BIODIVERSITAS
Volume 25, Number 9, September 2024
Pages: 3380-3389

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d250960

Characterization of *Trichoderma* sp. local isolate and antagonism assay against pathogen *Helminthosporium* sp.

PARLUHUTAN SIAHAAN♥, AGUSTINA MONALISA TANGAPO, STELLA DEIBY UMBOH
Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sam Ratulangi. Jl. Kampus Bahu, Manado 95115, North Sulawesi, Indonesia. Tel.: +62-431864386, Fax.: +62-431864386, ♥email: luhut.siahaan68@unsrat.ac.id

Manuscript received: 2 August 2024. Revision accepted: 30 September 2024.

Abstract. *Siahaan P, Tangpao AM, Umboh SD. 2024. Characterization of* Trichoderma *sp. local isolate and antagonism assay against pathogen* Helminthosporium *sp. Biodiversitas 25: 3380-3389. Trichoderma*, as a biological agent, plays an important role in controlling pathogens that cause plant diseases, so it needs to be utilized properly, especially for local isolates. This research aimed to analyze the morphological, molecular and secondary metabolic characteristics of local isolates of *Trichoderma* fungus and test their antagonism against the pathogenic fungus *Helminthosporium* sp. The results showed that Tomohon local isolate of *Trichoderma* was identified as *T. asperellum*. The results of GC-MS analysis show that *T. asperellum* produces secondary metabolites in the form of volatile compounds from the group of volatile aldehydes, esters and ketones, as well as four fatty acid compounds, namely n-hexadecanoic acid, hexadecanoic acid, methyl ester, octadecadienoic acid, methyl ester, 9,12-octadecadienoic acid (Z,Z), play an important role in the antagonistic ability of *Trichoderma*. *T. asperellum* was able to inhibit *Helminthosporium* sp. by 70% on the seventh day of observation, and had antagonistic mechanisms, namely antibiosis and competition. Based on these results, local isolates of *T. asperellum* have great potential for use as biological control agents.

Keywords: Antagonistic fungi, *Helmintosporium* sp., pathogen, *Trichoderma asperellum*, Tomohon City

INTRODUCTION

Leaf blight disease in corn plants, caused by the pathogenic fungus *Helminthosporium* sp. (Shan et al. 2023), has become a serious challenge for agriculture in many regions, including North Sulawesi, Indonesia. This pathogen is known for its ability to significantly damage corn plants. The presence of this pathogen can lead to diseases that harm the leaves, stems, and seeds of corn, thereby reducing the quality and quantity of the harvest, resulting in decreased production and productivity, and impacting the well-being of farmers (Priyashantha et al. 2023). In North Sulawesi, corn leaf blight has become one of the main problems in efforts to increase corn production and productivity. The geographical and climatic conditions in this region provide a highly conducive environment for the development of the *Helminthosporium* sp. pathogen, which in turn exacerbates its negative impact on local agriculture (Shabana et al. 2022). Factors such as warm temperatures, high humidity, and sufficient rainfall create ideal conditions for this pathogen to grow and spread rapidly (Gullino et al. 2022). Consequently, plants are vulnerable to infection, which can lead to reduced yields, economic losses for farmers, and threats to food security.

Efforts to control leaf blight disease in corn have often been conducted using synthetic chemical pesticides. However, the use of these pesticides not only has negative impacts on the environment but also poses significant risks to human health and non-target organisms. The increase in pesticide residues in soil and water can lead to contamination that endangers local ecosystems, while direct or indirect exposure to pesticides can result in serious health problems for humans (Boonupara et al. 2023). Moreover, excessive use of pesticides can affect the presence and balance of non-target organisms, such as pollinating insects, birds, and soil microorganisms that are essential for sustainable agriculture (Tudi et al. 2021). Therefore, there is an urgent need to find more environmentally friendly and sustainable solutions for controlling leaf blight disease in corn. Many environmentally friendly control methods have been implemented, and one approach that has gained attention is the use of biocontrol agents, such as antagonist fungi from the genus *Trichoderma* (Alwadai et al. 2022).

Trichoderma species are a group of fungi commonly found in soil and decaying wood. *Trichoderma* sp. can act as biocontrol agents, decomposers, and endophytes in various plant species (Woo et al. 2023). These species are easy to isolate, highly adaptive, and grow rapidly on various substrates. Additionally, *Trichoderma* spp. have a broad microparasitism capability and are non-pathogenic to plants (Mukherjee et al. 2022), making *Trichoderma* sp. one of the most widely used agents for controlling plant disease pathogens. *Trichoderma* sp. has been proven to has the potential to control plant pathogens and enhance plant resistance to diseases (Ferreira and Musumeci 2021). Several studies have demonstrated the antagonistic ability of *Trichoderma* sp. fungi against disease-causing pathogens, including the control of Vascular Streak Dieback disease caused by the pathogen *Oncobasidium theobromae* (Simamora et al. 2021), the control of *Sclerotinia minor* plant pathogens (Ramona et al. 2022), the control *Sclerotium rolfsii* causing collar rot of chili (Yadav et al. 2022), and

the control of sheath blight disease caused by the pathogen *Rhizoctonia solani* (Iswati et al. 2024).

The potential of the antagonist fungus *Trichoderma* sp. has been extensively researched. However, studies on the effectiveness and application of *Trichoderma* sp., particularly local isolates, in controlling leaf blight disease in corn are still limited, especially in the North Sulawesi region. With this understanding, this research aimed to explore, characterize, and analyze bioactive compounds and antagonistic tests of local isolates of *Trichoderma* sp. against the *Helminthosporium* sp. in controlling leaf blight disease in corn in North Sulawesi. This study not only provides new insights into the use of biocontrol agents in controlling plant diseases, but also has the potential to offer practical solutions that can be applied by farmers in North Sulawesi and other regions.

MATERIALS AND METHODS

Sample collection

Sampling was conducted in the corn fields in Tomohon City, North Sulawesi, Indonesia. Samples were collected directly by hand from plant parts infected with the fungus *Helminthosporium* sp. (coordinate: 1.29861, 124.84844). *Trichoderma* sp. was obtained by taking soil samples from banana plantations (coordinate: 1.299640, 124.846941). The samples were then brought to the Advanced Biology Laboratory at Universitas Sam Ratulangi, Manado, for further analysis.

Isolation of *Trichoderma* sp.

Trichoderma sp. was isolated using the serial dilution method. First, 25 g of the soil sample was placed in a beaker and mixed with 250 mL of sterile water. This mixture was then stirred with a stirring rod for 15-20 minutes. Second, dilution where 1 mL of the stock solution was taken and added to a test tube along with 9 mL of sterile distilled water. The mixture was then homogenized using a vortex. This process was repeated until a dilution of 10^{-4} was achieved. Subsequently, 1 mL of the solution was placed in a petri dish and mixed with PDA media. The plates were then left to incubate (25°C) for 7 days (Mayo-Prieto et al. 2020). The obtained fungi were re-cultured on fresh PDA media.

Isolation of *Helminthosporium* sp.

Diseased samples (leaf blight disease) of corn plants were taken from the leaf parts that showed brown spots scattered across the entire leaf blade. These samples were then placed in sterile plastic bags and transported to the Advanced Biology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sam Ratulangi. Isolation was performed by cutting infected leaf into 1x1 cm pieces, then sterilized by soaking in 70% alcohol for 1-3 minutes, followed by 5% NaOCl for 5-10 minutes, then rinsed twice with distilled water. After that, leaf samples were dried on sterile tissue paper. After drying, the leaf samples were then placed on PDA (Potato Dextrose Agar) medium and incubated at 25°C for 3 days (Hu et al. 2023).

Identification of *Trichoderma* and *Helmintosporium* isolates

The identification process of fungi after isolation was conducted in two stages: macroscopic and microscopic observations. Macroscopic morphological observation involved shape, size and color of colony, surface and edge of colony, while microscopic characters, included color and shape of colonies, as well as the shape of phialids, conidia and conidiophores (Senanayake 2020).

Molecular identification of *Trichoderma* sp.

Trichoderma was extracted using the standard procedures with the Plant Genomic DNA Mini Kit (Geneaid). DNA amplification was conducted using the T-Personal thermocycler (Biometra) with MyTaq HS Red Mix (Bioline) and universal primers ITS1 (5'- `TCC GTA GGT GAA CCT GCG G` -3') and ITS4 (5'- `TCC TCC GCT TAT TGA TAT GC` -3'). The PCR process involved an initial denaturation step at 94-95°C for 5 minutes, followed by 35 cycles consisting of three main steps: denaturation at 94-95°C for 30 seconds, annealing at 50-55°C for 30 seconds, and extension at 72°C for 1 minute. After all cycles were completed, final extension was performed at 72°C for 5-10 minutes. The PCR-amplified DNA was separated using 0.8% agarose gel electrophoresis. Sequencing was performed by First Base CO (Malaysia) using the PCR product and primers. The homology study with the comparison fungus *Trichoderma* in NCBI was conducted using the BLAST (Basic Local Alignment Search Tool) method. For analysis and dendrogram construction, the Mega X software was used (de Sousa et al. 2021).

Identification of bioactive compounds

The initial extraction steps involved homogenizing *Trichoderma* sp. with a mixture of methanol and acetic acid at 70°C for 4 hours with constant shaking. Subsequent extraction was conducted using 80% methanol at 70°C for 1 hour, followed by 70% acetone at 30°C for 1 hour, and finally with 100% methanol at 20°C for 4 hours. The extracted material was then purified by centrifugation at 2800 rpm for 10 minutes, followed by filtration of the supernatant using Whatman filter paper and the solvent was evaporated using a rotary evaporator. The resulting extract was then analyzed using GC-MS Agilent 8890 GC System (Alshuniaber et al. 2020).

Antagonism test (antagonistic activity, antibiosis, and microparasitism)

The experiment was performed in five replicates and conducted at the Advanced Biology Laboratory, Universitas Sam Ratulangi. Seven days old *Trichoderma* culture was used for this experiment. *Trichoderma* sample was taken using a cork borer from the edge of the colony and then placed on PDA medium labeled 'A'. Seven days old culture of *Helminthosporium* sp. was placed on PDA medium and labeled 'P' (Figure 1). Subsequently, all petri dishes were incubated at room temperature (Putri et al. 2022). Observations were made daily for one week to monitor the growth and development of the colonies. After one week, the radius of *Helminthosporium* sp. colony in the petri dish

was measured. This process aimed to quantitatively assess the extent of pathogen colony growth. These steps were designed to provide a deeper understanding of the interaction between *Trichoderma* sp. and *Helminthosporium* sp., as well as its impact on pathogenic colony growth.

Data analysis

The growth rate of fungi was determined by measuring the diameter of each colony daily after inoculation until the seventh day, using a ruler for measurements. The percentage of inhibition was calculated using the following formula (Skidmore and Dickinson 1976):

$$\text{Inhibitory activity}(\%)\ \frac{PR1 - PR2}{PR1}\ x\ 100\%$$

The metabolite profiling analysis involved reviewing the literature to obtain information on the compounds obtained from *Trichoderma* sp. fungi based on the GC-MS analysis results.

RESULTS AND DISCUSSION

Morphological characteristics of *Helminthosporium* sp. and *Trichoderma* sp.

Helminthosporium *sp.*

The fungus *Helminthosporium* sp. formed oval-shaped colonies with a white center that slightly turns brownish over time, also formed concentric circular rings on PDA. The growth type was concentric and round, with a cotton-like texture, dense compactness, and moderate thickness. After seven days, the colony size reached 6.8 cm. The colony surface appeared flat and fibrous (Figure 2.A). The conidia were single, elongated oval-shaped, slightly curved, with a swollen middle and tapering blunt ends. They were thick walled, slightly prominent hilum, and contain 5-7 septa, with a light brown color. Conidiophores were unbranched and septate, with a slightly prominent and distinct hilum (Figure 2.B). Overall, macroscopic and microscopic characteristics confirmed the fungus as *Helminthosporium* sp.

Trichoderma *sp.*

On PDA, media *Trichoderma* sp. initially formed white colonies. By the fourth and fifth days, the colonies began to turn whitish-green color, and expand in size. From sixth to seventh day, colonies turned in dark green, cover the entire surface of the petri dish with round colonies (Figure 3.A). Microscopic characteristics showed that hyphae were hyaline, septate, erect conidiophores arranged vertically and branching. Conidia were round, greenish-yellow in color, with phialides located beneath them oriented towards the apex, arranged 2-3 in parallel, ampulliform in shape, and slightly enlarged in the middle (Figure 3.B).

Molecular analysis of *Trichoderma* sp.

The PCR results for the fungal isolate using universal primers ITS1 and ITS4 showed excellent outcomes. The amplified DNA band matched the target size at 600 bp (Figure 4).

The sequence matching of isolate PSBT using BLAST (Basic Local Alignment Search Tool) on NCBI yielded results as shown in Table 1. The results indicated that query sequence had 100% similarities with sequences from species listed in the table. All results showed an e-value of 0.0, indicated that the similarity was highly significant. The PSBT isolate from molecular analysis showed similarity to four different species of *Trichoderma*: *T. asperellum*, *T. harzianum*, *T. yunnanense*, and *T. azevedoi*.

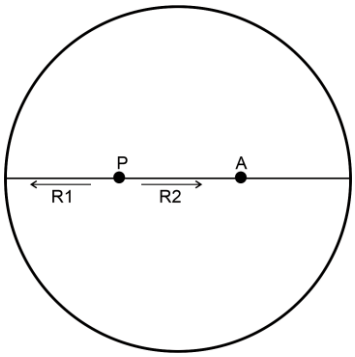


Figure 1. Antagonism test method. P: colony of *Helminthosporium* sp. A: colony of *Trichoderma* sp.; R1: radius of *Helminthosporium* sp. colony moving away from *Trichoderma* sp. colony; R2: radius of *Helminthosporium* sp. colony approaching *Trichoderma* sp. colony

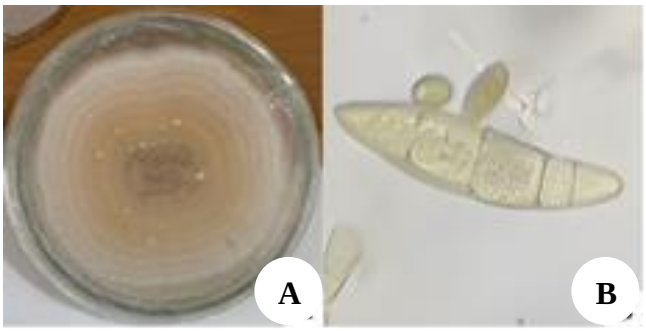


Figure 2. Colony of A. *Helmintosphorium* sp. on PDA media; B. Conidia (magnified 100×)

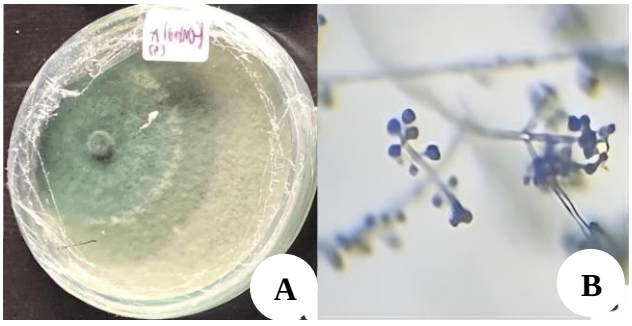


Figure 3. Colony of *Trichoderma* sp. on PDA media on A. Seventh day (A); B. Hyphae, conidiphores and conidia (magnified 100×)

The construction of phylogenetic tree (Figure 5) showed that *T. asperellum*, *T. harzianum*, *T. yunnanense*, and *T. azevedoi* were genetically related, with *T. asperellum* and *T. harzianum* exhibiting the closest relationship. This is consistent with the BLAST results from the previous table, which indicated 100% genetic similarity between the query sequence and sequences from these species. The visualization of cladogram provides insights into how these different species evolved from a common ancestor.

Matching the PSBT isolate with reference isolates and constructing the phylogenetic tree revealed that the PSBT isolate shares similarities with four different species, making it challenging to definitively assign a species name to PSBT. Conventional identification was crucial to compare the morphological characteristics of PSBT with those of the four species from GenBank, in order to establish its species classification. The macroscopic and microscopic morphological characteristics of *Trichoderma* PSBT isolate and the other four species are presented in Table 2. While the four *Trichoderma* species generally share similar morphological characteristics, upon closer examination, the *Trichoderma* PSBT isolate exhibits characteristics more similar to *T. asperellum.*

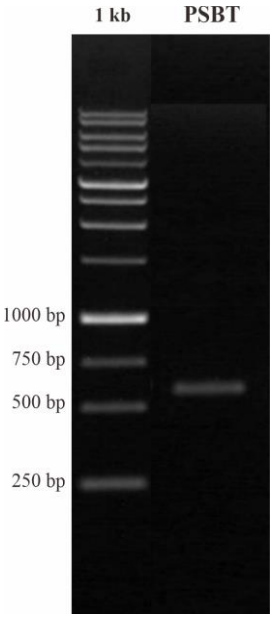


Figure 4. Products amplification of *Trichoderma* isolate

Table 1. Matching results of the isolate with reference isolates on NCBI

Species	Max score	Total score	Query cover	E-value	Per. Ident.	Acc. Len	Accession
Trichoderma asperellum	942	942	100%	0.0	100%	642	MN783048.1
Trichoderma asperellum	942	942	100%	0.0	100%	575	OR770586.1
Trichoderma harzianum	942	942	100%	0.0	100%	567	OP739100.1
Trichoderma yunnanense	942	942	100%	0.0	100%	602	OR775709.1
Trichoderma azevedoi	942	942	100%	0.0	100%	562	OR787381.1

Notes: Access Date: July 14, 2024

Table 2. Comparison of characteristics of the five *Trichoderma* fungal isolates

Species	Characteristics	References
Trichoderma isolate PSBT	Colonies were dark green with concentric rings. Hyphae were hyaline and septate, conidiophores were vertically arranged in an upright shape with branching. Conidia were green, subglobose to round in shape, beneath the conidia were phialides arranged 2-3 parallel towards the apex, ampulliform in shape and slightly enlarged in the middle.	(This study)
Trichoderma asperellum	The colonies were dark green and formed concentric rings. Green conidia were subglobose in shape. Phialides were single or form a circle of two to three and slightly enlarged in the middle. The entire conidiophore system appears pyramid-shaped with main branches emerging from the base to the tip.	Asis et al. (2020)
Trichoderma harzianum	The colony was round in shape with a dark green color, producing tufts or pustules that are bordered by sterile mycelium, with conidiophores often branching and vertical. Ampulliform and convergent phialides, conidia are subglobose to obovoid.	Manjur and Afia (2019)
Trichoderma azevedoi	The mycelium was cottony, with light green spores concentrated in the center of the plate and in a wide concentric ring about halfway to the edge of plate. Pyramid-shaped conidiophores with opposite or isolated branches, ending in groups of three to five phialides. Ampulliform phialides, shaped like a fask. The length of phialides was shorter than other species, narrowing under the tip to form a narrow neck. Conidia were spherical, subglobose to ovoid.	Inglis et al. (2020)
Trichoderma yunnanense	The colony was white to greenish-white. Hyaline conidiophores, macroconidiophores within flexible pustules. Irregularly branched, primary branches arise at acute angles or slightly curved towards the apex of conidiophore, solitary or paired. Phialides of macroconidiophores ranged from lageniform to ampulliform, appearing narrow at the base, with short conidia emerging separately or more commonly paired with branches, rarely forming three circles. Conidia were oval to ellipsoidal, with both ends rounded or slightly narrowed at the base.	Yu et al. (2007)

Antagonism test (antagonistic activity, antibiosis, and microparasitism)

The diameter of fungal colony continued to grow until the seventh day of observation (Table 3). Acting as an antagonist, *Trichoderma* grew rapidly, whether grown as a single isolate on PDA media or grown together with the pathogenic fungus *Helminthosporium* sp. This was different from the fungus *Helminthosporium* sp. which were grown together with *Trichoderma* sp. fungus, had an average colony diameter that was smaller than the control.

The percentage of inhibition of *Trichoderma* sp. against *Helminthosporium* sp. increased every day, where on the first day of observation, it reached 33% and on the seventh day it reached 71% (Figure 6). The results of T-test conducted on seventh day of observation obtained a significance (<0.05) value of 0.019, this indicated that the application of *Trichoderma* sp. provide influence or resistance to *Helminthosporium* sp.

The results of antagonist test showed that *Trichoderma* sp. inhibited the growth of *Helminthosporium* sp. through an antibiosis mechanism which was characterized by the formation of a clear zone between the two fungi (Figure 7.A). The competition mechanism can be seen from the growth of *Trichoderma* which grows faster and covers the growth zone of the pathogen *Helminthosporium* sp. (Figure 7.B).

Bioactive compounds from *Trichoderma* sp.

Analysis of secondary metabolites of *Trichoderma* using GC-MS produced a chromatogram with several main peaks representing the identified compounds. A total of 32 compounds were identified, where the first compound was identified at a retention time of 11.295 and the last compound was detected at a retention time of 31.273 (Figure 8).

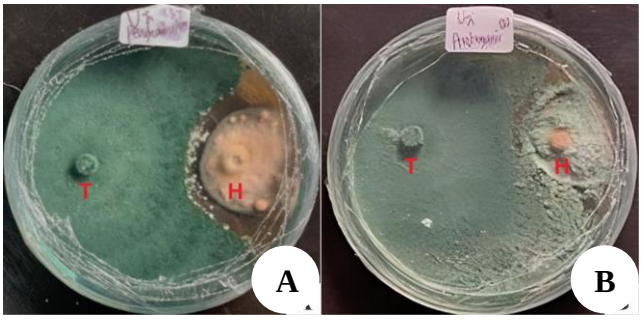


Figure 7. Antagonistic activity of *Trichoderma* sp. against *Helminthosporium* sp.. A. Antibiotics; B. Competition T: *Trichoderma* sp., H: *Helminthosporium* sp.

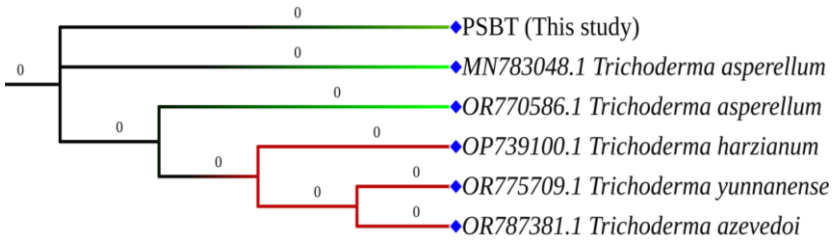


Figure 5. Cladogram of each isolate with five sequences from GenBank

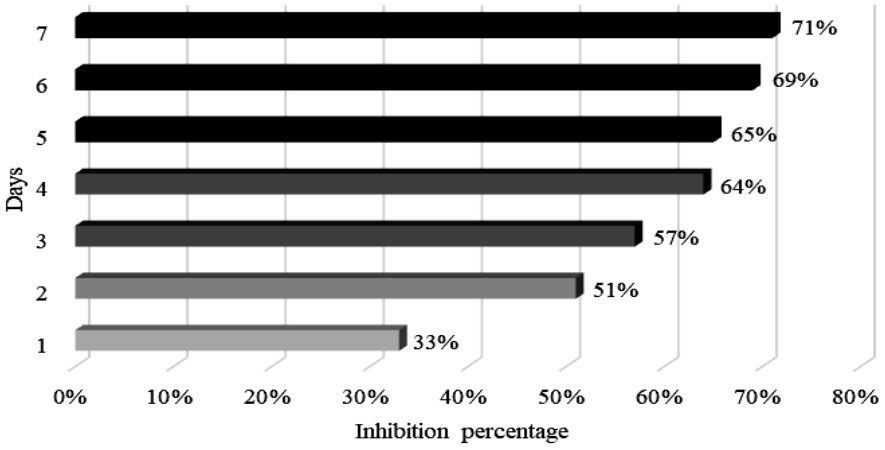


Figure 6. Percentage of inhibitory power of *Trichoderma* sp. against *Helmintosporium* sp.

Table 3. Average diameter of fungal colony growth

Fungi	Average mycelial growth diameter (cm)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Trichoderma sp. (control)	1.00±0.00	4.70±0.82	5.87±0.49	7.37±1.50	7.77±1.10	8.00±1.00	9.00±0.00
Trichoderma sp. (treatment)	1.10±0.14	4.18±0.67	6.02±1.56	6.42±1.42	6.72±1.30	8.06±0.52	8.82±0.35
Helminthosporium sp. (control)	0.67±0.57	1.17 ±0.57	2.30±0.43	2.80±0.43	3.50±0.50	3.83±0.57	5.00±1.56
Helminthosporium sp. (treatment)	0.44±0.24	1.18±0.29	1.70±0.39	1.96±0.52	2.28±0.44	2.70±0.67	2.96±0.84

The compound with the lowest abundance was hexacosanioc acid, methyl ester with a total area of 0.39%, while the compound with the highest abundance was 9,12-octadecadienoic acid (Z,Z) with a total area of 14.44% which was a combination of three of these compounds detected at different retention times, namely at 19.075, 20.100 and 23.321. Three compounds were included in the adehyde group, namely 4-hydoxy-3-methoxybenzaldehyde, butyrovanillone and 2-ethoxy-5-methoxybenzaldehyde. Five compounds were detected as sterols, namely campesterol ergost-5-en-3. beta,-ol, stigmasterol, .gamma.-sitosterol, 4-campestene-3-one, and 4,22-stigmastadiene-3-one. A total of 21 compounds belonged to the fatty acid group with several compounds with the highest abundance, namely n-hexadecanoic acid, hexadecanoic acid, methyl ester, octadecadienoic acid, methyl ester, 9,12-octadecadienoic acid (Z,Z) and the rest had an abundance below 5%. Two other compounds were detected as esters and terpenes, namely hexamethyl-pyranoindane and α-amyrin, respectively (Table 4).

Discussion

Exploration is the first step in efforts to utilize antagonistic fungi as biological agents and also detect diseases caused by pathogenic herbs. The results of the exploration revealed that *Helminthosporium* sp. caused leaf blight in corn plants, this is in accordance with Mahadevakumar and Sridhar (2021) who also reported that *Helminthosporium* sp. is a genus of fungal pathogens known to cause leaf blight on a variety of plants, especially cereal crops.

The conventional identification method involves morphological observations, such as colony shape, color, texture, and microscopic characteristics of conidia and conidiophores. In this study, PSBT isolate was morphologically identified as *Trichoderma* sp. with colonies characterized by dark green color and the presence of concentric rings. The hyphae were hyaline and septate, and conidiophores were vertically upright and branched. The conidia were green, ranging from subglobose to round in shape, with phialides beneath the conidia arranged 2-3 in parallel towards the apex, ampulliform in shape, and slightly swollen in the middle. The prominent feature of *Trichoderma* is the formation of concentric rings in the colony, resulting from the complex interaction between genetic, environmental, and biological factors that influence the fungus's growth and development in the medium (Jangir et al. 2017).

Molecular analysis revealed that the amplified DNA band matched the target size at 600 bp. This aligns with Antil et al. (2023), who stated that the length of ITS region typically ranges from 500 to 600 base pairs (bp), indicating that DNA fragments in this region are sized between 500 and 600 base pairs. This range allows for accurate differentiation between various fungal species, as variations in ITS sequence and length are often species-specific. It was also noted that PSBT isolate showed similarities with four different species of *Trichoderma* (i) *T. asperellum*; (ii) *T. harzianum*; (iii) *T. yunnanense*; and (iv) *T. azevedoi*. 100% identity with multiple different species, can be attributed to several factors. First, genetic conservation within the *Trichoderma* genus results in the genetic markers used having little variation between species. Second, convergent evolution can cause genetically unrelated species to exhibit similarities. Third, genetic polymorphism within species can add complexity to the identification process. Additionally, the use of markers with low resolution or limitations in the scope of reference databases can reduce the accuracy of species identification. Although molecular methods have become powerful tools for species identification, conventional methods remain highly relevant (Gautam et al. 2022). A more comprehensive analysis and the use of broader databases also help to achieve more accurate species identification (Dou et al. 2020; Cai et al. 2021; Brito et al. 2023).

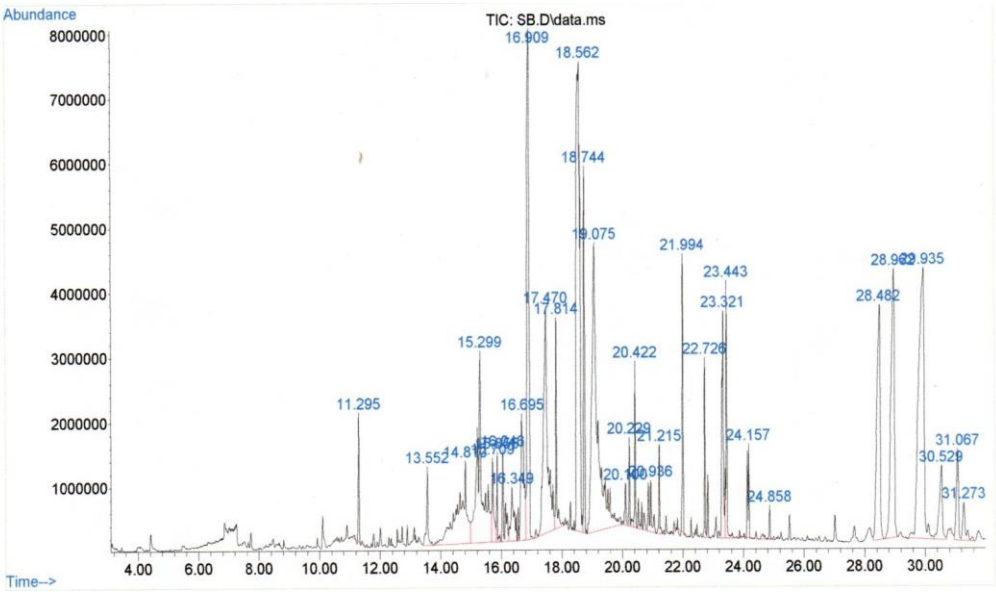
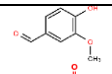
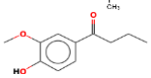
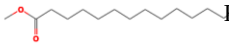
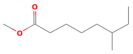
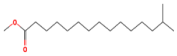
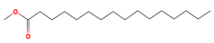
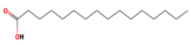
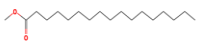
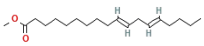
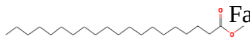
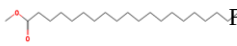
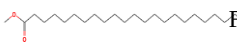
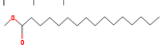
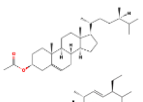
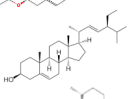
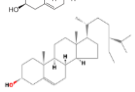
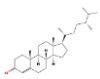
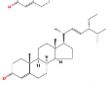
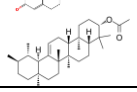


Figure 8. Chromatogram results by chromatography-mass spectrometry (GC-MS) separation

Table 4. Secondary metabolite compounds of *Trichoderma* sp.

Retention time	Compounds name	Area (%)	Formula	Chemical structure	Coumpounds nature
11.295	4-Hydoxy-3 metoxybenzaldehyde	0.77	$C_8H_8O_3$		Aldehyde
13.552	Butyrovanillone	0.79	$C_{11}H_{14}O_3$		Aldehyde
14.813	Methyl Tetradecanoate	4.66	$C_{15}H_{30}O_2$		Fatty acid
15.299	2-Ethoxy-5-methoxybenzaldehyde	6.44	$C_{10}H_{12}O_3$		Aldehyde
15.709	Methyl 6-methyloctanoate	0.95	$C_{10}H_{20}O_2$		Fatty acid
15.855	Pentadecanoic acid, methyl ester	0.56	$C_{16}H_{32}O_2$		Fatty acid
16.046	Gentisic acid	1.14	$C_7H_6O_4$		Fatty acid
16.349	Hexamethyl-pyranoindane	1.33	$C_{18}H_{24}O$		Ester
16.695	4-ethyl-5,6,7,8-tetrahydro	2.59	$C_{13}H_{17}N_3S_2$		NF
16.909	Hexadecanoic acid, methyl ester	8.00	$C_{17}H_{34}O_2$		Fatty acid
17.470	n-hexadecanoic acid	6.64	$C_{16}H_{32}O_2$		Fatty acid
17.814	Heptadecanoic acid, methyl ester	1.44	$C_{18}H_{36}O_2$		Fatty acid
18.562	10,13-Octadecadienoic acid, methyl ester	12.01	$C_{19}H_{34}O_2$		Fatty acid
18.744	Methyl stearate	2.61	$C_{19}H_{38}O_2$		Fatty acid
19.075	9,12-Octadecadienoic acid (Z,Z)	11.60	$C_{18}H_{32}O_2$		Fatty acid
20.100	9,12-Octadecadienoic acid (Z,Z)	0.31	$C_{18}H_{32}O_2$		Fatty acid
20.229	cis-Methyl 11-eicosenoate	0.59	$C_{21}H_{40}O_2$		Fatty acid
20.422	Eicosanoic acid, methyl ester	1.40	$C_{21}H_{42}O_2$		Fatty acid
20.936	9-Octadecyne	0.84	$C_{18}H_{34}$		Fatty
21.215	Heneicosanoic acid, methyl ester	0.55	$C_{22}H_{44}O_2$		Fatty acid
21.994	Docosanoic acid, methyl ester	1.65	$C_{23}H_{46}O_2$		Fatty acid
22.726	Tricosanoic acid, methyl ester	1.28	$C_{24}H_{48}O_2$		Fatty acid
23.321	9,12-Octadecadienoic acid (Z,Z),	2.53	$C_{18}H_{32}O_2$		Fatty acid
23.443	Tetracosanoic acid, methyl ester	1.85	$C_{25}H_{50}O_2$		Fatty acid
24.157	Squalene	0.91	$C_{30}H_{50}$		Fatty
24.858	Hexacosanioc acid, methyl ester	0.39	$C_{17}H_{34}O$		Fatty acid
28.482	Campesterol Ergost-5-en-3. Beta,-ol	5.46	$C_{30}H_{50}O_2$		Sterols
28.962	Stigmasterol	6.43	$C_{29}H_{48}O$		Sterols
29.935	.gamma.-sitosterol	10.49	$C_{29}H_{50}O$		Sterols
30.529	4-Campestene-3-one	1.64	$C_{28}H_{46}O$		Sterols
31.067	4,22-Stigmastadiene-3-one	1.37	$C_{29}H_{46}O$		Sterols
31.273	α-Amyrin	0.78	$C_{30}H_{50}O$		Terpenes

Results of inhibition revealed that *Trichoderma* isolate PSBT showed good inhibitory power against *Helminthosporium* sp. with inhibition reached 71% on the seventh day of observation. This level of inhibition comes into the category of moderately strong inhibition. At the first day *Trichoderma* sp. started to show an inhibitory effect against *Helminthosporium* sp., although it was relatively low. A significant increase (51%) on the second day indicated that *Trichoderma* sp. had the ability to inhibit the growth of *Helminthosporium* sp., this may be because it produces more inhibitory enzymes and metabolites. *Trichoderma* sp. produces enzymes, such as chitinase, glucanase, and protease, which can degrade the cell walls of pathogenic fungi, causing direct damage and inhibiting their growth (Tyśkiewicz et al. 2023). On the seventh day, it inhibited the maximum growth of *Helminthosporium* sp. This suggests that *Trichoderma* sp. produces maximum amounts of inhibitory metabolites that are effective against *Helminthosporium* sp.

The process of antagonism of *Trichoderma* sp. against pathogens is conducted in several ways such as mycoparasitism, antibiosis and competition (Mukherjee et al. 2022). A clear zone was formed in antagonism test of *Trichoderma* sp. this may be due to its antagonistic activity against *Helminthosporium* sp., such as mycoparasitism and metabolite compound activity. The metabolite compounds produced play an important role in the antagonistic ability of the *Trichoderma*. Volatile compounds, such as aldehydes, esters and terpenes have been linked to the mycoparasitism ability of *Trichoderma* (Bhardwaj and Kumar 2017), where GC-MS results found several volatile compounds such as 4-Hydoxy-3-methoxybenzaldehyde, Butyrovanillone, 2-Ethoxy-5 -methoxybenzaldehyde, Hexamethyl-pyranoindane and α-Amyrin. A total of four fatty acid compounds, namely n-hexadecanoic acid, Hexadecanoic acid, methyl ester, Octadecadienoic acid, methyl ester, 9,12-Octadecadienoic acid (Z,Z) is the compound with the greatest abundance produced by the fungus *Trichoderma* sp. is known to play an important role in the ability of antagonism. Several studies have reported that these four compounds have antimicrobial, antibacterial and antifungal activity (Srinivasa 2017; Yuef et al. 2018; Amaechi 2021; El-Nasr et al. 2023; Lakhdari et al. 2023). The other five compounds that fall into the sterol group were campesterol ergost-5-en-3. beta,-ol, stigmasterol, .gamma.-sitosterol, 4-campestene-3-one and 4,22-stigmastadiene-3-one were isoprenoid derivative compounds. These compounds are important for the growth and development of eukaryotic organisms, and can be found in animals, plants, and fungi (Ślusarczyk et al. 2021).

Trichoderma has been widely used to control pathogenic fungi causing disease in plants (Yadav et al. 2020; Alwadai et al. 2022; López-Valenzuela et al. 2022), however, the use of *Trichoderma*, especially local isolates, has become important to support sustainable agriculture. Local isolates of *Trichoderma* have specific advantages as they have adapted to local environmental conditions, such as temperature, humidity, and soil type, making them more effective in controlling pathogens that also originate from the same environment (Lahlali et al. 2022). This adaptation provides significant benefits, having high competitive ability against pathogens and other microorganisms, enhancing its effectiveness in disease control (Abdullah et al. 2021). Furthermore, the use of local isolates supports more environmentally friendly agricultural practices by reducing the need for chemical fungicides that can potentially harm soil and water, as well as pose risks to human and animal health. The use of antagonistic fungi like *Trichoderma* also contributes to soil health management by increasing soil microbiota activity, which ultimately enhances soil fertility and agricultural land productivity (Asghar et al. 2024). From an economic perspective, the use of local antagonistic fungi supports the local economy by reducing dependency on imported products and promoting the use of local resources. This not only lowers production costs for farmers but also creates opportunities for innovation and the development of local industries, such as the mass production of *Trichoderma* based biofungicides (Kumar et al. 2023).

In conclusion, the local Tomohon isolate was identified as *Trichoderma asperellum* after morphological and molecular analysis. The results of GC-MS analysis showed that *T. asperellum* produced secondary metabolites in the form of volatile compounds. The four fatty acid compounds were n-hexadecanoic acid, hexadecanoic acid, methyl ester, octadecadienoic acid, methyl ester, 9,12-octadecadienoic acid (Z,Z), play an important role in inhibiting the growth of *Helmintosporium*. From these results, the local isolates of the *T. asperellum* fungus have great potential to be used as a biological control agent.

ACKNOWLEDGEMENTS

The authors are grateful to the *Direktorat Riset, Teknologi, dan Pengabdian Kepada Masyarakat* (DRTPM) of the Ministry of Education, Culture, Research, and Technology, Republic of Indonesia for the funding provided through the basic research scheme for the 2024 fiscal year with contract number: 084/E5/PG.02.00.PL/2024. The authors claim that there are no conflicting interests involved in making this information public.

REFERENCES

Abdullah NS, Doni F, Mispan MS, Saiman MZ, Yusuf YM, Oke MA, Suhaimi NSM. 2021. Harnessing *Trichoderma* in agriculture for productivity and sustainability. Agronomy 11 (12): 2559. DOI: 10.3390/agronomy11122559.

Alshuniaber MA, Krishnamoorthy R, AlQhtan WH. 2020. Antimicrobial activity of polyphenolic compounds from *Spirulina* against food-borne bacterial pathogens. Saudi J Biol Sci 28 (1): 459-464. DOI: 10.1016/j.sjbs.2020.10.029.

Alwadai AS, Perveen K, Alwahaibi M. 2022. The isolation and characterization of antagonist *Trichoderma* spp. from the soil of Abha, Saudi Arabia. Molecules 27: 2525. DOI: 10.3390/molecules27082525.

Amaechi NC. 2021. Evaluation of bioactive compounds in *Moringa oleifera* flower using gas chromatography mass spectrometry/fourier transform infrared spectroscopy: The need for good postharvest handling. Acta Sci Nutr Health 5 (12): 112-122.

Antil S, Abraham JS, Sripoorna S, Maurya S, Dagar J, Makhija S, Bhagat P, Gupta R, Sood U, Lal R, Toteja R. 2023. DNA barcoding, an

effective tool for species identification: A review. Mol Biol Rep 50: 761-775. DOI: 10.1007/s11033-022-08015-7.

Asghar W, Craven KD, Kataoka R et al. 2024. The application of *Trichoderma* spp., an old but new useful fungus, in sustainable soil health intensification: A comprehensive strategy for addressing challenges. Plant Stress 12: 100455. DOI: 10.1016/j.stress.2024.100455.

Asis A, Shahriar SA, Naher L et al. 2021. Identification patterns of *Trichoderma* strains using morphological characteristics, phylogenetic analyses and lignocellulolytic activities. Mol Biol Rep 48 (4): 3285-3301. DOI: 10.1007/s11033-021-06321-0.

Bhardwaj NR, Kumar J. 2017. Characterization of volatile secondary metabolites from *Trichoderma asperellum*. J Appl Nat Sci 9 (2): 954-959. DOI: 10.31018/jans.v9i2.1303.

Boonupara T, Udomkun P, Khan E, Kajitvichyanukul P. 2023. Airborne pesticides from agricultural practices: A critical review of pathways, influencing factors, and human health implications. Toxics 11 (10): 858. DOI: 10.3390/toxics11100858.

Brito VN, Alves JL, Araújo KS, de Souza Leite T, de Queiroz CB, Pereira OL, de Queiroz MV. 2023. Endophytic *Trichoderma* species from rubber trees native to the Brazilian Amazon, including four new species. Front Microbiol 14: 1095199. DOI: 10.3389/fmicb.2023.1095199.

Cai F, Druzhinina IS. 2021. In honor of John Bissett: Authoritative guidelines on molecular identification of *Trichoderma*. Fungal Divers 107: 1-69. DOI: 10.1007/s13225-020-00464-4.

de Sousa TP, Chaibub AA, Cortes MVdB, Batista TFC, de Andrade Bezerra G, da Silva GB, de Filippi MMC. 2021. Molecular identification of *Trichoderma* sp. isolates and biochemical characterization of antagonistic interaction against rice blast. Arch Microbiol 203: 3257-3268. DOI: 10.1007/s00203-021-02307-5.

Dou K, Lu Z, Wu Q, Ni M, Yu C, Wang M, Li Y, Wang X, Xie H, Chen J, Zhang C. 2020. MIST: A multilocus identification system for *Trichoderma*. Appl Environ Microbiol 86: e01532-20. DOI: 10.1128/AEM.01532-20.

El-Nasr AA, Elaasser MM, Elsaba YM, Mokhtar FY. 2023. Antioxidant, antimicrobial, and anticancer cells line of *Aspergillus flavus* ON764430 extracts isolated from Al Mudawara Mountain, El Fayum governorate. Adv Basic Appl Sci 1 (1): 33-45. DOI: 10.21608/abas.2023.194936.1005.

Ferreira FV, Musumeci MA. 2021. *Trichoderma* as biological control agent: Scope and prospects to improve efficacy. World J Microbiol Biotechnol 37 (5): 90. DOI: 10.1007/s11274-021-03058-7.

Gautam AK, Verma RK, Avasthi S, Sushma, Bohra Y, Devadtha B, Niranjan M, Suwannarach N. 2022. Current insight into traditional and modern methods in fungal diversity estimates. J Fungi 8 (3): 226. DOI: 10.3390/jof8030226.

Gullino ML, Albajes R, Al-Jboory I, Angelotti F, Chakraborty S, Garrett KA, Hurley BP, Juroszek P, Lopian R, Khaled Makkouk K, Xubin Pan X, Pugliese M Tannecia Stephenson T. 2022. Climate change and pathways used by pests as challenges to plant health in agriculture and forestry. Sustainability 14: 12421. DOI: 10.3390/su141912421.

Hu YF, Liu JW, Xu ZH, Castañeda-Ruíz RF, Zhang K, Ma J. 2023. Morphology and multigene phylogeny revealed three new species of *Helminthosporium* (Massarinaceae, Pleosporales) from China. J Fungi 9 (2): 280. DOI: 10.3390/jof9020280.

Inglis PW, Mello SCM, Martins I et al. 2020. *Trichoderma* from Brazilian garlic and onion crop soils and description of two new species: *Trichoderma azevedoi* and *Trichoderma peberdyi*. PLoS One 15 (3): e0228485. DOI: 10.1371/journal.pone.0228485.

Iswati R, Aini LQ, Soemarno, Abadi AL. 2024. Exploration and characterization of indigenous *Trichoderma* spp. as antagonist of *Rhizoctonia solani* and plant growth promoter of maize. Biodiversitas 25 (4): 1375-1385. DOI: 10.13057/biodiv/d250405.

Jangir M, Pathak R, Sharma S. 2017. *Trichoderma* and its potential applications. In: Singh DP, Singh HB, Prabha R (eds). Plant-Microbe Interactions in Agro-Ecological Perspectives. Springer, Singapore. DOI: 10.1007/978-981-10-6593-4_13.

Kumar V, Koul B, Taak P, Yadav D, Song M. 2023. Journey of *Trichoderma* from pilot scale to mass production: A review. Agriculture 13 (10): 2022. DOI: 10.3390/agriculture13102022.

Lahlali R, Ezrari S, Radouane N, Kenfaoui J, Esmaeel Q, Hamss HE, Belabess Z, Barka EA. 2022. Biological control of plant pathogens: A global perspective. Microorganisms 10 (3): 596. DOI: 10.3390/microorganisms10030596.

Lakhdari W, Benyahia I, Bouhenna MM, Bendif H, Khelafi H, Bachir H, Ladjal A, Hammi H, Mouhoubi D, Khelil H, Alomar TS, AlMasoud N, Boufafa N, Boufahja F, Dehliz A. 2023. Exploration and evaluation of secondary metabolites from *Trichoderma harzianum*: GC-MS analysis, phytochemical profiling, antifungal and antioxidant activity assessment. Molecules 28 (13): 5025. DOI: 10.3390/molecules28135025.

López-Valenzuela B, Tzintzun-Camacho O, Armenta-Bojórquez A, Valenzuela-Escoboza F, Lizárraga-Sánchez G, Ruelas-Islas J, González-Mendoza D. 2022. Microorganisms of genus *Trichoderma* as phytohormone promoters and pathogen suppressors. Bioagro 34 (2): 163-172. DOI: 10.51372/bioagro342.6.

Mahadevakumar S, Sridhar KR. 2021. Diversity of pathogenic fungi in agricultural crops. In: Dubry SK, Verma SK (eds). Rhizosphere Biology. Springer, Singapore. DOI: 10.1007/978-981-16-3364-5_6.

Manjur SM, Afiya H. 2019. Introductory chapter: Identification and isolation of *Trichoderma* spp. - their significance in agriculture, human health, industrial and environmental application. In: Shah MM, Sharif U, Buhari TR (eds). *Trichoderma* - The Most Widely Used Fungicide. IntechOpen, London. DOI: 10.5772/intechopen.83528.

Mayo-Prieto S, Campelo MP, Lorenzana A et al. 2020. Antifungal activity and bean growth promotion of *Trichoderma* strains isolated from seed vs soil. Eur J Plant Pathol 158: 817-828. DOI: 10.1007/s10658-020-02069-8.

Mukherjee PK, Mendoza-Mendoza A, Zeilinger S, Horwitz BA. 2022. Mycoparasitism as a mechanism of *Trichoderma*-mediated suppression of plant diseases. Fungal Biol Rev 39: 15-33. DOI: 10.1016/j.fbr.2021.11.004.

Priyashantha AKH, Karunarathna SC, Lu L, Tibpromma S. 2023. Fungal endophytes: An alternative biocontrol agent against phytopathogenic fungi. Encyclopedia 3: 759-780. DOI: 10.3390/encyclopedia3020055.

Putri ND, Sulistyowati L, Aini LQ, Muhibuddin A, Trianti I. 2022. Screening of endophytic fungi as potential antagonistic agents of *Pyricularia oryzae* and evaluation of their ability in producing hydrolytic enzymes. Biodiversitas 23: 1048-1057. DOI: 10.13057/biodiv/d230248.

Ramona Y, Darmayasa IBG, Line MA. 2022. Biological control of *Sclerotinia minor* attack on pyrethrum plants by *Trichoderma harzianum* in glasshouse experiment. Biodiversitas 23: 3264-3269. 10.13057/biodiv/d230655.

Senanayake I. 2020. Morphological approaches in studying fungi: Collection, examination, isolation, sporulation, and preservation. Mycosphere 11 (1): 2678-2754. DOI: 10.5943/mycosphere/11/1/20.

Shabana YM, Ghoneem KM, Rashad YM et al. 2022. Distribution and biodiversity of seed-borne pathogenic and toxigenic fungi of maize in Egypt and their correlations with weather variables. Plants 11 (18): 2347. DOI: 10.3390/plants11182347.

Shah J, Ramzan U, Naseer S, Khalid MN, Amjad I, Majeed T, Sabir W, Shaheen MK, Ali B, Shamim F, Nazeer S. 2023. Chemical control of southern leaf blight of maize caused by *Helminthosporium maydis*. Biol Clin Sci Res J (1): 225-225. DOI: 10.54112/bcsrj.v2023i1.225.

Simamora M, Basyuni M, Lisnawita. 2021. Potency of secondary metabolites of *Trichoderma asperellum* and *Pseudomonas fluorescens* in the growth of cocoa plants affected by vascular streak dieback. Biodiversitas 22: 2542-2547. DOI: 10.13057/biodiv/d220511.

Skidmore AM, Dickinson CH. 1976. Colony interactions and hyphal interference between *Septoria nodorumand* phylloplane fungi. Trans Br Mycol Soc 66 (1): 57-64. DOI: 10.1016/S0007-1536(76)80092-7.

Ślusarczyk J, Adamska E, Czerwik-Marcinkowska J. 2021. Fungi and algae as sources of medicinal and other biologically active compounds: A review. Nutrients 13 (9): 3178. DOI: 10.3390/nu13093178.

Srinivasa N, Sriram S, Singh C, Shivashankar KS. 2017. Secondary metabolites approach to study the bio-efficacy of *Trichoderma asperellum* isolates in India. Intl J Curr Microbiol App Sci 6 (5): 1105-1123. DOI: 10.20546/ijcmas.2017.605.120.

Tudi M, Daniel RH, Wang L, Lyu J, Sadler R, Connell D. 2021. Agriculture development, pesticide application and its impact on the environment. Intl J Environ Res Public Health 18 (3): 1112. DOI: 10.3390%2Fijerph18031112.

Tyśkiewicz R, Nowak A, Ozimek E, Jaroszuk-Ściseł J. 2023. *Trichoderma*: The current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. Intl J Mol Sci 23 (4): 2329. DOI: 10.3390/ijms23042329.

Woo SL, Hermosa R, Lorito M, Monte E. 2023 *Trichoderma*: A multipurpose, plant-beneficial microorganism for eco-sustainable agriculture. Nat Rev Microbiol 21: 312-326. DOI: 10.1038/s41579-022-00819-5.

Yadav GK, Yadav RS, Singh G, Khilari K, Mishra P, Singh H. 2020. Evaluate the inhibitory ability of fungicides and biocontrol agents against *Pyricularia oryzae* and *Helminthosporium oryzae* in vitro. Intl J Curr Microbiol App Sci 9 (8): 3569-3575. DOI: 10.20546/ijcmas.2020.908.411.

Yadav D, Adhikari A, Dhuingana B, Gurung H, Khatri N, Pandit S. 2022. In-vitro efficacy of *Trichoderma* isolates on *Sclerotium rolfsii* causing collar rot of chili. Asian J Agric 6 (2): 97-102. DOI: 10.13057/asianjagric/g060206.

Yu ZF, Qiao M, Zhang Y, Zhang KQ. 2007. Two new species of *Trichoderma* from Yunnan, China. Antonie van Leeuwenhoek 92 (1): 101-108. DOI: 10.1007/s10482-006-9140-4.

Yuef MH, Ariel TJ, Raúl R, Alberto LJ, Benigno E, Eduardo O. 2018. Identification and evaluation of secondary metabolites by gas chromatography-mass spectrometry (GC-MS) in native strains of *Trichoderma* species. Afr J Biotechnol 17 (37): 1162-1171. DOI: 10.5897/AJB2018.16546.