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Trichoderma asperellum (NST-009): A potential native antagonistic fungus to control Cercospora leaf spot and promote the growth of 'Green Oak' lettuce (*Lactuca sativa* L.) cultivated in the commercial NFT hydroponic system

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Abstract: Leaf spot caused by *Cercospora lactucae-sativae* is one of the most damaging diseases of 'Green Oak' lettuce in Thailand. This study was conducted to estimate the effectiveness of *Trichoderma asperellum* NST-009, a native strain in Thailand, to manage the leaf spot disease and enhance the growth of 'Green Oak' lettuce in a nutrient film technique (NFT) hydroponic system. *In vitro* tests showed that *T. asperellum* NST-009 significantly inhibited the mycelial growth of *C. lactucae-sativae* by 72.50%, and its antifungal metabolite from the culture filtrate of *T. asperellum* NST-009 inhibited the mycelial growth of *C. lactucae-sativae* by 93.26%. In the hydroponics experiment, *T. asperellum* NST-009 reduced the disease severity index by 67.51% compared to the inoculated control and significantly stimulated the growth of the 'Green Oak' lettuce in terms of the plant height (8.62%), canopy width (16.67%), leaf number (18.39%), shoot fresh weight (25.71%), root fresh weight (39.26%), and total P in the leaves (31.45%) compared to the control. In addition, *T. asperellum* NST-009 was found to survive in both the lettuce leaves and roots at 100.00%.

Keywords: biological control; beneficial microoganism; plant growth promoting fungi; PGPF; soilless culture

A hydroponic system is one of the modern agricultural technologies that has been used for planting in numerous countries such as the United States, Israel, Canada, China, the Czech Republic, Japan, Italy, India, Iran as well as Thailand (Kabiri et al. 2014; Chen et al. 2016; Chairin et al. 2017; Thongkamngam & Jaenaksorn 2017). In Thailand, this technology has officially been recognised since 1988, and many farmers have used hydroponic systems for planting on a commercial scale. Three techniques of hydroponics have been established in several areas for plant products, including the nutrient film technique (NFT), deep flow technique (DFT), and dynamic root floating technique (DRFT). Nonetheless, NFT is a marketable system that is effective for large-scale commercial vegetable production (Koohakan et al. 2008).

'Green Oak' lettuce (*Lactuca sativa* L.) is one of the most common leafy vegetables grown in the NFT hydroponic system. This vegetable is rich in nutrients, including fibre, folate, carotenoids, phenolic and antioxidant compounds, minerals,

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and vitamins A, C, and K (Camejo et al. 2020). However, the leaf spot disease caused by *Cercospora lactucae-sativae* is one of the most significant problems in the production of 'Green Oak' leaf lettuce, which affects both the quantity and quality of the product (Srimai & Akarapisarn 2014; Khamun et al. 2017). The disease occurs on the lower older leaves and spreads to the younger ones. Lesions are light-dark brown, enclosed by a yellow halo, and further characterised by a light ash to white-coloured dot of about a pinhole in size (1 mm wide) in the lesion's centre (Thomas & Saravanakumar 2019). In general, farmers regularly manage *Cercospora* plant diseases using fungicides such as mancozeb, carbendazim, tetraconazole, and pyraclostrobin (Khan & Smith 2005; Khunti et al. 2005). Nevertheless, these chemicals may remain in the product that affects consumers, and the plant pathogen may be resistant to these chemicals (Suwan et al. 2012; Trkulja et al. 2015). The use of beneficial microorganisms in the *Trichoderma* group is an alternative solution to this problem.

Trichoderma strains are known as biological control agents against various genera of plant pathogenic fungi, such as *Alternaria*, *Botrytis*, *Bipolaris*, *Cercospora*, *Colletotrichum*, *Corynespora*, *Curvularia*, *Phomopsis*, *Pythium*, *Phytophthora*, *Rigidoporus*, *Ganoderma*, *Rhizoctonia*, *Fusarium*, *Sclerotium*, *Sclerotinia*, *Rosellinia* (Brožová 2004; Izzati & Abdullah 2008; Abo-Elyousr et al. 2014; Nawrocka et al. 2018; Redda et al. 2018; Baiyee et al. 2019; Wonglom et al. 2019). The *Trichoderma* strains' mechanisms for controlling plant diseases are nutrient and space competition, antibiosis, parasitism, and the induction of resistance in host plants (Waghunde et al. 2016). Additionally, *Trichoderma* strains can also improve plant productivity through phytohormone production, phosphate synthesis, increased carbohydrate metabolism and photosynthesis, an enhanced nutrient uptake, and control of minor pathogens (Stewart & Hill 2014).

Trichoderma asperellum NST-009 (formerly identified as *T. harzianum* FR-NST-009) is a native fungus isolated from the forest soil in Nakhon Si Thammarat, southern Thailand. In previous studies, this strain was shown to be effective in controlling *Phytophthora* leaf fall in a rubber tree and solubilising the insoluble phosphate (Promwee et al. 2014; Promwee et al. 2017). Currently, *T. asperellum* NST-009 has been suggested to farmers for controlling plant diseases and growth promotion in several plants such as rice, durian, oil palm, and vegetables through the Agricultural Microbial Production and Service Center, Walailak University, Nakhon Si Thammarat, Thailand, which has also been distributed to farmers in 77 provinces of Thailand.

Although Baiyee et al. (2019) used *T. asperellum* T1 to control the leaf spot of lettuce in hydroponic systems, there are no reports on the use of the native *T. asperellum* NST-009 in controlling plant diseases in commercial hydroponic systems. In consequence, the objectives of this study were to test the efficacy of *T. asperellum* NST-009 and its antifungal metabolite to inhibit the mycelial growth of *C. lactucae-sativae*, and to determine the effectiveness of *T. asperellum* NST-009 to control the leaf spot disease and stimulate the growth of 'Green Oak' leaf lettuce in a commercially NFT-hydroponic system.

MATERIAL AND METHODS

Trichoderma strains and the *Cercospora* pathogen

Three strains of *T. asperellum* (formally identified as *T. harzianum*) were used in this study, including two native Nakhon Si Thammarat strains (*T. asperellum* NST-009, *T. asperellum* NST-028) and the commercial Thai strain (*T. asperellum* CB-Pin-01) (Promwee et al. 2017; Charoenrak et al. 2019; Unartngam et al. 2020). The *Cercospora* pathogen (*C. lactucae-sativae* Tha-02) identified both morphologically and molecularly as described by To-Anun et al. (2011) and Nguanhom et al. (2015) was obtained from the culture collection of microorganisms, Agricultural Microbial Production and Service Center, Walailak University, Thailand. All the *Trichoderma* strains and *Cercospora* pathogen were sub-cultured on potato dextrose agar (PDA) at room temperature (28 ± 2 °C) for five days and 14 days, respectively, before use in the experiments. The symptoms of 'Green Oak' lettuce leaf spot caused by *C. lactucae-sativae* were confirmed by Koch's postulates. The 35-day-old 'Green Oak' lettuce was sprayed with a *C. lactucae-sativae* spore suspension at 10 mL/plant (1×10^6 spores/mL) on the leaves compared to the control (sprayed with distilled water). The morphological and reproductive characteristics of *C. lactucae-sativae* were observed under a light compound microscope and a scanning electron microscope.

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Dual culture assessment

All the strains of *T. asperellum* were determined for their ability to inhibit the mycelial growth of *C. lactucae-sativae* using a dual culture technique. Five-day-old *T. asperellum* on the PDA was cut with a sterile cork borer (3 mm in diameter), and the agar plug of *T. asperellum* was placed on one side of the PDA Petri dish (9-cm diameter). Then, a 3-mm-diameter plug of the 14-day-old *C. lactucae-sativae* was placed on the opposite side of the PDA Petri dish. The plates were incubated at 28 ± 2 °C for 14 days. The experiment was conducted and repeated twice using a completely randomised design (CRD) with four replicates. The radial growth of *C. lactucae-sativae* was measured, and the percent inhibition of mycelial growth was calculated using the following Formula (1):

$$Inhibition\ (\%) = \frac{RC - RT}{RC} \times 100 \quad (1)$$

where: *RC* – represents the radial growth of *C. lactucae-sativae* in the untreated control; *RT* – represents the radial growth of *C. lactucae-sativae* in the treatment (Baiyee et al. 2019).

Scanning electron microscopy assay

The high-efficiency strain of *T. asperellum*, which was able to inhibit the mycelia of *C. lactucae-sativae* in the dual culture test, was studied for its ability to parasitise the mycelia of *C. lactucae-sativae* under a Field Emission Scanning Electron Microscope (FE-SEM) (Merlin Compact; Zeiss, Germany), energy dispersive X-ray spectrometer (Aztec; Oxford Instruments, UK), electron backscatter diffraction (Nordlys Max; Oxford Instruments, UK). *Trichoderma asperellum* and *C. lactucae-sativae* were cultured using a dual culture technique on a PDA. After a colony of *C. lactucae-sativae* was attacked by the mycelia of *T. asperellum*, the samples of the activity zone were cut into small pieces (0.5 × 0.5 cm), fixed in 2.5% glutaraldehyde at 4 °C for 24 h, and rinsed with distilled water before dehydration in a 30–100% alcohol series. The samples were dried in a critical point dryer machine K850 (Quorum, UK) followed by gold coating using a Sputter Coater 108 (Cressington, UK). The coated samples were then immediately examined by FE-SEM. This item was studied at the Center for Scientific and Technological Equipment, Walailak University, Thailand.

Antifungal metabolite determination

The antifungal metabolites of *T. asperellum* were tested to inhibit the mycelial growth of *C. lactucae-sativae*. Twenty-five mycelial agar plugs (7 mm in diameter) obtained from the margins of the growing colonies of *T. asperellum* grown on the PDA were inoculated into a 3 L Erlenmeyer flask containing 1 L of 1/5 strength potato dextrose broth. The flask was then incubated at 28 ± 2 °C for 28 days. After that, the spores and mycelia of *T. asperellum* were removed from the broth culture by filtration through 0.45 µm Whatman No. 1 filter paper. The culture filtrates were extracted with ethyl acetate ($CH_3–COO–CH_2–CH_3$) before evaporation at 40 °C using a rotary vacuum evaporator (EYELA, Japan). The antifungal metabolites (crude extracted substances) were kept in sterile glass bottles and tested to inhibit the mycelial growth of *C. lactucae-sativae* on the PDA using the agar dilution method. Each antifungal metabolite was dissolved in 2% dimethyl sulfoxide (DMSO), mixed with PDA to a final concentration of 250 and 500 mg/L, and poured into the Petri dish. A mycelial agar disc of *C. lactucae-sativae* was then placed in the middle of the solidified agar plate and incubated at 28 ± 2 °C for 14 days. The experiment was conducted twice using a CRD with four replicates. The colony diameter of *C. lactucae-sativae* was measured, and the inhibition percentage of mycelial growth was calculated using the following Formula (2):

$$Inhibition\ (\%) = \frac{DC - DT}{DC} \times 100 \quad (2)$$

where: *DC* – the mean of the mycelial diameter of *C. lactucae-sativae* in the control treatment (*C. lactucae-sativae* grown on the media mixed with 2% DMSO); *DT* – the mean mycelial diameter of *C. lactucae-sativae* in the tested treatment.

Hydroponic system test

The efficacy of *T. asperellum* to control the Cercospora leaf spot and growth promotion of 'Green Oak' lettuce was conducted in a commercial NFT hydroponic system (1.8 m wide × 6.0 m long). The 14-day-old 'Green Oak' seedling (Vizir®, Enza Zaden, the Netherlands) planted in a sponge (2.5 cm × 2.5 cm × 2.5 cm) was transplanted into the NFT-hydroponic system containing the nutrient solution that was prepared according to the method described by Charoenrak et al. (2019). The EC and

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pH of the nutrient solution were adjusted to 1.8–2.0 mS/cm and 5.5–6.5 mS/cm, respectively.

The spore suspension of the *Trichoderma* strain was prepared by filtering the cultures to separate the conidia, adjusted to around 1×10^8 spores/mL, mixed in the nutrient solution (1 L/200 L nutrient solution), and used to spray the 'Green Oak' lettuce (10 mL/plant) at seven, 14, and 21 days after transplanting.

The inoculum of *C. lactucae-sativae* was prepared according to the method of Kham-um et al. (2017). The *C. lactucae-sativae* spore suspension was adjusted to 1×10^6 spores/mL using a haemocytometer and sprayed on the 'Green Oak' lettuce at 14 days after transplanting (28-day-old 'Green Oak' lettuce).

The experiment was designed in a CRD with six treatments, four replications/treatment, 30 plants/replication. The treatments included the following: treatment 1: *T. asperellum* NST-009 + *C. lactucae-sativae*; treatment 2: *T. asperellum* NST-028 + *C. lactucae-sativae*; treatment 3: *T. asperellum* CB-Pin-01 + *C. lactucae-sativae*; treatment 4: chemical fungicide (mancozeb) + *C. lactucae-sativae*; treatment 5: Control 1 (only *C. lactucae-sativae*); and treatment 6: Control 2 (without *C. lactucae-sativae*).

Disease severity. Cercospora leaf spot was observed after inoculation with *C. lactucae-sativae* for 14 days (42-day-old 'Green Oak' lettuce) at six severity levels (0–5) as follows: 0 = no spots; 1 = 1–20% spots; 2 = 21–40% spots; 3 = 41–60% spots; 4 = 61–80% spots; and 5 = 81–100% spots. The disease severity index (*DSI*) was then determined using the Formula (3):

$$DSI\ (\%) = \frac{\Sigma\ (scale \times amount\ of\ plants)}{maximum\ level \times total\ plants} \times 100 \qquad (3)$$

Root and leaf colonisation. The root and leaf colonisation of the *Trichoderma* strains were observed after harvesting the 42-day-old 'Green Oak' lettuce. The plant roots and leaves were cut into a size of 1 cm in length and 0.5 cm × 0.5 cm, respectively, soaked in a 0.53% solution of sodium hypochlorite for 5 min and washed with sterile water three times. The pieces of 'Green Oak' lettuce root or leaf were dried with sterile paper and put on Martin's medium. The experiment was designed in a CRD with four replications per treatment. The colonisation percentage of the 'Green Oak' lettuce root and leaf was estimated after incubation at room temperature for four days using the following Formula (4):

$$Root\ \text{or}\ leaf\ colonisation\ (\%) = \frac{PC}{PT} \times 100 \qquad (4)$$

where: *PC* – the number of root or leaf pieces colonised by the *Trichoderma* strain; *PT* – the total root or leaf pieces.

Trichoderma population in nutrient solution. The population of the *Trichoderma* strain was evaluated using the dilution plate technique after application of *T. asperellum* for 21 days. Ten millilitres of nutrient solution in each treatment was added to a 250-mL flask containing 90 mL of sterile water and was mixed using a shaker at 120 rpm for 30 minutes. The nutrient suspension was then diluted with sterile water at 10^{-1} to 10^{-5}-fold, and 0.1 mL of the diluted nutrient solution was dropped onto the surface of Martin's medium with a micropipette and spread over the nutrient solution with a sterile glass rod. The experiment was designed in a CRD with four replications per treatment. The number of *Trichoderma* strains was counted after incubation at room temperature for four days.

Plant growth parameter. In the 42-day-old 'Green Oak' lettuce, the plant growth parameters, including the plant height, canopy width, leaf number, shoot fresh weight, and root fresh weight were recorded. Moreover, the total phosphorus (P) in the leaves was determined using the vanadomolybdate method (AOAC 2000).

Statistical analysis

All the data were subjected to an analysis of variance (ANOVA), followed by a comparison using Duncan's multiple range test. The significance level was set at $P \leq 0.05$.

RESULTS

Cercospora pathogen

The symptoms of 'Green Oak' lettuce leaf spot caused by *C. lactucae-sativae* was confirmed by Koch's postulates. The results showed that this pathogen caused leaf spot on 'Green Oak' lettuce leaves seven days after inoculation when compared with the control (Figure 1). The morphological and reproductive characteristics of *C. lactucae-sativae* were observed using a scanning electron microscope. The character-

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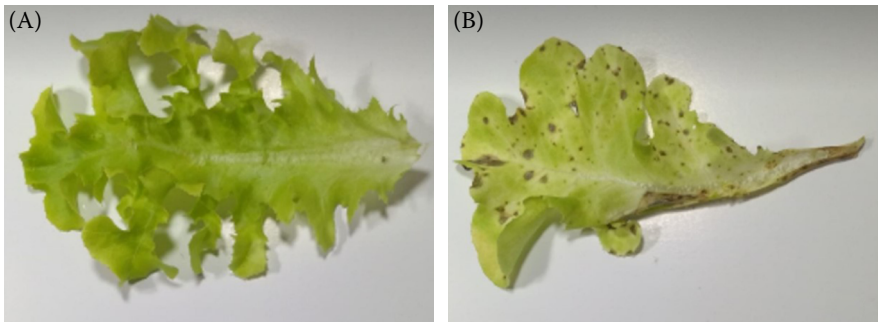

Figure 1. Comparison between a normal leaf (A) and an abnormal leaf (B) of 'Green Oak' lettuce destroyed by *C. lactucae sativae*

istics of the conidiophores and conidia of *C. lactucae-sativae* are shown in Figure 2 and Figure 3.

Dual culture test

This study aimed to find the indigenous *T. asperellum* strains that have antagonistic properties to control *C. lactucae-sativae*. In a dual culture experiment, all strains of *T. asperellum* effectively inhibited the mycelia of *C. lactucae-sativae* on the PDA (68.36–72.50%), especially *T. asperellum* NST-009, which gave the highest percentage of mycelia growth inhibition (Figure 4, Table 1).

Mycoparasitism assessment

The ability of *T. asperellum* to induce mycoparasitism on the mycelia of *C. lactucae-sativae* under FE-SEM showed that the selected strain of *T. asperellum* NST-009 was able to parasitise the my-

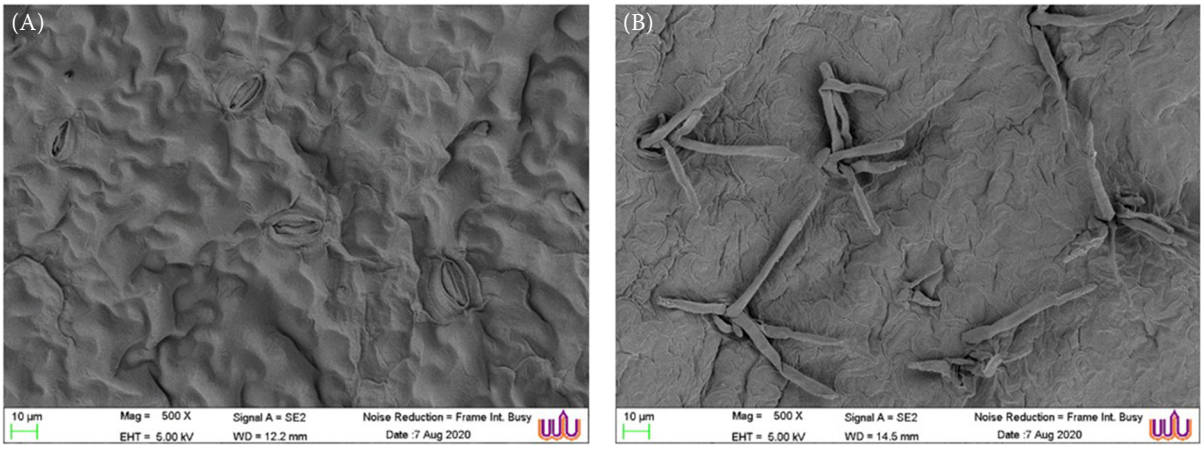

Figure 2. Comparison between a normal stomata of 'Green Oak' lettuce leaf (A), an abnormal stomata of 'Green Oak' lettuce leaf destroyed by *C. lactucae-sativae* (B)

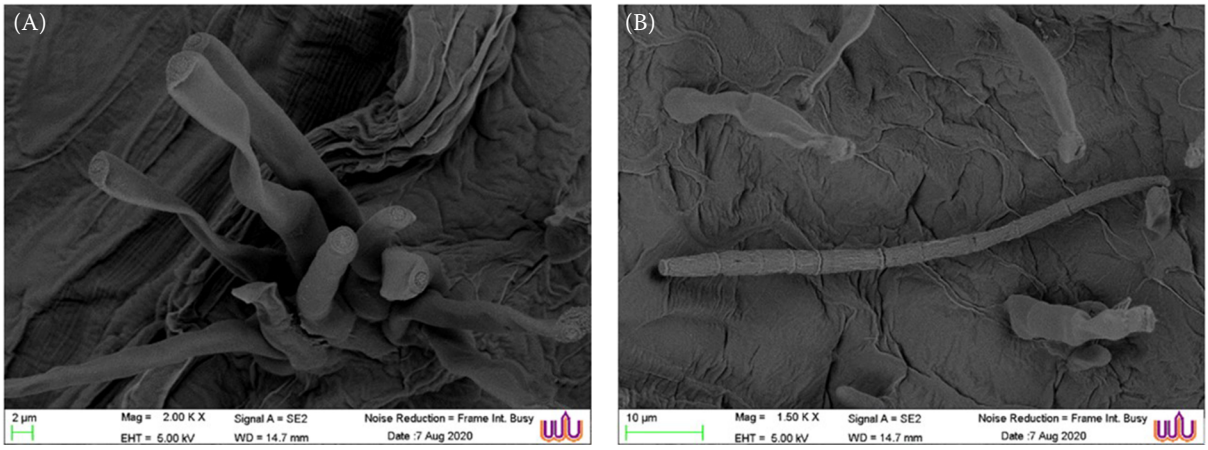

Figure 3. Characteristics of the conidiophore (A) and conidia (B) of *C. lactucae-sativae*

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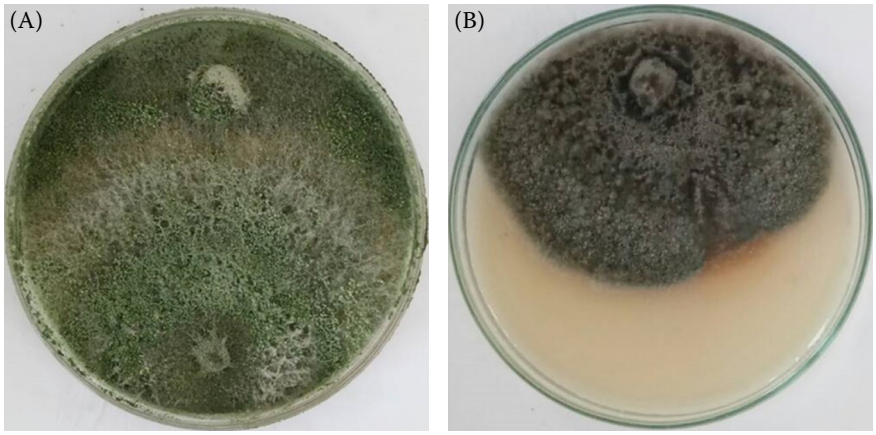

Figure 4. Efficiency of *T. asperellum* NST-009 to inhibit the mycelial growth of *C. lactucae-sativae* on potato dextrose agar after incubation for 14 days
(A) Dual culture, (B) only pathogen

Table 1. Effect of *Trichoderma asperellum* on the mycelial growth inhibition of *Cercospora lactucae-sativae* after incubation at room temperature for 14 days

Treatments	*C. lactucae-sativae* inhibition (%)
T. asperellum NST-009	72.50[a]
T. asperellum NST-028	68.36[b]
T. asperellum CB-Pin-01	70.71[a]
C. lactucae-sativae	–

[a,b]Mean values within the same columns followed by the same latter are not significantly different according to Duncan's multiple range test ($P < 0.05$)

celia of *C. lactucae-sativae* through colonisation of *Cercospora* hypha, drilling holes of *Cercospora* hypha, and the conidia reproduction on mycelia of *C. lactucae-sativae* (Figure 5).

Inhibitory activity of crude extract

The antifungal metabolites of *T. asperellum* were tested to inhibit the mycelial growth of *C. lactucae-sativae* on a PDA for 14 days. The results showed that the antifungal metabolites of all strains of *T. asperellum* at 500 µg/mL effectively reduced the mycelial growth of *C. lactucae-sativae* (76.72–93.26%), especially the antifungal metabolites of the *T. asperellum* strain NST-009, which gave the highest percentage of mycelial growth inhibition (Table 2).

NFT hydroponics experiment

Disease severity. The efficacy of *T. asperellum* in controlling the leaf spot disease in 'Green Oak' lettuce was tested under NFT hydroponic conditions. The results indicated that 21 days after 'Green Oak' lettuce plants were inoculated with the inoculum of *C. lactucae-sativae*, all strains of *T. asperellum* had high efficacy in controlling the leaf spot disease at a low disease severity index (18.05–30.94%), especially the *T. asperellum* strain NST-009, which presented the lowest disease severity index, whereas the Control 1 treatment inoc-

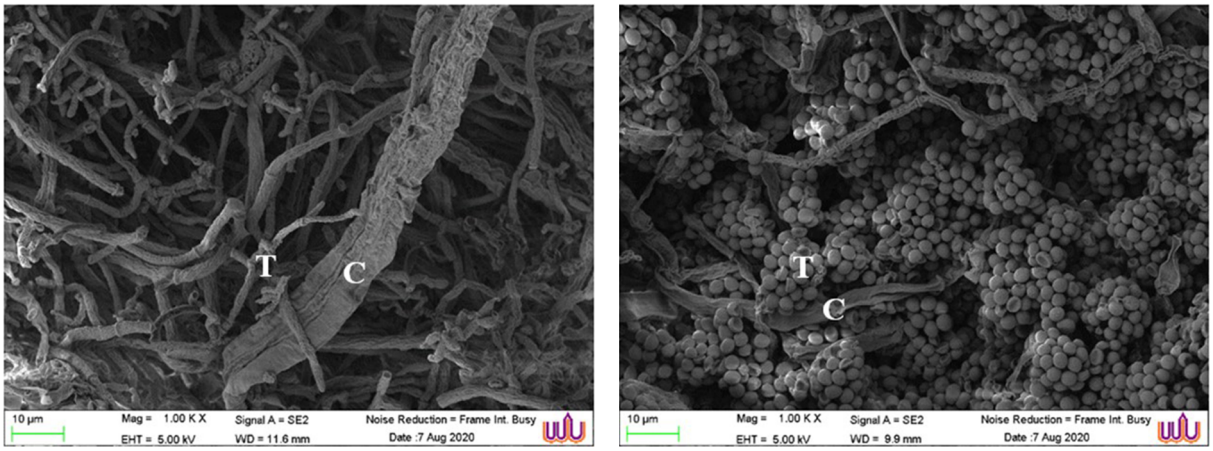

Figure 5. Scanning electron micrographs of the *Trichoderma asperellum* strain NST-009 hyphae (T) interaction with the mycelium of *Cercospora lactucae-sativae* (C) from the dual culture test

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Table 2. Effect of the crude extract of the *Trichoderma* cultures on the mycelial growth inhibition of *Cercospora lactucae-sativae* after incubation at room temperature for 14 days

Treatments	*C. lactucae-sativae* inhibition (%)	
	250 µg/mL	500 µg/mL
T. asperellum NST-009	64.15[a]	93.26[a]
T. asperellum NST-028	48.26[b]	76.72[b]
T. asperellum CB-Pin-01	50.54[b]	78.64[b]

[a,b]Mean values within the same columns followed by the same latter are not significantly different according to Duncan's multiple range test ($P < 0.05$)

ulated with only *C. lactucae-sativae* had the highest disease severity index, at 85.56% (Table 3).

Trichoderma root and leaf colonisation. The root and leaf colonisations of *T. asperellum* were determined after application of *T. asperellum* for 21 days. The treatments with the *T. asperellum* strains NST-009, NST-028, and CB-Pin-01 had the highest root and leaf colonisation percentages (100%), which were significantly different from the Control 1, Control 2 and mancozeb treatments, which did not find *Trichoderma* strains colonised on the roots and leaves (Table 4).

Trichoderma population in nutrient solution

The treatments of *T. asperellum* induced a population of 1.63×10^4–1.78×10^4 colony-forming units per mL nutrient solution (CFU/mL nutrient solution) after application for 21 days, whereas the treatments with the fungicide (mancozeb), Control 1 (with the pathogen) and Control 2 (without the pathogen) did not find the *Trichoderma* species in the nutrient solution (Table 4).

Table 3. Disease severity index (*DSI*) of the 'Green Oak' lettuce leaf spot and population of *Cercospora lactucae-sativae* under nutrient film technique hydroponic conditions after inoculation for 21 days

Treatments	*DSI* (%)	Disease reduction (%)
T. asperellum NST-009	18.05[c]	67.51[a]
T. asperellum NST-028	30.94[b]	54.62[b]
T. asperellum CB-Pin-01	28.05[b]	57.51[b]
Mancozeb	26.56[b]	59.00[b]
Control 1 (only *C. lactucae-sativae*)	85.56[a]	0.00[c]
Control 2 (without *C. lactucae-sativae*)	5.32[d]	–

[a–d]Mean values within the same columns followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$)

Growth promotion. At 21 days after inoculation with the *Trichoderma* strain in the nutrient solution, the treatments with *T. asperellum* showed significant differences in the growth and development parameters, and the chemical analysis when compared with the Control 1, Control 2, and mancozeb treatments. The treatments with *T. asperellum* NST-009 increased the plant height (8.62%), canopy width (16.67%), leaf number (18.39%), shoot fresh weight (25.71%), root fresh weight (39.26%), and total P in the leaves (31.45%) when compared with the control (Table 5, Table 6).

DISCUSSION

Cercospora lactucae-sativae is one of the pathogens that causes leaf spots in lettuce (Kham-un et al. 2017). In the present study, effective *T. asperellum* strains were tested to control Cercospora leaf spot and the growth promotion of 'Green Oak' lettuce in the NFT hydroponic system. An *in vitro* and hydroponic system experiment clear up the multifaceted actions of *T. asperellum* NST-009, including competition, parasitism, antibiosis, leaf and root colonisation, reducing the disease severity index, and plant growth promotion, with details as follows.

Trichoderma strains can compete for nutrients (carbon, nitrogen, and other growth factors) and space or at specific infection sites with plant pathogens. In this study, the competition for nutrients and space was confirmed by the dual culture method. *T. asperellum* NST-009 provided the highest percentage of *C. lactucae-sativae* mycelial growth inhibition of 77.10%. This result is supported by several research reports that *Trichoderma* strains are highly effective in inhibiting the growth on the mycelia of *Cercospora* species such as *C. beticola*, *C. traversiana*, and *C. arachidicola* (Galletti et al. 2008; Ramesh & Zacharia 2017).

The *Trichoderma* strains' parasitism is a complex process that consists of several steps, including recognition of the host, attack and subsequent penetration, and killing the host. During this process, *Trichoderma* strains secrete cell wall degrading enzymes (CWDEs) that hydrolyse the plant pathogen's cell wall, subsequently releasing oli-

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Table 4. Root colonisation percentage at 42 days after planting, the population of *Trichoderma* species in the nutrient solution at 14 and 21 days after planting under nutrient film technique-hydroponic conditions

Treatments	Root colonization (%)	Leaf colonization (%)	*Trichoderma* population (CFU/ml nutrient solution)	
			14 days	21 days
T. asperellum NST-009	100.00[a]	100.00[a]	3.50×10^{4a}	1.78×10^{4a}
T. asperellum NST-028	100.00[a]	100.00[a]	3.20×10^{4a}	1.63×10^{4a}
T. asperellum CB-Pin-01	100.00[a]	100.00[a]	3.35×10^{4a}	1.68×10^{4a}
Mancozeb	0.00[b]	0.00[b]	0.00[b]	0.00[b]
Control 1 (only *C. lactucae-sativae*)	0.00[b]	0.00[b]	0.00[b]	0.00[b]
Control 2 (without *C. lactucae-sativae*)	0.00[b]	0.00[b]	0.00[b]	0.00[b]

[a,b]Mean values within the same columns followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$)

Table 5. Plant height, canopy width, leaf number and total phosphorus in the leaves of the 'Green Oak' lettuce after inoculation with the *Trichoderma* strains in the nutrient solution for 21 days under nutrient film technique-hydroponic conditions

Treatments	Plant height		Canopy width		Leaf number	
	(cm)	(%)	(cm)	(%)	leaf/plant	(%)
T. asperellum NST-009	17.33[a]	8.62	21.00[a]	16.67	25.75[a]	18.39
T. asperellum NST-028	17.08[a]	7.05	20.75[a]	15.28	24.25[a]	11.49
T. asperellum CB-Pin-01	17.18[a]	7.68	20.50[a]	13.89	25.00[a]	14.94
Mancozeb	16.30[b]	2.19	18.50[b]	2.78	22.25[b]	2.30
Control 1 (only *C. lactucae-sativae*)	15.95[b]	0.00	18.00[b]	0.00	21.75[b]	0.00
Control 2 (without *C. lactucae-sativae*)	16.18[b]	1.41	18.25[b]	1.39	22.00[b]	1.15

[a,b]Mean values within the same columns followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$)

Table 6. Shoot and root fresh weight, shoot and root dry weight, and total phosphorus in the leaves of the 'Green Oak' lettuce after inoculation with the *Trichoderma* strains in the nutrient solution for 21 days under nutrient film technique-hydroponic conditions

Treatments	Shoot fresh weight		Root fresh weight		Total P in leaves	
	(g/plant)	(%)	(g/plant)	(%)	mg/kg	(%)
T. asperellum NST-009	130.56[a]	25.71	24.04[a]	39.26	3.55[a]	31.45
T. asperellum NST-028	125.52[a]	20.85	23.38[a]	35.41	3.49[a]	29.05
T. asperellum CB-Pin-01	129.82[a]	24.99	23.82[a]	38.00	3.41[a]	25.99
Mancozeb	118.41[b]	14.01	21.04[b]	21.85	2.90[b]	7.22
Control 1 (only *C. lactucae-sativae*)	103.86[c]	0.00	17.26[c]	0.00	2.70[b]	0.00
Control 2 (without *C. lactucae-sativae*)	108.58[c]	4.55	18.39[c]	6.53	2.91[b]	7.68

[a–c]Means values within the same columns followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$)

gomers from the plant pathogen cell wall (Vinale et al. 2008). Many researchers have reported that *Trichoderma* strains produce many CWDEs such as β-1,3-glucanase, β-1,4-glucanase, β-1,6-glucanase, chitinase, cellulase, protease, and xylanase (Baiyee et al. 2019; Wonglom et al. 2019;

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Wonglom et al. 2020). Many researchers used light compound microscopy, scanning electron microscopy (SEM), and fluorescence microscopy to study the *Trichoderma* strains' parasitism on the mycelia of several plant pathogens (Ruangwong et al. 2021). This study revealed that *T. asperellum* NST-009 could parasitise the mycelia of *C. lactucae-sativae*. The hyphae of *T. asperellum* NST-009 could colonise and produce conidia on the mycelia of *Cercospora* pathogen. The SEM study confirmed that mycoparasitism was one of the most critical mechanisms of *T. asperellum* NST-009 in controlling the 'Green Oak' lettuce leaf spot disease.

Antibiosis is the process of secreting antimicrobial compounds by antagonistic fungi to suppress or kill pathogenic fungi in the vicinity of its growth area. The antibiosis of *Trichoderma* strains has long been suggested to be involved in biocontrols. Forty-three substances produced by *Trichoderma* have antibiotic activities. Seven out of 43, alkyl pyrones, isonitriles, polyketides, peptaibols, diketopiperazines, sesquiterpenes, and steroids have frequently been associated with the biocontrol activity of *Trichoderma* strains (Vinale et al. 2008). This study showed that the antifungal metabolite of *T. asperellum* NST-009 effectively inhibits the mycelial growth of *C. lactucae-sativae* with previously supported that this *Trichoderma* strain produced alkyl pyrone (6-*n*-pentyl-*2H*-pyran-2-one), which is highly effective against plant pathogens (Promwee et al. 2017).

Root and leaf colonisation is another mechanism by *Trichoderma* strains to control plant diseases. This study indicated that *T. asperellum* NST-009 has the ability to colonise 'Green Oak' lettuce roots and leaves as a result of the protection from the *Cercospora* pathogen. Harman et al. (2004) reported that the root colonisation by *Trichoderma* strains results in increased levels of defence-related plant enzymes, such as peroxidase, chitinase, and β-1-3-glucanase. *Trichoderma* strains could be used to induce resistance in both induced systemic resistance, and systemic acquired resistance pathways. On the other hand, this phenomenon depends on the *Trichoderma* spp., plant species, as well as the biotic and abiotic conditions (Nawrocka & Malolepsza 2013).

Using *Trichoderma* strains in a hydroponic system not only controls plant diseases, but also stimulates plants when compared with the control. Yedidia et al. (2001) reported that the *T. harzianum* strain T-203 increased the cucumber plant growth under axenic hydroponic growth conditions. Plants stimulated by *Trichoderma* spp. have a higher chlorophyll content than those not using the *Trichoderma* method (Azarmi et al. 2011; Wonglom et al. 2020). Phosphate solubilisation is one of the mechanisms how *Trichoderma* strains promote plant growth. Other mechanisms of *Trichoderma* strains that increase the growth and yield of plants have been reported. *Trichoderma* strains produced growth-regulating factors, which enhanced the seed germination and plant growth. In addition, increasing the plant growth and development may result from the control of minor pathogens or the increased nutrient uptake through the enhanced root growth and the promoted the availability of necessary nutrients (Stewart & Hill 2014). Additionally, *T. harzianum* could produce indole acetic acid, which affects the plant growth (Janardan et al. 2011; Nieto-Jacobo et al. 2017).

In conclusion, the *Trichoderma asperellum* strain NST-009 is indigenous to southern Thailand. *T. asperellum* NST-009 effectively inhibited the mycelial growth of *C. lactucae-sativae* more than the Thai commercial strain *T. asperellum* CB-Pin-01 in both the dual culture and crude extract tests. *T. asperellum* NST-009 exhibited mycoparasitism under a scanning electron microscope. In the NFT-hydroponics experiment, *T. asperellum* NST-009 showed a disease severity index equivalent to the mancozeb fungicide. Moreover, the *T. asperellum* strains exhibited root colonisation protection against a lettuce infection with *C. lactucae-sativae* and could survive in the nutrient solution.

REFERENCES

Abo-Elyousr K.A., Abdel-Hafez S.I., Abdel-Rahim I.R. (2014): Isolation of *Trichoderma* and evaluation of their antagonistic potential against *Alternaria porri*. Journal of Phytopathology, 162: 567–574.

AOAC (2000): Official Methods of Analysis. Gaithersburg, AOAC International.

Azarmi R., Hajieghrari B., Giglou A. (2011): Effect of *Trichoderma* isolates on tomato seedling growth response and nutrient uptake. African Journal of Biotechnology, 10: 5850–5855.

Baiyee B., Ito S., Sunapapo A. (2019): *Trichoderma asperellum* T1 mediated antifungal activity and induced defense response against leaf spot fungi in lettuce (*Lactuca sativa* L.). Physiological and Molecular Plant Pathology, 106: 96–101.

https://doi.org/10.17221/69/2021-PPS

Brožová J. (2004): Mycoparasitic fungi *Trichoderma* spp. in plant protection – Review. Plant Protection Science, 40: 63–74.

Camejo D., Frutos A., Mestre T.C., del Carmen Piñero M., Rivero R.M., Martínez V. (2020): Artificial light impacts the physical and nutritional quality of lettuce plants. Horticulture, Environment, and Biotechnology, 61: 69–82.

Chairin T., Pornsuriya C., Thaochan N., Sunpapao A. (2017): *Corynespora cassiicola* causes leaf spot disease on lettuce (*Lactuca sativa*) cultivated in hydroponic systems in Thailand. Australasian Plant Disease Notes, 12: 16. doi: 10.1007/s13314-017-0241-x

Charoenrak P., Chamswarng C., Intanoo W., Keawprasert N. (2019): The effects of vermicompost mixed with *Trichoderma asperellum* on the growth and Pythium root rot of lettuces. International Journal of Geomate, 17: 215–221.

Chen Z., Cuervo D.P., Müller J.A., Wiessner A., Köser H., Vymazal J., Kuschk P. (2016): Hydroponic root mats for wastewater treatment – A review. Environmental Science and Pollution Research, 23: 15911–15928.

Galletti S., Burzi P.L., Cerato C., Marinello S., Sala E. (2008): *Trichoderma* as a potential biocontrol agent for *Cercospora* leaf spot of sugar beet. BioControl, 53: 917–930.

Harman G.E., Howell C.R., Viterbo A., Chet I., Lorito M. (2004): *Trichoderma* species – Opportunistic, avirulent plant symbionts. Nature Reviews Microbiology, 2: 43–56.

Izzati M.Z.N.A., Abdullah F. (2008): Disease suppression in *Ganoderma*-infected oil palm seedlings treated with *Trichoderma harzianum*. Plant Protection Science, 44: 101–107.

Janardan Y., Verma J.P., Tiwari K.N. (2011): Plant growth promoting activities of fungi and their effect on chickpea plant growth. Asian Journal of Biological Sciences, 4: 291–299.

Kabiri R., Nasibi F., Farahbakhsh H. (2014): Effect of exogenous salicylic acid on some physiological parameters and alleviation of drought stress in *Nigella sativa* plant under hydroponic culture. Plant Protection Science, 50: 43–51.

Kham-un P., Cheewangkoon R., To-anun C. (2017): Controlling *Cercospora lactucae-sativae* causes lettuce leaf spot disease using antagonistic yeasts. International Journal of Agricultural Technology, 13: 153–162.

Khan M.F., Smith L.J. (2005): Evaluating fungicides for controlling *Cercospora* leaf spot on sugar beet. Crop Protection, 24: 79–86.

Khunti J.P., Bhoraniya M.E., Vora V.D. (2005): Management of powdery mildew and *Cercospora* leaf spot of mungbean by some systemic fungicides. Legume Research, 28: 65–67.

Koohakan P., Jeanaksorn T., Nuntagij I. (2008): Major diseases of lettuce grown by commercial nutrient film technique in Thailand. Current Applied Science and Technology, 8: 56–63.

Nawrocka J., Małolepsza U. (2013): Diversity in plant systemic resistance induced by *Trichoderma*. Biological Control, 67: 149–156.

Nawrocka J., Szczech M., Małolepsza U. (2018): *Trichoderma* atroviride enhances phenolic synthesis and cucumber protection against *Rhizoctonia solani*. Plant Protection Science, 54: 17–23.

Nguanhom J., Cheewangkoon R., Groenewald J.Z., Braun U., To-anun C., Crous P.W. (2015): Taxonomy and phylogeny of *Cercospora* spp. from Northern Thailand. Phytotaxa, 233: 27–48.

Nieto-Jacobo M.F., Steyaert J.M., Salazar-Badillo F.B., Nguyen D.V., Rostás M., Braithwaite M., De Souza J.T., Jimenez-Bremont J.F., Ohkura M., Stewart A., Mendoza-Mendoza A. (2017): Environmental growth conditions of *Trichoderma* spp. affects indole acetic acid derivatives, volatile organic compounds, and plant growth promotion. Frontiers in Plant Science, 8: 102. doi: 10.3389/fpls.2017.00102

Promwee A., Issarakraisila M., Intana W., Chamswarng C., Yenjit P. (2014): Phosphate solubilization and growth promotion of rubber tree (*Hevea brasiliensis* Muell. Arg.) by *Trichoderma* strains. Journal of Agricultural Science, 6: 8–20.

Promwee A., Yenjit P., Issarakraisila M., Intana W., Chamswarng C. (2017): Efficacy of indigenous *Trichoderma harzianum* in controlling *Phytophthora* leaf fall (*Phytophthora palmivora*) in Thai rubber trees. Journal of Plant Diseases and Protection, 124: 41–50.

Ramesh A.M., Zacharia S. (2017): Efficacy of bio-agents and botanicals against leaf spot (*Cercospora arachidicola* Hori) of groundnut (*Arachis hypogaea* L.). Journal of Pharmacognosy and Phytochemistry, 6: 504–506.

Redda E.T., Ma J., Mei J., Li M., Wu B., Jiang X. (2018): Antagonistic potential of different isolates of *Trichoderma* against *Fusarium oxysporum*, *Rhizoctonia solani*, and *Botrytis cinerea*. European Journal of Experimental Biology, 8: 1–8.

Ruangwong O.U., Wonglom P., Phoka N., Suwannarach N., Lumyong S., Ito S.I., Sunpapao A. (2021): Biological control activity of *Trichoderma asperelloides* PSU-P1 against gummy stem blight in muskmelon (*Cucumis melo*). Physiological and Molecular Plant Pathology, 115: 101663. doi: 10.1016/j.pmpp.2021.101663

Srimai K., Akarapisarn A. (2014): *Bacillus subtilis* LBF02 as biocontrol agent against leaf spot diseases caused by *Cercospora lactucae-sativae* in lettuce. Journal of Agricultural Science, 6: 151–158.

Stewart A., Hill R. (2014): Applications of *Trichoderma* in plant growth promotion. In: Gupta V.K., Schmoll M., Herrera-Estrella A., Upadhyay R.S., Druzhinina I., Tuohy M.G. (eds). Biotechnology and Biology of Trichoderma. Amsterdam, Elsevier: 415–428.

https://doi.org/10.17221/69/2021-PPS

Suwan N., Nuandee N., Akimitsu K., Nalumpang S. (2012): Analysis of β-tubulin gene from carbendazim resistant isolates of *Cercospora lactucae-sativae* on lettuce in Thailand. Journal of Agricultural Technology, 8: 711–723.

Thomas A., Saravanakumar D. (2019): Effect of host extract on growth and sporulation of *Cercospora lactucae-sativae*. Australasian Plant Disease Notes, 14: 1–4.

Thongkamngam T., Jaenaksorn T. (2017): *Fusarium oxysporum* (F221-B) as biocontrol agent against plant pathogenic fungi *in vitro* and in hydroponics. Plant Protection Science, 53: 85–95.

To-Anun C., Hidayat I., Meeboon J. (2011): Genus *Cercospora* in Thailand: Taxonomy and phylogeny (with a dichotomous key to species). Plant Pathology and Quarantine, 1: 11–87.

Trkulja N., Milosavljević A., Stanisavljević R., Mitrović M., Jović J., Toševski I., Bošković J. (2015): Occurrence of *Cercospora beticola* populations resistant to benzimidazoles and demethylation-inhibiting fungicides in Serbia and their impact on disease management. Crop Protection, 75: 80–87.

Unartngam J., Srithongkum B., Intanoo W., Charoenrak P., Chamswarng C. (2020): Morphological and molecular based identification of *Trichoderma* CB-Pin-01 biological control agent of plant pathogenic fungi in Thailand. International Journal of Agricultural Technology, 16: 175–188.

Vinale F., Sivasithamparam K., Ghisalberti E.L., Marra R., Woo S.L., Lorito M. (2008): *Trichoderma*–plant–pathogen interactions. Soil Biology and Biochemistry, 40: 1–10.

Waghunde R.R., Shelake R.M., Sabalpara A.N. (2016): *Trichoderma*: A significant fungus for agriculture and environment. African Journal of Agricultural Research, 11: 1952–1965.

Wonglom P., Daengsuwan W., Ito S., Sunpapao A. (2019): Biological control of *Sclerotium* fruit rot of snake fruit and stem rot of lettuce by *Trichoderma* sp. T76-12/2 and the mechanisms involved. Physiological and Molecular Plant Pathology, 107: 1–7.

Wonglom P., Ito S.I., Sunpapao A. (2020): Volatile organic compounds emitted from endophytic fungus *Trichoderma asperellum* T1 mediate antifungal activity, defense response and promote plant growth in lettuce (*Lactuca sativa*). Fungal Ecology, 43: 100867. doi: 10.1016/j.funeco.2019.100867

Yedidia I., Srivastva A.K., Kapulnik Y., Chet I. (2001): Effect of *Trichoderma harzianum* on microelement concentrations and increased growth of cucumber plants. Plant and Soil, 235: 235–242.

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