., Zhou, J., Yang, G., Wilson, G.W.T., Zhang, Y., 2018. *Trichoderm*a biofertilizer links to altered soil chemistry, altered microbial communities, and improved grassland biomass. Front. Microbiol. 9, 848.