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RESEARCH PAPER

Isolating, characterizing, and utilizing *Trichoderma asperellum* to antagonize *Neurospora* spp. causing scab disease on King mandarin fruits (*Citrus sinensis*)

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ABSTRACT

The research aimed to (i) isolate, select, and assess the pathogenic potential of fungi that pose a risk of causing scab disease on King mandarin fruits (*Citrus sinensis*), and (ii) evaluate the antagonistic potential of *Trichoderma* spp. against *Neurospora* spp., , which cause scab disease on King mandarin fruits under *in vitro* conditions. The isolation process identified four fungal strains from ten King mandarin fruits showing scab symptoms, collected from ten orchards in Vung Liem District, Vinh Long Province. The three most virulent scab-causing fungal strains were KMS-01, KMS-02, and KMS-04, with growth diameters of 7.60–7.63 cm after 72 hours of culture (HoC). Additionally, the antagonistic ability of 50 *Trichoderma* spp. strains against *Neurospora* spp. ranged from 49.8% to 87.6% at 72 HoC. Among these, three *Trichoderma* spp. strains—T-SP03, T-SP26, and T-SP41—exhibited high antagonistic efficiency (86.1%–87.7%) against all three scab-causing strains. Based on the ITS region, the pathogenic fungal strains were identified as *Neurospora intermedi*a KMS-01, *N. intermedia* KMS-02, and *N. crassa* KMS-04, while the *Trichoderma* spp. strains were identified as *Trichoderma asperellum* T-SP03, T-SP26, and T-SP41 with 100% similarity. *T. asperellum* shows potential as a biological control agent against *Neurospora* spp., the causative agents of scab disease in King mandarin.

Key words: King mandarin, *Neurospora* spp., scab disease, *Trichoderma* spp.

INTRODUCTION

Citrus scab disease primarily occurs in regions with humid climates and affects citrus trees such as King mandarin (*Citrus sinensis*), lime (*C. limon*), grapefruit (*C. paradisi*), tangerine (*C. reticulata, C. unshiu*, and *C. clementina*), and rootstock plants like sour orange (*C. aurantium*), rough lemon (*C. jambhiri*), and Cleopatra mandarin (*C. reshni*) (Pham et al., 2025). The main causative agents of citrus scab are *Elsinoë fawcettii* (affecting citrus trees) and *E. australis* (affecting oranges) (Shanmugam et al., 2020; Choi et al., 2020, Elliott et al., 2023). This fungal disease affects fruit, leaves, and branches. The disease symptoms include small, irregular, and rough-textured lesions. The infection spreads across the host plant tissues, forming scabs composed of the fungus and host plant cells (Caserta et al., 2019).

Although scab damage on fruit (scarring and distortion) does not affect the internal quality of the fruit, it reduces the fruits' marketability (EFSA Panel on Plant Health (PLH) et al., 2017). Scab is one of the most common citrus diseases worldwide, including in Southeast Asia (Alam et al., 2020). In Korea, scab was found to cause losses amounting to 10.04 million dollars, equivalent to 5,497 ton of yield reduction (Kwon et al., 2015). Scab on citrus can lead to a 66–72.2% drop in fruitlets (Huang & Huang, 1999) and a 25–55% reduction in yield (Siddiquee et al., 2011).

Elsinochrom, a secondary metabolite produced by *E. fawcettii,* causes cell membrane peroxidation and rapid electrolyte leakage from citrus tissues. The biosynthesis and regulation of Elsinochrom are influenced by environmental and physiological factors (Liu et al., 2024). Other scab-causing pathogens are also diverse. For example, *Venturia inaequalis* causes scab on apple, pecan, and almond (Khajuria et al., 2022; Reuveni et al., 2022), *V. nashicola* causes scab on pear (Takeuchi et al., 2023; Won et al., 2023), and *E. perseae* appears on avocado (Gañán-Betancur & Gazis, 2023).

Scab disease on King mandarin has been locally reported in citrus-growing regions of Ben Tre, Vinh

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Long, and Hau Giang. However, it has not been comprehensively investigated. Moreover, despite numerous studies on citrus scab, scab on mandarin specifically has not been thoroughly examined.

To prevent scab disease, biological approaches are preferred over chemical methods (Li et al., 2021). *Trichoderma* spp. and *Bacillus* spp. have been widely applied to control the disease due to their high adaptability under diverse conditions (Porto et al., 2022). For instance, *Bacillus* spp. have been recently used on apple (Leconte et al., 2022) and potato (Shuang et al., 2022). However, in the study by Kulimushi et al. (2021), *Trichoderma* spp. resulted in greater crop yields compared to *B. subtilis* and *Pseudomonas fluorescens*. Moreover, fungal isolates exhibited better biocontrol efficacy than bacterial isolates (Mannai & Boughalleb-M'Hamdi, 2023). Thus, *Trichoderma* spp. were chosen for investigation in the current study.

Trichoderma spp., a group of ascomycete fungi, are recognized as effective biocontrol agents against plant pathogens. These fungi utilize antagonistic and competitive mechanisms to suppress harmful microorganisms (Woo et al., 2023). Various *Trichoderma* species, such as *T. asperellum, T. atroviride, T. harzianum, T. koningii, T. longibrachiatum, T. virens,* and *T. viride*, have shown potential in controlling pathogenic fungi that damage crops (Kubiak et al., 2023). Specific strains, such as *T. yunnanense* T-02B1, *T. lentiforme* T-18B2, *T. asperellum* T-20B1, *T. hamatum* T-28B1, and *T. hamatum* T-29A1, have been used to control Fusarium wilt on sesame (*Fusarium oxysporum*), while *T. asperellum* has been utilized to manage fruit rot in pomelo (*Lasiodiplodia theobromae*) in the Mekong Delta, Vietnam (Khuong et al., 2023a, Khuong et al., 2023b).

For scab disease, *Trichoderma* spp. have been applied to apple (Gouit et al., 2024). Recently, *Trichoderma* spp. have been identified as biocontrol agents against soft rot on black nightshade (Arasu et al., 2023), *Fusarium oxysporum* causing root rot with a 68.1% reduction in rubber trees (Liu et al., 2023), *F. oxysporum* F.28.1A causing wilt disease with a 35% reduction in sesame (Khuong et al., 2023a), and *F. xylarioides* causing coffee wilt (Mulatu et al., 2023).

On citrus, Ferreira et al. (2020) showed that *T. harzianum* IC-30 effectively controlled the spread of green mold and other pathogens, reducing decay caused by *Penicillium digitatum* A21 by approximately 80% after three weeks. Additionally, *T. amatum* K01 produces secondary metabolites, including pyrone, organic compounds, fatty acids, and sorbicillin, which inhibit *Colletotrichum gloeosporioides* C01, the causative agent of anthracnose in citrus (Phal et al., 2023). The antagonistic effectiveness of *T. harzianum* T22 in controlling citrus scab was recorded at 62.4% (Alam, 2006). A study by Hieu et al. (2010) indicated that citrus scab caused by *E. fawcettii* is the most prevalent in lemon orchards in Tien Giang, Vietnam.

Therefore, this study aimed to (i) isolate and characterize fungal strains that cause scab disease on King mandarin fruits, and (ii) evaluate the effectiveness of *Trichoderma* spp. in antagonizing scab-causing fungi under laboratory conditions.

MATERIALS AND METHODS

Research Site. The experiment was conducted from January 2023 to July 2023 at the Fungal Laboratory, Faculty of Crop Science, College of Agriculture, and the Institute of Food and Biotechnology, Can Tho University.

Pathogenic Fungi Source. Ten King mandarin fruits infected with scab disease were collected from 10 orchards in Vung Liem District, Vinh Long Province, Vietnam, in January 2023. The sample sites had similar climate conditions. The orchards and samples were collected randomly. The disease progressed rapidly, causing the fruit peel to become brown and hard, with scabs covering the entire fruit within a few days.

Antagonistic Fungi Source. A total of 50 strains of *Trichoderma* spp. were isolated from soil samples taken from a depth of 0–20 cm in pomelo orchards in Ben Tre, Vietnam (Table S1). The fungal strains were isolated and stored at the Fungal Laboratory, Faculty of Crop Science, College of Agriculture, Can Tho University. These *Trichoderma* strains were labeled from T-SP01 to T-SP50.

Culture Medium. Two culture medium were used: 1) Potato Dextrose Agar (PDA)–200.0 g potato, 20.0 g D-glucose, 25.0 g chloramphenicol, and 20.0 g agar in 1 L of deionized water (Ahmed et al., 2022). This medium was used for general fungal experiments; 2) *Trichoderma* Selective Medium (TSM)–1.0 g NH_4NO_3, 0.90 g K_2HPO_4, 0.15 g KCl, 0.20 g $MgSO_4 \cdot 7H2O$, 0.25 g chloramphenicol, and 20.0 g agar in 1 L of deionized water (Mirzaeipour et al., 2023). This medium was used specifically for *Trichoderma* isolation.

Isolation of Scab-causing Fungi on King Mandarin. The isolation method followed Shivas & Beasley (2005) and Burgess et al. (2008). All symptomatic fruits

were collected, surface-sterilized, and used for fungal isolation. Diseased spots were cut into small pieces (2 × 2 mm), placed on PDA medium, and incubated in the dark at room temperature (28–30 ºC). When fungal mycelium appeared on the plate surface, pure cultures were obtained by transferring the mycelium to fresh PDA plates. This process was repeated until a uniform mycelium was established. After 8 days of growth, the fungal morphology, characteristics, and color were observed and described. The isolated fungal colonies were labeled as KMS (King Mandarin Scab), followed by sequential numbers for each isolate.

Assessment of Fungal Growth. The growth of scab-causing fungal isolates was assessed on PDA medium (Mubashir et al., 2024) at 24, 48, and 72 hours after inoculation (HAI), with three replicates for each isolate. Fungal growth was evaluated by measuring the mycelial colony diameter from the center using a ruler.

Assessment of the Antagonistic Ability of *Trichoderma* spp. Against Scab-causing Fungi.

***Materials Preparation*.** The TSM and PDA media were prepared as described in the "Culture Medium" section. The pathogenic fungal strains selected from the "Assessment of Fungal Growth" results and the 50 *Trichoderma* spp. strains from the "Antagonistic Fungi Source" section were used. The *Trichoderma* strains were cultured on TSM for 72 hours before the antagonistic experiment.

***Experimental Procedure*.** The experiment was arranged in a completely randomized design on PDA medium with 50 treatments (corresponding to 50 *Trichoderma* spp. strains) and three replicates per treatment. The dual culture method was conducted following Bell et al. (1982).

On each culture plate, an agar block (Ø = 5 mm) containing a *Trichoderma* strain was placed 3.0 cm from the edge. Similarly, an agar block containing the pathogenic fungus was placed opposite the *Trichoderma* block, maintaining a 3 cm distance between them. Control treatments included plates with either the pathogenic fungus or *Trichoderma* spp. cultured separately. The dual cultures were incubated in the dark at room temperature (28–32 ºC) and observed for antagonism interactions at 48 and 72 HAI.

***Evaluation Criteria*.** The diameters of *Trichoderma* spp. and scab-causing fungal colonies were measured at 48 and 72 HAI. Antagonistic efficiency (AE) was determined following Kakraliya et al. (2017):

$$AE(\%) = \frac{C - T}{C} \times 100$$

AE = Antagonistic efficiency;
C = Represents the radius of the pathogenic fungal colony on the PDA medium;
T = Radius of the pathogenic fungal colony in dual culture with *Trichoderma* spp.

The AE was classified into four categories (Kakraliya et al., 2017): High antagonism (≥ 60%), Moderate antagonism (40% ≤ AE ≤ 59%), Weak antagonism (20% ≤ AE ≤ 39%), and No antagonism (≤ 19%).

***Identification of Pathogenic Fungi and* Trichoderma *spp*.** The selected pathogenic fungi strains (KMS-01, KMS-02, and KMS-04) and *Trichoderma* strains (T-SP03, T-SP26, and T-SP41) were identified using Internal Transcribed Spacer (ITS) sequencing.

DNA was extracted from fungal mycelia cultured for 7–10 days on PDA medium. Spores from fungal colonies were placed into 2.2 mL Eppendorf tubes, shaken well, and incubated at room temperature for 10 min. The solution was centrifuged at 13,000 rpm for 5 min, and the supernatant was transferred to a new tube. The pellet was washed with 500 µL of 70% ethanol, centrifuged at 13,000 rpm for 5 min, and vacuum-dried. The DNA was then dissolved in 100 µL of 0.1× TE buffer.

The polymerase chain reaction (PCR) was performed using the primer pair: ITS 1: 5'-TCCGTAGGTGAACCTGCGG-3' and ITS 4: 5'-TCCTCCGCTTATTGATATGC-3' (White et al., 1990). The PCR reaction was conducted in a total volume of 50 µL with the following conditions: initial denaturation at 95 ºC for 5 min, followed by denaturation at 95 ºC for 90 sec (30 cycles), annealing at 52 ºC for 60 sec (30 cycles), extension at 72 ºC for 90 sec (30 cycles), and a final extension at 72 ºC for 5 sec, then termination at room temperature. PCR products were purified and sequenced using an automated sequencing system. The sequences were compared against the GenBank database (NCBI) using BLASTN. A phylogenetic tree was constructed based on the sequences using MEGA 6.0 software.

Statistical Analysis. Data were analyzed using one-way ANOVA, and mean differences between fungal strains were determined by Duncan's test at a 5% significance level using SPSS 13.0 software.

RESULTS AND DISCUSSION

Isolation, Morphological Characteristics, and Identification of the Scab-causing Fungi on King Mandarin in Vinh Long

Isolation and Morphological Characteristics of Scab-causing Fungi on King Mandarin. From 10 fruit samples showing scab symptoms collected in Vung Liem, Vinh Long, four scab-causing fungal strains —KMS-01, KMS-02, KMS-03, and KMS-04—were isolated and purified on PDA medium (Figure 1). All isolated strains covered the entire surface of the Petri dish by 72 HAI. Initially, the fungal mycelium appeared white and gradually turned light yellow by the fifth day of incubation in all strains. The pathogenic fungal mycelium exhibited rapid growth, forming evenly circular colonies. The mycelium structure varied, appeared fluffy and evenly spread. All four strains initially developed white colonies that later turned light yellow (Table 1).

Growth Ability of Scab-causing Fungi on PDA Medium. The growth rates of the fungal colonies at 24, 48, and 72 HAI on PDA medium showed statistically significant differences at the 5% level. At 24 HAI, strain KMS-04 exhibited the fastest growth, reaching 3.37 cm in diameter compared to the other strains. However, at 48 and 72 HAI, strains KMS-01, KMS-02, and KMS-04 grew faster than strain KMS-03, with diameters of 5.60–5.90 cm versus 4.57 cm at 48 HAI and 7.60–7.63 cm versus 6.50 cm at 72 HAI (Table 2). Based on the growth performance on PDA medium, three strains—KMS-01, KMS-02, and KMS-04—were selected for testing the antagonistic ability of 50 *Trichoderma* spp. strains under *in vitro* conditions.

Identification of Selected Scab-causing Fungal Strains on King Mandarin. The pathogenic fungal strains KMS-01, KMS-02, and KMS-04 were identified as *Neurospora intermedia* KMS-01, *N. intermedia* KMS-02, and *N. crassa* KMS-04 based on ITS region sequencing, showing 99% similarity (Figure 2).

Evaluation of Antagonistic Ability of *Trichoderma* spp. Against *Neurospora* spp. scab-causing fungi on King mandarin in vitro conditions

Antagonistic Ability of* Trichoderma *spp. Against Scab-causing Fungi KMS-01 on PDA Medium. At 48 HAI, the antagonistic efficiency of strains T-SP03 (52.10%), T-SP41 (56.30%), and T-SP26 (52.60%) was the highest and was classified as moderate antagonism. However, the remaining *Trichoderma* spp. strains exhibited lower antagonistic efficiency, ranging from 20.0% to 26.0%, which was rated as weak antagonism. At 72 HAI, strains T-SP41, T-SP26, and T-SP04 demonstrated high antagonistic efficiency, reaching

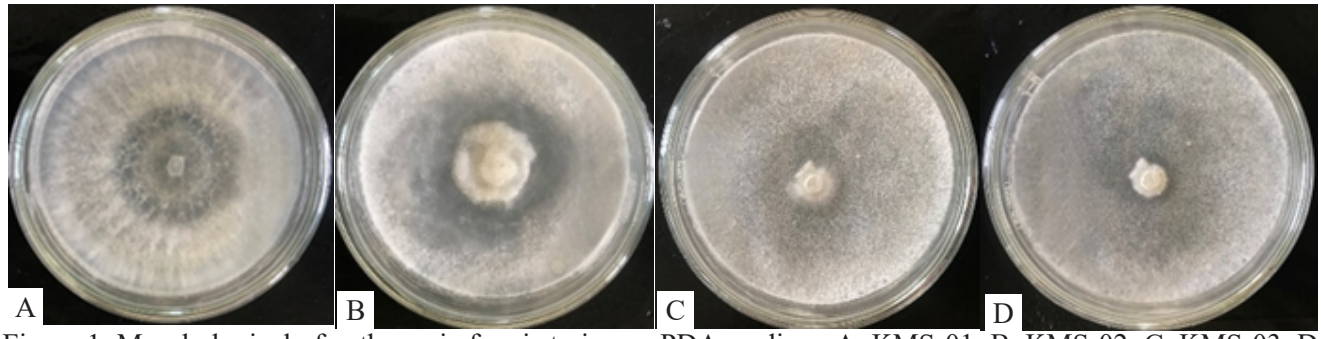

Figure 1. Morphological of pathogenic fungi strains on PDA medium. A. KMS-01; B. KMS-02; C. KMS-03, D. KMS-04.

Table 1. Colony characteristics of fungal strains causing scabies on PDA medium at 7 days after inoculation.

Strain	Morphology	Surface characteristics of mycelium	Color of mycelium
KMS-01	Radial arrangement	All strains had dense, water-resistant mycelium. The colonies were white to off-white, with a fuzzy texture and many aerial hyphae.	The translucent white mycelium gradually changed to yellow or light orange on the underside.
KMS-02	Radial arrangement		The opaque white mycelium gradually changed light yellow on the underside.
KMS-03	Radial arrangement		The opaque white color transited to light yellow and then to light orange.
KMS-04	Radial arrangement		The opaque white color transited to light yellow and then to light orange.

86.90–87.60%, while the remaining *Trichoderma* spp. strains exhibited moderate antagonistic efficiency, ranging from 49.80% to 56.90% (Table 3). In summary, the strains with the highest antagonistic efficiency were T-SP03, T-SP26, and T-SP41 (Figure 3).

Antagonistic Ability of* Trichoderma *spp. Against Scab-causing Fungi KMS-02 on PDA Medium. As shown in Table 4, at 48 HAI, the antagonistic efficiency strains T-SP03 (52.10%), T-SP26 (50.60%), and T-SP41 (55.20%) was classified as moderate antagonism. At 72 HAI, the strains with the highest antagonistic efficiency (≥ 60.00%) were T-SP03 (86.20%), T-SP26 (87.70%), and T-SP41 (86.10%), outperforming the other strains. Strains T-SP21, T-SP29, and T-SP39 exhibited the lowest antagonistic efficiency, with values of 49.8%, 47.5%, and 49.8%, respectively. In summary, the *Trichoderma* spp. strains with the highest antagonistic ability against the scab-causing strain KMS-02 were T-SP03 (86.20%), T-SP26 (87.70%), and T-SP41 (86.10%) (Figure 4).

Antagonistic Ability of* Trichoderma *spp. Against Scab-causing Fungi KMS-04 on PDA Medium. At 48 HAI, *Trichoderma* spp. strains T-SP03, T-SP26, and T-SP41 were classified as exhibiting moderate antagonism, with efficiencies of 56.30%, 52.10%, and 54.80%, respectively, while the remaining strains were rated as weak antagonists, with efficiencies ranging from 19.00% to 26.00%. At 72 HAI, the strains with the highest antagonistic efficiency were T-SP03 and T-SP41, both achieving 87.60%, while T-SP26 achieved 86.90%. The remaining strains were classified as having moderate antagonistic ability, with an average efficiency of 50.60% (Table 4). In summary, *Trichoderma* spp. strains T-SP03, T-SP26, and T-SP41 exhibited very strong antagonistic abilities over time (Figure 5).

Identification of Selected *Trichoderma* spp. Strains. The selected *Trichoderma* spp. strains were identified as *Trichoderma asperellum* T-SP03, T-SP26, and T-SP41, with 100% similarity (Figure 6).

The three fungal strains KMS-01, KMS-02, and KMS-04 were identified as *N. intermedia* KMS-01, *N. intermedia* KMS-02, and *N. crassa* KMS-04 (Figure 2). These strains exhibited rapid growth and a circular spreading morphology on the plates. All strains initially appeared white and gradually turned light yellow, with diverse mycelial structures that were fluffy and spread

Table 2. Average growth of pathogenic fungi on PDA medium

Strains	Growth of fungal colony (cm)		
	24 HAI	48 HAI	72 HAI
KMS-01	1.90 c	5.90 a	7.63 a
KMS-02	1.57 d	5.60 a	7.60 a
KMS-03	2.40 b	4.57 b	6.50 b
KMS-04	3.37 a	5.80 a	7.60 a
F	*	*	*
CV (%)	0.63	0.89	0.38

Note: Within the same column, entries with the same letter following them are not significantly different according to Duncan's test, while (*) denotes significant differences at the 5% level. HAI: hours after inoculation

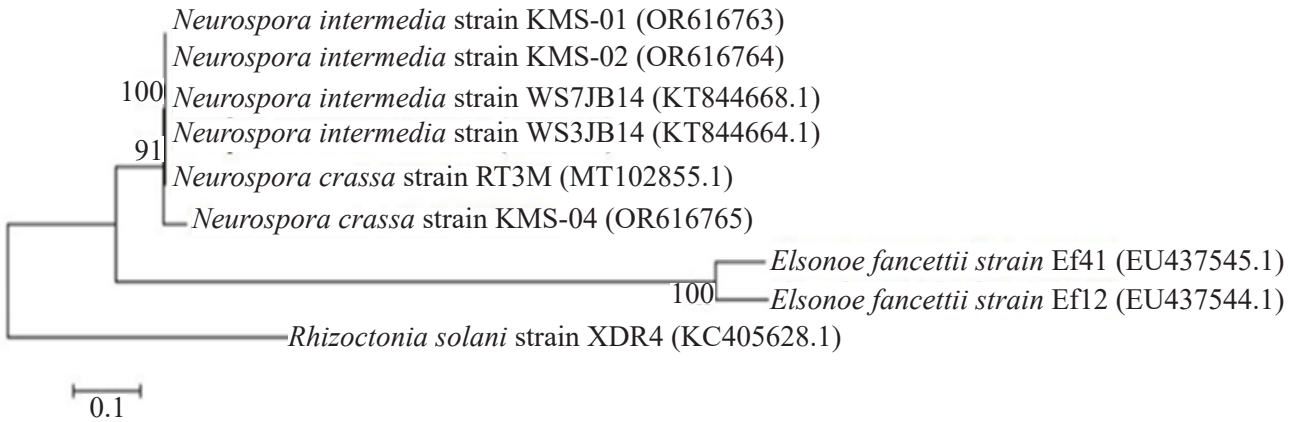

Figure 2. Phylogenetic tree of *Neurospora* spp. causing scab on King mandarin fruits (*Citrus sinensis*).

Table 3. Antagonistic efficiency of *Trichoderma* spp. against the pathogenic KMS-01 at 48 and 72 HAI

No.	Strain	Antagonistic efficiency (%)		Antagonistic diameter of strain KMS-01 (cm)	
		48 HAI	72 HAI	48 HAI	72 HAI
1	T-SP01	25.60 b	56.90 b	2.37 a ± 0.06	1.87 c ± 0.15
2	T-SP02	21.90 bc	55.60 cd	2.43 a ± 0.12	1.93 bc ± 0.21
3	T-SP03	52.10 a	87.00 a	1.53 b ± 0.06	0.57 d ± 0.06
4	T-SP04	21.90 bc	53.10 bcd	2.50 a ± 0.10	2.03 abc ± 0.06
5	T-SP05	23.90 bc	51.70 bcd	2.43 a ± 0.06	2.10 ab ± 0.10
6	T-SP06	22.50 bc	51.70 bcd	2.47 a ± 0.06	2.10 ab ± 0.10
7	T-SP07	21.50 bc	52.50 bcd	2.50 a ± 0.10	2.07 abc ± 0.15
8	T-SP08	24.00 bc	53.30 bcd	2.43 a ± 0.06	2.03 abc ± 0.06
9	T-SP09	21.90 bc	51.70 bcd	2.50 a ± 0.10	2.10 ab ± 0.10
10	T-SP10	24.60 bc	54.00 bcd	2.40 a ± 0.10	2.00 abc ± 0.10
11	T-SP11	21.10 bc	51.10 cd	2.50 a ± 0.10	2.13 ab ± 0.06
12	T-SP12	22.70 bc	54.20 bcd	2.50 a ± 0.06	2.00 abc ± 0.10
13	T-SP13	22.10 bc	54.40 bcd	2.47 a ± 0.15	1.97 abc ± 0.15
14	T-SP14	20.60 bc	51.30 cd	2.50 a ± 0.10	2.10 ab ± 0.10
15	T-SP15	20.00 bc	52.10 bcd	2.53 a ± 0.06	2.07 abc ± 0.06
16	T-SP16	24.00 bc	51.70 bcd	2.43 a ± 0.15	2.10 ab ± 0.10
17	T-SP17	20.40 bc	53.20 bcd	2.53 a ± 0.06	2.03 abc ± 0.06
18	T-SP18	20.00 bc	52.40 bcd	2.53 a ± 0.12	2.07 abc ± 0.12
19	T-SP19	20.40 bc	51.70 bcd	2.53 a ± 0.12	2.10 ab ± 0.10
20	T-SP20	22.40 bc	54.40 bcd	2.43 a ± 0.15	1.97 abc ± 0.15
21	T-SP21	21.50 bc	51.30 cd	2.50 a ± 0.10	2.10 ab ± 0.10
22	T-SP22	21.30 bc	52.90 bcd	2.47 a ± 0.12	2.03 abc ± 0.06
23	T-SP23	26.00 b	56.80 b	2.37 a ± 0.06	1.87 c ± 0.15
24	T-SP24	21.90 bc	53.50 bcd	2.50 a ± 0.10	2.00 abc ± 0.10
25	T-SP25	19.00 c	52.00 bcd	2.57 a ± 0.06	2.07 abc ± 0.06
26	T-SP26	52.60 a	87.60 a	1.50 ab ± 0.10	2.57 d ± 0.06
27	T-SP27	20.40 bc	52.10 bcd	2.53 a ± 0.06	2.07 abc ± 0.06
28	T-SP28	21.00 bc	51.30 cd	2.50 a ± 0.10	2.10 ab ± 0.10
29	T-SP29	23.60 bc	52.90 bcd	2.43 a ± 0.06	2.03 abc ± 0.06
30	T-SP30	22.10 bc	54.40 bcd	2.47 a ± 0.15	1.90 acb ± 0.06
31	T-SP31	21.10 bc	51.40 cd	2.50 a ± 0.10	2.10 ab ± 0.10
32	T-SP32	23.60 bc	54.80 bcd	2.43 a ± 0.15	1.97 abc ± 0.15
33	T-SP33	20.40 bc	54.30 bcd	2.53 a ± 0.12	1.97 abc ± 0.25
34	T-SP34	20.00 b	52.00 bcd	2.53 a ± 0.06	2.07 abc ± 0.06
35	T-SP35	23.20 bc	53.50 bcd	2.43 a ± 0.15	2.00 abc ± 0.20
36	T-SP36	21.70 bc	53.00 bcd	2.47 a ± 0.15	2.03 abc ± 0.12
37	T-SP37	23.20 bc	52.90 bcd	2.43 a ± 0.06	2.03 abc ± 0.06
38	T-SP38	22.50 bc	51.30 cd	2.47 a ± 0.06	2.10 ab ± 0.10
39	T-SP39	21.50 bc	49.80 d	2.50 a ± 0.10	2.17 a ± 0.06
40	T-SP40	23.00 bc	51.40 cd	2.47 a ± 0.06	2.10 ab ± 0.10

Table 3. Continued.

No.	Strain	Antagonistic efficiency (%)		Antagonistic diameter of strain KMS-01 (cm)	
		48 HAI	72 HAI	48 HAI	72 HAI
41	T-SP41	56.30 a	86.90 a	1.40 b ± 0.10	0.57 d ± 0.06
42	T-SP42	21.90 bc	51.30 cd	2.50 a ± 0.10	2.10 ab ± 0.10
43	T-SP43	22.10 bc	52.10 bcd	2.47 a ± 0.12	2.07 abc ± 0.12
44	T-SP44	23.20 bc	52.10 bcd	2.43 a ± 0.06	2.07 abc ± 0.12
45	T-SP45	22.90 bc	52.10 bcd	2.47 a ± 0.06	2.07 abc ± 0.06
46	T-SP46	21.90 bc	51.20 cd	2.50 a ± 0.10	2.10 ab ± 0.10
47	T-SP47	22.90 bc	52.00 bcd	2.47 a ± 0.06	2.07 abc ± 0.06
48	T-SP48	21.10 c	51.20 cd	2.50 a ± 0.10	2.10 ab ± 0.10
49	T-SP49	23.10 bc	51.20 cd	2.43 a ± 0.06	2.10 ab ± 0.10
50	T-SP50	22.10 bc	51.20 cd	2.47 a ± 0.12	2.10 ab ± 0.10
	F	*	*	*	*
	CV (%)	6.18	3.31	0.63	0.78

Within the same column, entries with the same letter following them are not significantly different according to Duncan's test, while (*) denotes significant differences at the 5% level. HAI: hours after inoculation.

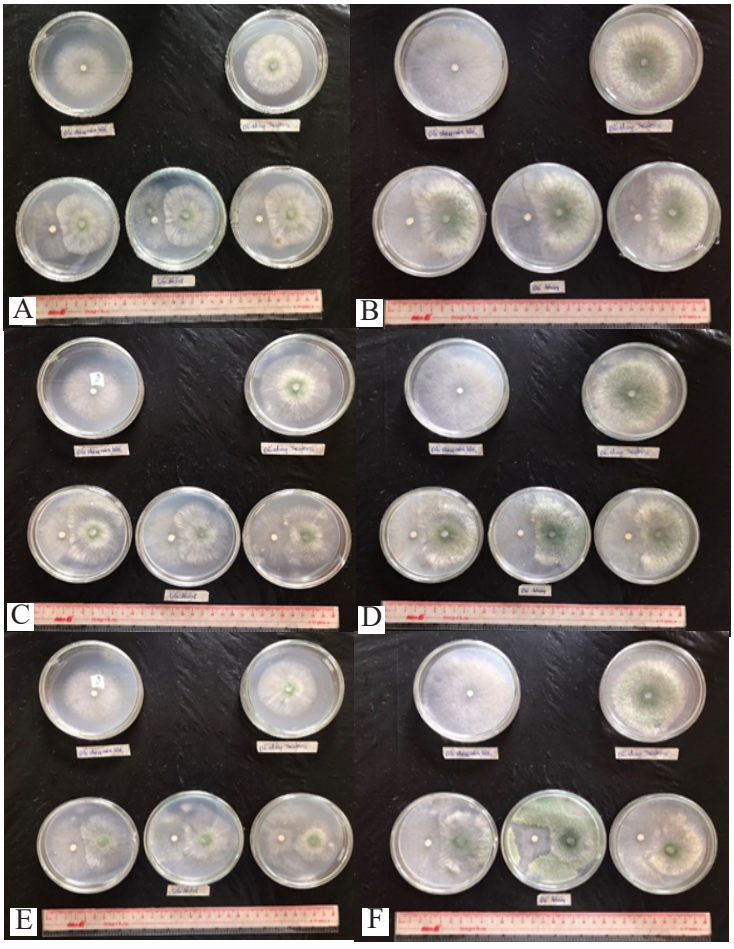

Figure 3. Antagonistic ability of *Trichoderma* spp. A, B. T-SP03; C, D. T-SP26; E, F. T-SP41 with pathogenic fungus strain KMS-01 on PDA medium at 48 and 72 HAI, respectively.

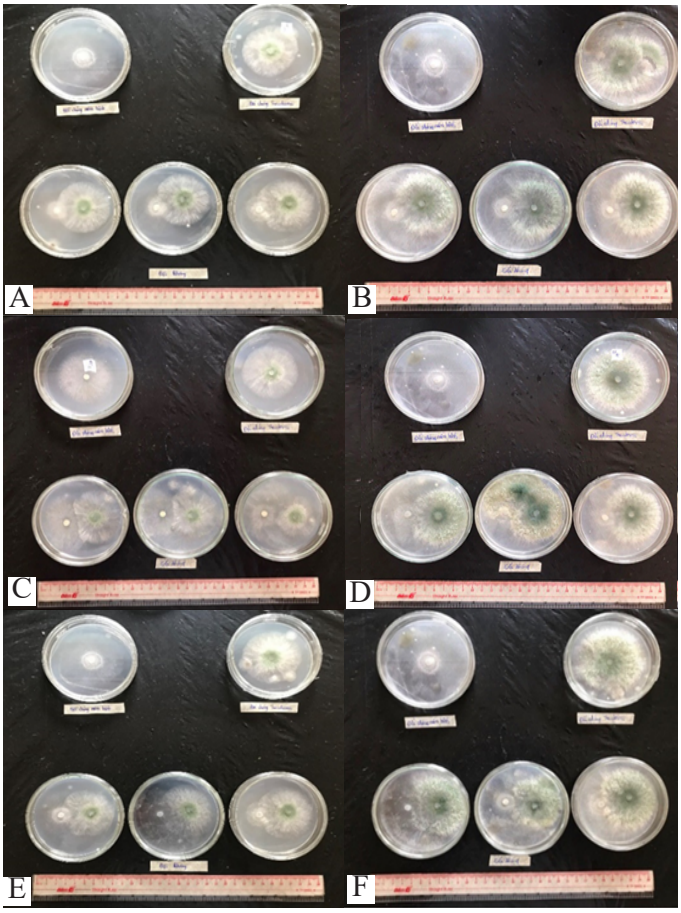

Figure 4. Antagonistic ability of *Trichoderma* spp. A, B. T-SP03; C, D. T-SP26; E, F. T-SP41 with pathogenic fungus strain KMS-02 on PDA medium at 48 and 72 HAI, respectively.

uniformly (Table 1).

According to Tam et al. (2021), *N. intermedia* DX2C forms a broad colony with fine, cotton-like hyphae, lacks spores and exudates, and has a fluffy structure with hyphae that are initially white before turning brick orange. The hyphae are relatively long, wide in cross-section, with spikes and a rough texture, distributed similarly to tangled fibers. *N. intermedia* is found in tropical and subtropical regions; for instance, *N. intermedia* has been identified in nonburned substrates with a saffron-yellow appearance, while *N. crassa* and *N. sitophila* are found in burned substrates with a salmon-orange coloration (Turner, 1987). In Vietnam, *Neurospora* spp. have been identified on pomelo peels (Tam et al., 2021), a fruit tree related to sour oranges, indicating potential risks of *Neurospora* spp. affecting citrus plants.

However, citrus scab is commonly caused by *Elsinoë* spp. Gopal et al. (2014) reported that *Elsinoë* colonies in culture media grow rapidly, ranging in color from light yellow to tan or black, with raised, short, erect hyphal tufts. Most *E. fawcettii* strains produce red pigments after 10–15 days of incubation under light conditions. *Elsinoë* is a genus that causes scab and anthracnose on crops. *E. fawcettii* Bitanc. & Jenkins and *E. australis* Bitanc. & Jenkins cause scab on *Citrus* spp. (Shanmugam et al., 2020; Elliott et al., 2023). *E. fawcettii* is more common than *E. australis* and is more economically significant due to its impact on high-value citrus species. Although the damage is primarily aesthetic, it can result in severe economic losses (Liu et al., 2024).

Moreover, strains KMS-01, KMS-02, and KMS-04 exhibited higher growth rates than KMS-03, with respective growth diameters of 5.90 cm, 5.80 cm, and 5.80 cm, compared to 4.75 cm at 72 HAI. These strain were used to test resistance against 50 *Trichoderma* spp. strains (Table 2).

At 72 HAI, *T. asperellum* strains T-SP03, T-SP26, and T-SP41 demonstrated strong antagonism against all three pathogenic strains (*N. intermedia* KMS-01, KMS-02, and *N. crassa* KMS-04), with antagonistic

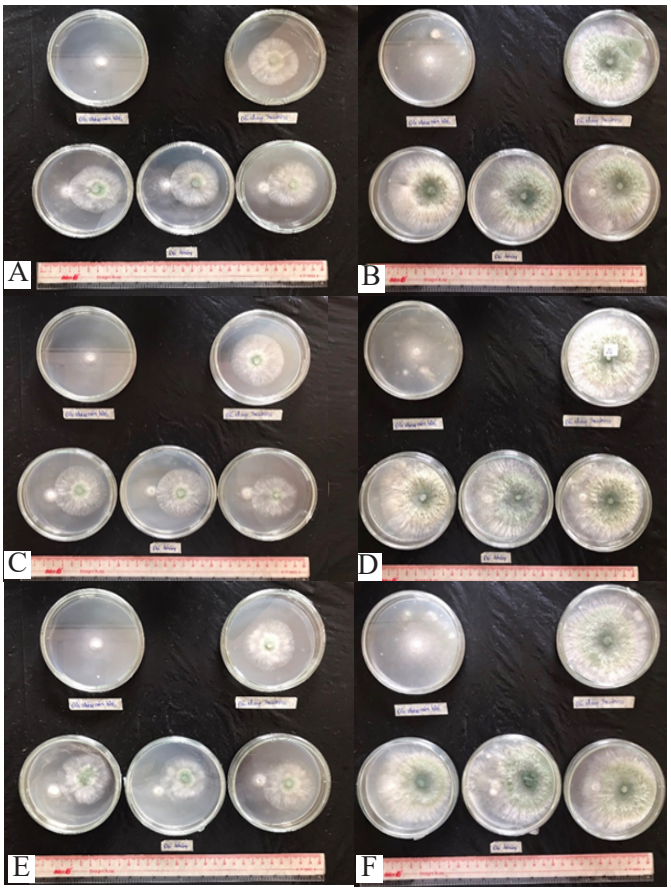

Figure 5. Antagonistic ability of *Trichoderma* spp. A, B. T-SP03; C, D. T-SP26; E, F. T-SP41 with pathogenic fungus strain KMS-04 on PDA medium at 48 and 72 HAI, respectively.

efficiencies ranging from 86.1% to 87.7% (Tables 3, 4, and 5). This is the first study to use *Trichoderma* spp. against *Neurospora* spp. While the antagonistic mechanism of *Trichoderma* spp. against *Neurospora* spp. was not identified in this study, the antagonism follows the general pattern of fungal antagonism. Future studies should focus on elucidating the antagonistic mechanisms of *Trichoderma* spp. against *Neurospora* spp.

Although most research on citrus scab focuses on *Elsinoë* spp., the antagonism of *Trichoderma* spp. against *Elsinoë* spp. has not been extensively studied. However, the use of *T. harzianum* to manage citrus scab has reduced disease incidence by 17.8%. Despite the benefits of foliar application of *Trichoderma* spp. in reducing disease severity under field conditions, this approach remains technically challenging due to increased dosage requirements and low economic efficacy (Kumar et al., 2023).

Trichoderma spp. compete ecologically and nutritionally with other fungi by producing antibiotics and toxins. Certain species, such as *T. harzianum*, are capable of parasitizing other fungi, making them promising biocontrol agents due to their ability to invade and degrade the structural defenses of plant pathogens (Lyubenova et al., 2023). Several mechanisms enable *Trichoderma* spp. to antagonize pathogens, including the production of volatile compounds and enzymes such as β-1,4-N-acetylglucosaminidase and endochitinase, which contribute to the breakdown of pathogenic fungal cell structure (Sharma et al., 2024). Chitinase and β-1,3-glucanase play crucial roles in *Trichoderma* parasitism by degrading cell walls and opposing pathogenic fungi (Silva et al., 2019; Ye et al., 2023).

According to De la Cruz-Quiroz et al. (2018), chitinases from *T. asperellum* T2-31 and exoglucanase from *T. harzianum* and *T. asperellum* have reduced the growth of *F. oxysporum, F. fujikuroi, F. tricinctum,* and *F. cantenulatum*, which cause Panama wilt in bananas, by 65.0%–74.0% and spore germination by 30.0%–75.0% (Win et al., 2021). Additionally, endochitinase, β-1,3-

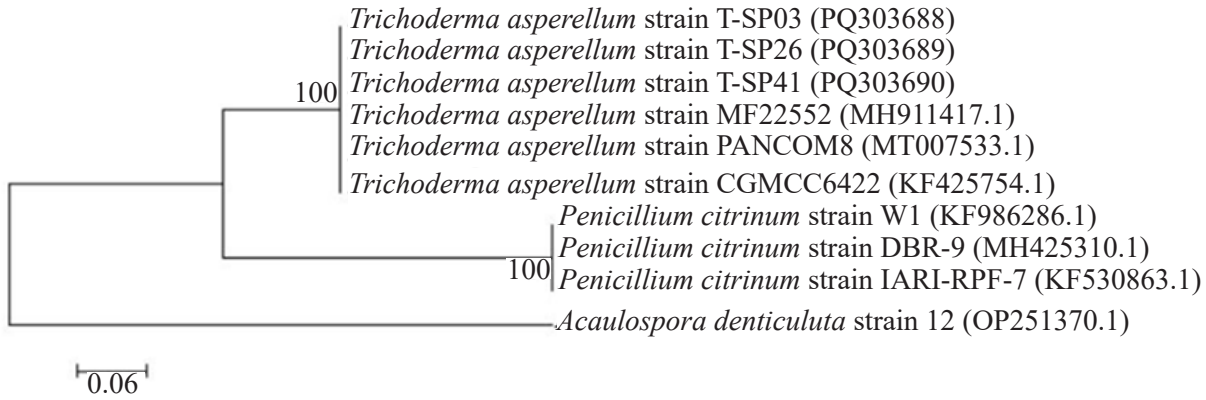

Figure 6. Phylogenetic tree of *Trichoderma* spp.

Table 4. Antagonistic efficiency of *Trichoderma* spp. against the pathogenic KMS-02 at 48 and 72 HAI

No.	Strain	Antagonistic efficiency (%)		Antagonistic diameter of strain KMS-02 (cm)	
		48 HAI	72 HAI	48 HAI	72 HAI
1	T-SP01	23.00 b	56.10 c-f	2.47 a ± 0.06	1.90 d-g ± 0.10
2	T-SP02	22.90 b	50.60 g-i	2.47 a ± 0.15	2.17 ab ± 0.05
3	T-SP03	52.10 a	86.20 a	1.53 b ± 0.12	0.60 i ± 0.10
4	T-SP04	22.90 b	53.90 d-h	2.47 a ± 0.06	2.00 b-f ± 0.10
5	T-SP05	22.50 b	53.20 e-h	2.47 a ± 0.06	2.03 b-e ± 0.15
6	T-SP06	20.40 b	52.40 e-h	2.47 a ± 0.06	2.07 a-e ± 0.15
7	T-SP07	19.80 b	51.70 f-i	2.53 a ± 0.06	2.10 a-d ± 0.12
8	T-SP08	22.90 b	54.00 d-h	2.57 a ± 0.06	2.00 b-f ± 0.10
9	T-SP09	19.40 b	53.30 e-h	2.47 a ± 0.06	2.00 b-e ± 0.06
10	T-SP10	22.10 b	58.60 b-d	2.57 a ± 0.06	2.03 fgh ± 0.10
11	T-SP11	20.60 b	54.90 c-g	2.47 a ± 0.06	1.97 b-g ± 0.06
12	T-SP12	21.00 b	61.10 b	2.50 a ± 0.10	1.70 h ± 0.10
13	T-SP13	23.80 b	52.90 e-h	2.50 a ± 0.10	2.00 b-e ± 0.06
14	T-SP14	20.00 b	57.10 b-e	2.40 a ± 0.10	1.87 e-h ± 0.05
15	T-SP15	22.90 b	52.10 e-i	2.53 a ± 0.06	2.07 a-e ± 0.06
16	T-SP16	23.50 b	53.20 e-h	2.47 a ± 0.06	2.03 b-e ± 0.06
17	T-SP17	20.00 b	53.20 e-h	2.43 a ± 0.12	2.03 b-e ± 0.15
18	T-SP18	23.50 b	51.70 f-i	2.53 a ± 0.06	2.10 a-d ± 0.10
19	T-SP19	21.30 b	54.80 c-h	2.43 a ± 0.12	1.97 b-g ± 0.15
20	T-SP20	21.50 b	54.4 d-h	2.47 a ± 0.06	1.97 b-g ± 0.15
21	T-SP21	18.10 b	49.80 f-i	2.50 a ± 0.10	2.17 ab ± 0.06
22	T-SP22	23.90 b	51.40 f-i	2.57 a ± 0.06	2.10 a-d ± 0.10
23	T-SP23	22.90 b	54.40 d-h	2.43 a ± 0.12	1.97 b-g ± 0.15
24	T-SP24	19.00 b	52.70 e-h	2.47 a ± 0.06	2.03 b-e ± 0.06
25	T-SP25	21.40 b	55.80 c-f	2.57 a ± 0.06	1.90 d-g ± 0.10
26	T-SP26	50.60 a	87.70 a	1.57 b ± 0.06	0.53 i ± 0.06
27	T-SP27	22.10 b	51.30 f-i	2.50 a ± 0.10	2.10 a-d ± 0.10
28	T-SP28	20.40 b	59.50 bc	2.47 a ± 0.06	1.77 ghi ± 0.05
29	T-SP29	21.10 b	47.50 i	2.53 a ± 0.06	2.17 a ± 0.06
30	T-SP30	22.10 b	53.70 e-h	2.50 a ± 0.10	2.00 b-f ± 0.10

Table 4. Continued.....

No.	Strain	Antagonistic efficiency (%)		Antagonistic diameter of strain KMS-02 (cm)	
		48 HAI	72 HAI	48 HAI	72 HAI
31	T-SP31	22.40 b	52.90 e-h	2.47 a ± 0.06	2.03 b-e ± 0.06
32	T-SP32	22.50 b	54.80 c-h	2.47 a ± 0.15	1.97 b-g ± 0.06
33	T-SP33	20.00 b	55.80 c-f	2.47 a ± 0.12	1.90 d-g ± 0.10
34	T-SP34	22.10 b	53.50 e-h	2.53 a ± 0.06	2.00 b-f ± 0.17
35	T-SP35	20.60 b	52.70 e-h	2.47 a ± 0.06	2.03 b-e ± 0.06
36	T-SP36	22.10 b	52.90 e-h	2.50 a ± 0.10	2.03 b-e ± 0.15
37	T-SP37	22.50 b	56.00 c-f	2.47 a ± 0.12	1.90 d-g ± 0.17
38	T-SP38	19.40 b	55.20 c-g	2.47 a ± 0.06	1.93 c-g ± 0.15
39	T-SP39	21.90 b	49.80 hi	2.57 a ± 0.06	2.17 ab ± 0.12
40	T-SP40	21.90 b	50.60 ghi	2.50 a ± 0.10	2.13 abc ± 0.06
41	T-SP41	55.20 a	86.10 a	1.43 b ± 0.06	0.60 i ± 0.10
42	T-SP42	19.00 b	52.10 e-i	2.50 a ± 0.10	2.07 a-e ± 0.06
43	T-SP43	22.10 b	52.10 e-i	2.57 a ± 0.06	2.07 a-e ± 0.15
44	T-SP44	20.80 b	52.10 f-i	2.47 a ± 0.06	2.07 a-e ± 0.12
45	T-SP45	24.00 b	49.80 hi	2.53 a ± 0.06	2.17 ab ± 0.15
46	T-SP46	22.9 0b	52.70 e-h	2.43 a ± 0.06	2.03 b-e ± 0.06
47	T-SP47	20.00 b	51.20 f-i	2.47 a ± 0.06	2.10 a-d ± 0.10
48	T-SP48	22.10 b	50.40 ghi	2.53 a ± 0.06	2.13 abc ± 0.06
49	T-SP49	22.10 b	50.40 ghi	2.47 a ± 0.06	2.13 abc ± 0.12
50	T-SP50	23.00 b	51.20 f-i	2.47 a ± 0.12	2.10 a-d ± 0.10
	F	*	*	*	*
	CV (%)	16.2	30.2	0.52	0.75

Within the same column, entries with the same letter following them are not significantly different according to Duncan's test, while (*) denotes significant differences at the 5% level. HAI: hours after inoculation.

Table 5. Antagonistic efficiency of *Trichoderma* spp. against the pathogenic KMS-04 at 48 and 72 HAI

No.	Strain	Antagonistic efficiency (%)		Antagonistic diameter of strain KMS-04 (cm)	
		48 HAI	72 HAI	48 HAI	72 HAI
1	T-SP01	21.90 bc	53.30 cd	2.50 bc ± 0.10	2.03 bc ± 0.12
2	T-SP02	22.50 bc	50.60 cde	2.47 bc ± 0.15	2.17 ab ± 0.06
3	T-SP03	56.30 a	87.60 a	1.40 c ± 0.10	0.53 e ± 0.06
4	T-SP04	22.50 bc	52.40 cde	2.47 bc ± 0.06	2.07 abc ± 0.12
5	T-SP05	21.10 bc	52.40 cde	2.50 bc ± 0.10	2.07 abc ± 0.23
6	T-SP06	23.50 bc	51.70 cde	2.43 bc ± 0.12	2.10 abc ± 0.10
7	T-SP07	21.50 bc	51.30 cde	2.50 bc ± 0.10	2.10 abc ± 0.10
8	T-SP08	19.10 c	52.90 cde	2.53 ab ± 0.12	2.03 bc ± 0.21
9	T-SP09	26.00 b	59.50 b	2.37 b ± 0.06	1.77 d ± 0.06
10	T-SP10	21.90 bc	51.20 cde	2.50 bc ± 0.10	2.10 abc ± 0.10
11	T-SP11	19.00 c	51.20 cde	2.57 a ± 0.06	2.10 abc ± 0.10
12	T-SP12	22.50 bc	52.30 cde	2.47 bc ± 0.15	2.07 abc ± 0.06
13	T-SP13	22.10 bc	53.50 cd	2.47 bc ± 0.06	2.00 bc ± 0.10

Table 5. Continued.

No.	Strain	Antagonistic efficiency (%)		Antagonistic diameter of strain KMS-04 (cm)	
		48 HAI	72 HAI	48 HAI	72 HAI
14	T-SP14	20.40 bc	51.40 cde	2.53 ab ± 0.06	2.10 abc ± 0.10
15	T-SP15	21.00 bc	53.70 cd	2.50 bc ± 0.10	2.00 bc ± 0.10
16	T-SP16	23.60 bc	52.90 cde	2.43 bc ± 0.06	2.03 bc ± 0.06
17	T-SP17	21.10 bc	52.10 cde	2.50 bc ± 0.10	2.07 abc ± 0.12
18	T-SP18	21.10 bc	51.40 cde	2.50 bc ± 0.10	2.10 abc ± 0.10
19	T-SP19	21.90 bc	52.90 cde	2.50 bc ± 0.10	2.03 bc ± 0.06
20	T-SP20	22.10 bc	52.10 cde	2.47 ab ± 0.12	2.07 abc ± 0.12
21	T-SP21	23.20 bc	52.10 cde	2.43 bc ± 0.06	2.07 abc ± 0.12
22	T-SP22	25.00 bc	55.20 c	2.40 bc ± 0.10	1.93 c ± 0.12
23	T-SP23	21.90 bc	51.20 cde	2.50 bc ± 0.10	2.10 abc ± 0.10
24	T-SP24	21.00 bc	52.10 cde	2.50 bc ± 0.10	2.07 abc ± 0.12
25	T-SP25	24.60 a	52.50 cde	2.40 bc ± 0.10	2.07 abc ± 0.06
26	T-SP26	52.10 a	86.90 a	1.53 c ± 0.06	0.57 e ± 0.06
27	T-SP27	20.80 bc	50.60 cde	2.53 ab ± 0.06	2.13 abc ± 0.12
28	T-SP28	24.00 bc	51.90 cde	2.43 bc ± 0.06	2.07 abc ± 0.12
29	T-SP29	22.90 bc	51.20 cde	2.47 bc ± 0.06	2.10 abc ± 0.10
30	T-SP30	21.50 bc	48.30 e	2.50 bc ± 0.10	2.27 a ± 0.06
31	T-SP31	22.10 bc	52.70 cde	2.47 bc ± 0.06	2.07 abc ± 0.12
32	T-SP32	20.60 bc	51.90 cde	2.50 bc ± 0.10	2.10 abc ± 0.10
33	T-SP33	21.00 bc	50.60 cde	2.50 bc ± 0.10	2.13 abc ± 0.12
34	T-SP34	23.80 bc	53.70 cd	2.40 bc ± 0.10	2.00 bc ± 0.10
35	T-SP35	20.00 bc	50.60 cde	2.53 ab ± 0.06	2.13 abc ± 0.06
36	T-SP36	21.90 bc	53.20 cd	2.50 bc ± 0.10	2.03 bc ± 0.06
37	T-SP37	21.40 bc	51.30 cde	2.50 bc ± 0.00	2.10 abc ± 0.10
38	T-SP38	22.10 bc	52.10cde	2.47 bc ± 0.06	2.07 abc ± 0.12
39	T-SP39	20.40 bc	49.00 de	2.53 ab ± 0.06	2.20 ab ± 0.10
40	T-SP40	22.50 bc	51.90 cde	2.47 bc ± 0.12	2.07 abc ± 0.12
41	T-SP41	54.80 a	87.60 a	1.43 c ± 0.06	0.53 e ± 0.06
42	T-SP42	21.70 bc	51.40 cde	2.47 bc ± 0.15	2.10 abc ± 0.10
43	T-SP43	22.10 bc	53.70 cd	2.47 bc ± 0.12	2.00 bc ± 0.10
44	T-SP44	23.50 bc	52.90 cde	2.43 bc ± 0.06	2.03 bc ± 0.06
45	T-SP45	19.40 c	50.60 cde	2.57 a ± 0.06	2.13 abc ± 0.06
46	T-SP46	21.00 bc	52.40 cde	2.50 bc ± 0.10	2.07 abc ± 0.12
47	T-SP47	23.50 bc	50.60 cde	2.43 bc ± 0.12	2.17 ab ± 0.06
48	T-SP48	21.30 bc	51.40 cde	2.47 bc ± 0.06	2.10 abc ± 0.10
49	T-SP49	21.50 bc	49.80 de	2.50 bc ± 0.10	2.17 ab ± 0.06
50	T-SP50	23.50 bc	51.30 cde	2.43 bc ± 0.15	2.10 abc ± 0.10
	F	**	**	**	**
	CV (%)	6.47	3.25	0.60	0.72

Within the same column, entries with the same letter following them are not significantly different according to Duncan's test, while (**) denotes significant differences at the 1% level. HAI: hours after inoculation.

glucanase, and chitobiosidase are chitinolytic enzymes secreted by *T. harzianum* that are effective against crop pathogens. Chitobiosidase and endochitinase inhibit the spore production of pathogenic fungi and degrade fungal hyphae, while endochitinase and β-1,3-glucanase decompose fungal spores, thalli, and hyphal tips (Nygren et al., 2018; Lakhdari et al., 2023).

Esparza-Reynoso et al. (2021) reported that the volatile compound 6-pentyl-α-pyrone from *T. atroviride* enhances plant growth and regulates sugar transport in *Arabidopsis*, along with other volatiles produced by fungi. The antagonistic effect of *Trichoderma* spp. is primarily due to extracellular enzymes rather than similar metabolite activities observed in *Aspergillus* sp. and *Penicillium* sp. (Awad et al., 2018). Rajendiran et al. (2010) noted that non-volatile compounds from *Trichoderma* spp. inhibit *Aspergillus* growth by up to 64%.

T. hamatum K01 inhibited both colony growth and spore production of *C. gloeosporioides* C01, which causes anthracnose on citrus, by 70.6% and 79.1%, respectively, through the synthesis of the secondary metabolite pyrone (6-pentyl-2H-pyran-2-one) (Phal et al., 2023). Similarly, *T. harzianum* CT-566, CT-862, CT-634, CT-873, *T. afroharzianum* CT-55, and *T. aureoviridis* CT-936 have destroyed the hyphae of *Phytophthora citrophthora*, a root and shoot rot pathogen in citrus, through a coiling and degradation process of fungal cell walls (Barahoei et al., 2023).

Guzmán-Guzmán et al. (2023) reviewed the potential of *Trichoderma* spp. in inhibiting various fungal pathogens, including *Rhizoctonia, Alternaria, Curvularia,* and *Fusarium* spp. under laboratory conditions. Martanto et al. (2015) demonstrated that *Trichoderma* sp. could inhibit *E. batatas,* which causes scab in sweet potatoes, by 68.7%. *T. harzianum* has shown 17.8% efficacy in controlling scab disease in field experiments (Singh et al., 2000). Therefore, *T. asperellum* T-SP03, T-SP26, and T-SP41 exhibit strong antagonism against *N. intermedia* KMS-01, *N. intermedia* KMS-02, and *N. crassa* KMS-04.

CONCLUSION

Three strains of scab-causing fungi on King mandarin fruits were identified as the most virulent: KMS-01, KMS-02, and KMS-04. These were classified as *Neurospora intermedia* KMS-01, *N. intermedia* KMS-02, and *N. crassa* KMS-04, respectively. Additionally, three *Trichoderma* strains—namely T-SP03, T-SP26, and T-SP41—were selected for their strong antagonistic activity against these pathogens, with antagonistic efficiencies ranging from 86.9% to 87.6%. These strains were identified as *Trichoderma asperellum*. Further research is needed to elucidate the mechanisms by which *T. asperellum* T-SP03, T-SP26, and T-SP41 antagonize *N. intermedia* KMS-01, *N. intermedia* KMS-02, and *N. crassa* KMS-04.

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AUTHORS' CONTRIBUTIONS

NQK and LNTX reviewed and developed the methodology and planned the experiments. LYN and VMT isolated and tested the pathogenicity of *Neurospora* spp. strains on King mandarin fruits. TCN, LTQ, and NDT described the pathogen's characteristics and evaluated the growth and development of *Neurospora* spp. strains on PDA medium. PCH and NGH conducted antagonistic experiments of *Trichoderma* spp. strains against *Neurospora* spp. strains. LTMT performed molecular identification of the pathogenic *Neurospora* spp. strains and *Trichoderma* spp. strains. DTX was responsible for data processing, research analysis, data interpretation, and manuscript preparation. All authors provided feedback on the research process, data analysis, interpretation, and manuscript formatting. All authors have read and approved the final manuscript.

COMPETING INTEREST

The author declare no competing interests in the research process or the creation of this manuscript.

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