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The Biocontrol Potential of *Trichoderma* Species for Combatting Seedling Blight in Flax

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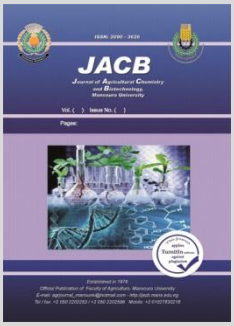

ABSTRACT

In this study, samples of seedlings infected with damping-off symptoms were obtained from different Governorates. A total 102 pathogenic fungal isolates were collected from samples. The pathogenic isolates were frequency percentage as *Rhizoctonia solani* (9.80%), *Fusarium* sp. (29.41%), *Pythium* sp. (7.84%), *Penicillium* sp. (8.82%), *Alternaria* spp. (20.59%), *Trichoderma* sp. (2.94%), *Aspergillus* sp. (9.80%), *Chaetomium* sp. (0.98%), *Sphaerosorium* sp. (0.98%) and unknown (8.82%). Under glasshouse circumstances, the pathogenicity of all 102 fungal isolates was assessed on flax cultivar Giza 12 seedlings. The most effective fungal isolates that decreased survival percentage were F81, RS68, PY97 and Pen58. *In vitro*, the antifungal activity of six *Trichoderma* isolates were isolated and tested against four pathogenic fungi (Rs68), (F81), (Py97) and (Pen58). The *Trichoderma* isolate (T14) was the most effective as it decreased (F81) growth by 78.67%, isolate (T23) inhibited growth by 87% on *Pythium* (Py97). Also, the isolates of *Trichoderma* efficiency in controlling damping off of flax seedlings caused by all chosen pathogenic isolates (Rs68), (F81), (Py97) and (Pen58) when tested under glasshouse conditions. In the case of *R. solani* (Rs68) and *Pythium* (P97), T14 and T23 showed maximum disease control efficacy (0.00% suppression). *In vitro*, on differentiate media were used to evaluate the growth performance of *Trichoderma* spp. Only three as the most potential of *Trichoderma* isolates were identified as *Trichoderma harzianum* (T12), *T. longibrachiatum* (T14) and *T. viride* (T23). Finally, in flax Trichoderma , could be a safe strategy to use as biocontrol agents for control fungal disease.

***Keywords*:** biocontrol; damping-off symptoms; flax blight; *Trichoderma* spp

INTRODUCTION

Flax (*Linum usitatissimum* L.) is a bluish-flowered plant, in mainly the cooler regions of the world has been used for fiber and food purposes. Several benefits for animal and human health were provided furthermore, linseed is the highest omega- 3 oleaginous crop (Moyse *et al.* 2023). A bout many years ago, flax is considered one of the earliest crops and significant cash fiber crop in Egypt. Also, it positions second crop plants after cotton in terms of financial importance and production. Several of soilborne fungi are causes seedling blight of flax by the most important of which are *Rhizoctonia solani* and *Fusarium* spp. and *Pythium* spp. (Aly *et al.* 2013). The most common flax diseases in flax are wilt, root rots, mildew, seedling blight and flax rust (Perryman *et al.* 2000). Since 1996 in Lithuania were stated flax diseases: Fusarium wilt, anthracnose (seedling blight), seedling spotting, stem break and browning (Gruzdevienė *et al.* 2008).

Biological control of plant pathogens is very important for environment and health issues attributed to the use of fungicides in agriculture. The interest in biological control using *Trichoderma* is in line with ensuring environmental sustainability and sustainable agriculture by applying the principles of ecology to disease control (Cumagun, 2012). The first step in the biocontrol study is the identification of promising biocontrol agent. In the case of *Trichoderma* as a biocontrol agent both solid and liquid formulations are used to produce suitable quantities of active and viable inocula. There are three kinds of propagules can be used in formulations: hyphae, chlamydospores and conidia (Howell, 2003). To elevate the biocontrol ability, *Trichoderma* species are available as fungal agents for the control of plant diseases by several mechanisms including competition, antibiosis, antagonism and mycoparasitism (Rahman *et al.*, 2023). The majority of commercial preparations consist of asexual reproductive spores (conidia) of genus *Trichoderma* therefore, is an essential feature of a successful biocontrol agent (Steyaert *et al.*, 2010). Mycoparasitism, competition for nutrients and/or space, antibiosis, and induction of systemic resistance are effective biocontrol tools is achieved. Results showed that seed coating enhanced growth, reduced root symptoms, and made plants more resistant to flax scorch until 8 weeks old. (Cariou - Pham and Bonnan, 2006). A number of reports demonstrated that *Trichoderma* isolates could be effectively used for controlling soil-borne fungi on flax. The *Trichoderma* spp. are the most beneficial because they proved a higher level of safety with the least amount of environmental damage (Nofal *et al.* 2021). *Trichoderma* spp., are typical aerobic, facultative and cosmopolitan fungi that can be found in large numbers in agricultural soils and in other substrates such as decaying wood (Irina and Christian 2004). They belong to the subdivision Deuteromycetes, members a determinate sexual state as most strains are adapted to an asexual life cycle (Harman 2004).

This article aims to provide a comprehensive overview of the potential of *Trichoderma* spp. as a biological control agent for managing seedling blight in flax crops, with the goal of enhancing crop productivity and sustainability in agricultural systems. This research is screening of *Trichoderma* spp. with antagonistic activity against flax soil pathogens. The control

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effect of selected strains against seedling blight in flax was further assessed *In vitro* and in the glasshouse.

MATERIALS AND METHODS

Source of bioagents as *Trichoderma* isolates

Six isolates of *Trichoderma* spp. (T12, T14, T20, T21, T23 and T34) were selected from the *Trichoderma* culture collection of Cotton and Fiber Crops Disease Research Section; Plant Pathology Research Institute., Agricultural Research Center, Giza, Egypt. For morphological appearance *Trichoderma* isolates were grown in differentiate media Nutrient Agar medium (NA) (Devika *et al*. 2021), Sabouraud Dextrose Agar medium (SDA) (Scognamiglio *et al*. 2010), Potato Dextrose Agar medium (PDA) (Sneh *et al*. 1991) and Carrot Agar medium (CA) (Mustafa *et al*. 2009 & Jahan *et al*. 2013). In addition, *Trichoderma* species were identified based on morphological techniques in the Regional Center of Mycology and Biotechnology Al-Azhar University, Egypt (Samuels and Hebbar, 2015).

Source of phytopathogens

Isolates of pathogenic fungi found in the present study were obtained from samples of flax seedlings with typical damping-off symptoms and the samples were collected from different regions of four flax producing Governorates (Garbia, Dakahliya, Kafr El-sheikh and Giza). At Cotton and the Fiber Crops Diseases Research Section, Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt, the developing colonies were identified according to Gilmen (1966) or Barnett and Hunter (1972). The isolates were cultured and maintained on PDA slants for further studies.

Pathogenicity tests:

Preparation of fungal inoculum

Fifty g of sorghum grains and 40 ml of tap water were combined in 500 mL glass bottles that served as the growing medium for each chosen isolate. The bottles' contents were autoclaved. After being removed from a one-week-old PDA culture, the isolated inocula was aseptically added to the bottle and given three weeks to colonize the sorghum.

Pathogenicity of isolated fungi under glasshouse

The soil used in the current experiments is autoclaved clay loam. Separate batches of soil were inoculated 1 g/kg of soil for *R. solani*, 50 g/kg of soil for other fungi. Clay pots with a diameter of 10 cm were filled with infested soil and filled with twenty flax seeds cultivar Giza 12. Sterilized sorghum grains (cv. Balady) were well mixed with soil at a rate of 1 g/kg of soil for *R. solani* and 50 g/kg of soil for other fungi in the control treatment. For every fungal isolate, there were five replicates. Plants were arranged haphazardly on benches within the glasshouse. The glasshouse had a heating system installed to guarantee that the lowest temperature within the glasshouse was kept at 28°C, but because there was no cooling system, the highest temperature could not be regulated and would instead fluctuate between 30 and 35°C depending on the daytime high. (The experiments were conducted in Novamber and Decamber 2020). Forty-five days following planting, dead seedlings (including pre-emergence and post-emergence damping off) were noted (Aly *et al.* 1996).

Soil analysis

Soil particle size analysis was made by the pipette method according to piper (1945). Physico-chemical properties of soil were analyzed according to Jackson (1973).

***In vitro* antagonism**

In vitro, the six isolates of *Trichoderma* spp. were evaluated as antagonists toward pathogenic isolates of *R. solani* (RS68), *Fusarium* (F81), *Pythium* (Py97) and *Penicillium* (Pen58) obtained from pathogenicity test. According to (Skidmore and Dickinson, 1976), dual cultures technique was carried out by using one-week-old cultures of pathogenic fungi and *Trichoderma* spp. on PDA medium. In the control treatment, a disc of the pathogen only was placed in dish. There were three plates (replicates) for each treatment. The antagonistic effects of the *Trichoderma* isolates were determined by measuring the free inhibition zone (mm), at the end of the incubation period.

Preparation of *Trichoderma* -sorghum mixture for glasshouse conditions

Fifty g of sorghum grains and 40 ml of tap water were placed in 500 ml bottles as a carefully prepared medium for the growth of *Trichoderma* isolates. The contents of each bottle were sterilized for 30 minutes to disinfect. Vaccine isolates from a week-old culture were grown on PDA and aseptically placed in bottles to colonize sorghum for 21 days. The dry *Trichoderma* -sorghum mixture was homogenized to a fine powder in a mixer (Papvizas and Lewis, 1981).

Glasshouse experiment

In a glasshouse pots, containing autoclaved soil, were infested with two-week old pathogen-sorghum cultures of *R. solani* (RS68), *Fusarium* (F81), *Pythium* (Py97) and *Penicillium* (Pen58) at rates of 1, 50, 50, and 50 g/kg soil, respectively. *Trichoderma* -sorghum mixture of T12, T14, T20, T21, T23 and T34 were applied to surface sterilized flax seeds (Giza 12) at a rate of 7 g/kg seeds. Every isolate was inoculated into slightly moist seeds, which were then thoroughly shaken in plastic bags for five minutes before being planted into 10-cm-diameter clay pots containing 0.5 kg of autoclaved soil (20 seeds/ pot). In the control treatments, sterilized sorghum grains were carefully mixed with soil and untreated flax seeds were planted. In negative control, infested soil at the rate of 1 g/kg of soil for *R. solani* (RS68) and 50 g/kg of soil for *Fusarium* sp. (F81), *Pythium* (Py97) and *Penicillium* (Pen58) and flax seeds without any treatment were planted. In glasshouse bench, pots were distributed randomly under a temperature range 23-27°C. Plant height (cm/plant), dry weight (mg/plant), and survival percentage were all measured 45 days after planting. The treatments in the glasshouse experiment are presented in Table (1).

Table 1. The treatments in the glasshouse experiment

No.	Treatment	Rate of application
1	Autoclaved soil	1 g for *R. solani* and 50 g for *Fusarium*, *Pythium* and *Penicillium* sterilized sorghum/kg soil
2	Infested soil	1 g for *R. solani* and 50 g for *Fusarium*, *Pythium* and *Penicillium* infested sorghum/kg soil
3	T 12+infested soil	7 g/kg seeds
4	T14 +infested soil	7 g/kg seeds
5	T20+infested soil	7 g/kg seeds
6	T21+infested soil	7 g/kg seeds
7	T23+infested soil	7 g/kg seeds
8	T34+infested soil	7 g/kg seeds

Statistical analysis

The pathogenicity tests were designed as a randomized complete block consisting of five replicates. The MSTAT-C Statistical Package was used to do an analysis of variance (ANOVA) on the data. The isolate means were compared using the least significant difference (LSD) at $P \leq 0.05$. After converting % data into arc-sine angles, where x is

the percentage data, the data were normalized, and variances were stabilized over the data range using the ANOVA.

RESLTUS AND DISCUSSION

Isolation, identification, and quantification of fungal pathogens from flax roots

From different regions of four flax producing Governorates (Gharbia, Dakahliya, Kafr El-sheikh and Giza), samples of blighted flax seedlings plants were collected. A bout of 102 isolates were collected and identified as *Fusarium* spp. (29.41%), *Alternaria* spp. (20.59%), *Rhizoctonia solani* (9.80%), *Pythium* spp. (7.84%), *Penicillium* spp. (8.82%), *Trichodirma* spp. (2.94%), *Aspergillus* spp. (9.80%), *Chaetomium* spp. (0.98%), *Sphaerosorium* (0.98%) and other species (8.82%). Samples obtained from Dakahliya showed the highest isolation frequency (43.14%), while those obtained from Gharbia showed the lowest isolation frequency (10.78%). Table (2) showed isolation frequency of fungi isolated from flax seedlings showing typical damping-off of plants symptoms collected.

Table 2. Frequency of fungal pathogens isolated from flax seedlings damping-off

Fungal pathogens	Total no. of isolates	frequency of isolates(%)	frequency of geographic origin (%) of isolates			
			Dakahliya	Kafr El-sheikh	Gharbia	Giza
Fusarium spp.	30	29.41	33.33	43.33	13.33	10.00
Alternaria spp.	21	20.59	52.38	28.57	19.05	0.00
R. solani	10	9.80	70.00	30.00	0.00	0.00
Pythium spp.	8	7.84	0.00	37.50	12.50	50.00
Penicillium spp.	9	8.82	33.33	55.55	11.11	0.00
Trichoderma spp.	3	2.94	100.00	0.00	0.00	0.00
Aspergillus spp.	10	9.80	30.00	50.00	10.00	10.00
Chaetomium spp.	1	0.98	0.00	0.00	0.00	100.00
Sphaerosorium spp.	1	0.98	0.00	0.00	0.00	100.00
Other isolates	9	8.82	77.78	11.11	11.11	0.00
Total	102	100	43.14	34.31	10.78	11.77

Based on the findings of pathogenicity tests, which included 38.60% of the pathogenic isolates in both tests, it was concluded that *Fusarium* sp. constitute the primary cause of flax seedling blight (Table 3). Total number of pathogenic isolates was 57 which represented 62.86% of total isolated fungi. It was found that 73.33% of obtained *Fusarium* spp. were pathogenic isolates representing 21.56% of total isolates. Both *Penicillium* spp. and *Alternaria* spp. represented 15.79 % of the pathogenic isolates. *Pythium* spp. represented 12.28 % and *R. solani* accounted 8.77% of the pathogenic isolates. Unidentified isolates were 5.26% of the pathogenic isolates. Finally, both *Sphaerosorium* spp. and *Trichoderma* spp. represented 1.75% of the pathogenic isolates.

Table 3. Distribution of pathogenic isolates from flax roots

Fungus	Total number of pathogenic isolates	Isolates within fungus (%)	Total isolates (%)	Pathogenic isolates (%)
Pythium spp.	7	87.50	6.86	12.28
Penicillium spp.	9	100.00	8.82	15.79
Alternaria spp.	9	42.86	8.82	15.79
Fusarium spp.	22	73.33	21.56	38.60
R. solani	5	50.00	4.90	8.77
Trichoderma spp.	1	33.33	0.98	1.75
Sphaerosorium spp.	1	100.00	0.98	1.75
Chaetomium spp.	0.00	0.00	0.00	0.00
Others fungi	3	33.33	9.94	5.26
Total	57		62.86	100.00

This study aimed to identify and determine the distribution of fungal pathogens associated with blight in flax seedlings and adult plants in Egypt. Among the different fungal species identified, *Fusarium* spp. was the most prevalent, accounting for 29.41% of the isolates. *Alternaria* spp. was the second most prevalent (20.59%), followed by *Rhizoctonia solani* (9.80%), *Pythium* spp. (7.84%), *Penicillium* spp. (8.82%), and others. The specific distribution and prevalence of these pathogens in each governorate will be described in more detail in four Governorates. Our random sample's prevalence of *F. oxysporum* is consistent with prior results that show this species comprises a significant component of the fungal flora. For instance, *F. oxysporum* accounted for almost 67% of all *Fusarium* spp. in Canadian soil, according to Gordon's (1956) research, making it by far the most common species. In the rhizosphere, its relative abundance can reach 43% of the total microfungal population (Meyer, 1967). It is highly likely that *F. oxysporum* and *F. solani* are the most significant pathogenic fusaria implicated in the etiology of seedling blight and root rot flax in Egypt due to their high frequency and capacity to inflict significant losses during the seedling stage (Aly *et al.* 2017). *Aspergillus* spp., *Fusarium* spp. and *Rhizoctonia solani* were isolated from the infected flax seedlings (Zayed *et al.* 2019).

When application koch postulates, fungal isolates recorded same symptoms of pre- and post-emeregence damping-off as well as seedling survival of cotton seedlings (Ashour and Afify 2025).

Effect of *Trichoderma* isolates on fungal pathogens caused damping-off of flax seedlings

Six isolates of *Trichoderma* spp. (T12, T14, T20, T21, T23 and T34) were randomly selected from the *Trichoderma* cultures collection. Isolates were distributed as T14, T20, T21, T34 (66.6%), T12 (16.7%) and T23 (16.7%). Samples obtained from Dakahliya and Gharbia showed the highest isolation frequency (100.0%), while those obtained from Giza showed the moderate isolation frequency (50.0%), but others from Menofia and Gharbia showed the lowest isolation frequency (25.0%). Table (4) showed isolation frequency of *Trichoderma* isolates collected from four Governorates. Fungi as *Trichoderma* spp. are the most widely used to control damping off caused by pathogenic fungi in the field (Papavizas 1985).

Table 4. Frequency of *Trichoderma* isolated

Trichoderma isolates	Total no. of isolates	Isolation frequency of isolates (%)	Isolation frequency of geographic origin (%)			
			Dakahliya	Menofia	Gharbia	Giza
T14, T20, T21, T34	4	66.6	0.00	25.0	25.0	50.0
T12	1	16.7	0.0	0.0	100.0	0.0
T23	1	16.7	100.0	0.0.	0.0	0.0
Total	6	100	16.7	16.7	33.3	33.3

In vitro antagonism of _Trichoderma_ isolates against pathogenic fungi

Six isolates of *Trichoderma* spp., (T12), (T14), (T20), (T21), (T23) and (T34) were evaluated In vitro as antagonists against four pathogenic isolates of *R. solani* (Rs68), *Fusarium* (F81), *Pythium* (Py97) and *Penicillium* (Pen58) obtained from pathogenicity test, see Table (5) and Figs (1,2,3&4). On *R. solani* (Rs68), all *Trichoderma* isolates were effective in decreasing the growth compared to the control, and T20 was the most effective *Trichoderma* isolate as it decreased growth to 1.00 cm compared to control (9 cm) i.e. it inhibited growth by 88.89% (Fig.1). On *Fusarium* (F81), all *Trichoderma* isolates were effective in decreasing the growth compared to the control, and T14 was the most effective *Trichoderma* isolate as it decreased growth to 1.92 cm compared to control (9 cm) i.e. it inhibited (F81) growth by 78.67% (Fig. 2). On *Pythium* (Py97), Isolates of *Trichoderma* were effective in decreasing the growth compared to the control and T23 was the most effective *Trichoderma* isolate as it decreased growth to 1.17 cm compared to control (9 cm) i.e. it inhibited growth by 87% (Fig. 3). On *Penicillium* (pen58), T23, and T20 was the most effective *Trichoderma* isolate as it decreased growth to 5.67 cm compared to control (9 cm) i.e. it inhibited growth by 37% (Fig. 4). The degree of inhibition varied depending on the specific Trichoderma isolate and the pathogenic isolate being challenged

Table 5. Antagonistic activity of _Trichoderma_ isolates against pathogenic fungi (Rs68), (F81), (Py97) and (Pen58)

Trichoderma spp.	growth of pathogens(cm)[a]			
	R. solani (Rs68)	*Fusarium* (F81)	*Penicillium* (pen58)	*Pythium* (Py97)
T12	1.83	3.08	6.92	3.35
T14	1.25	1.92	6.00	3.42
T20	1.00	2.00	5.67	3.00
T21	1.07	2.08	7.08	3.52
T23	1.03	2.58	7.83	1.17
T34	1.33	2.08	6.42	3.33
C[(b)](only pathogen)	9.00	9.00	9.00	9.00
LSD	0.81	1.13	1.39	1.04

[(a)]Mean of three replicates[(b)]Control *Trichoderma* was absent

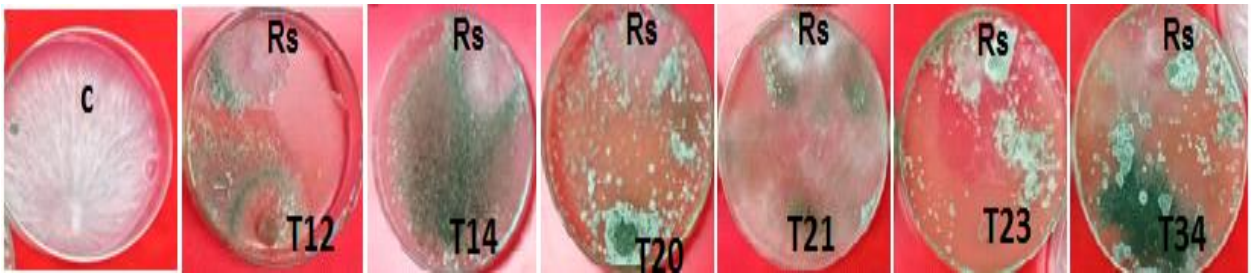

Fig. 1. Antagonistic activity of _Trichoderma_ isolates on growth of Rs68

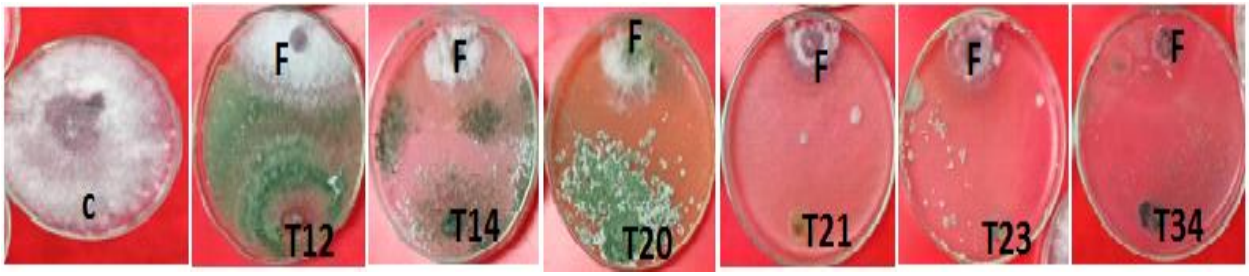

Fig. 2. Antagonistic activity of _Trichoderma_ isolates on growth of F81

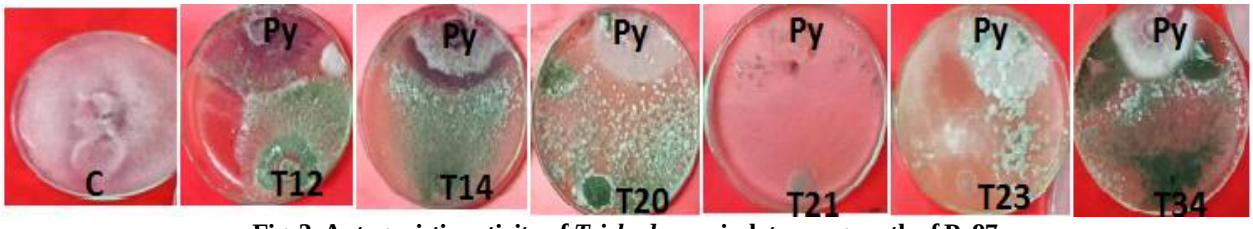

Fig. 3. Antagonistic activity of _Trichoderma_ isolates on growth of Py97

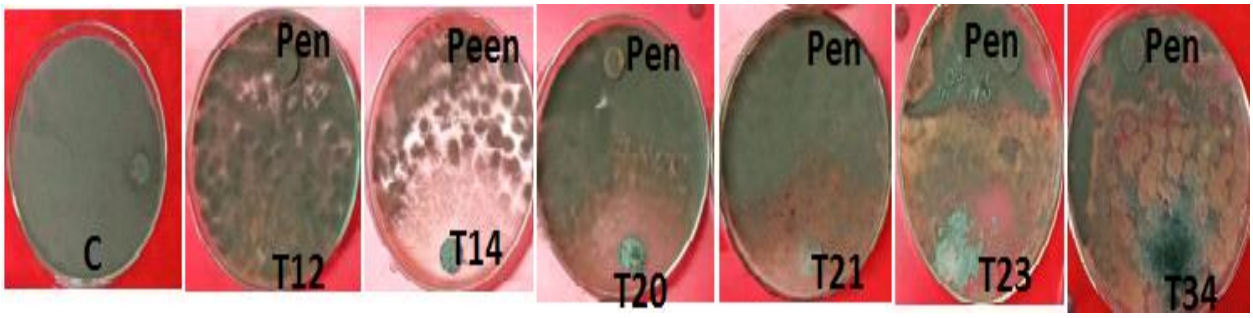

Fig. 4. Antagonistic activity of _Trichoderma_ isolates on growth of Pen58

The degree of inhibition varied depending on the specific *Trichoderma* isolate and the pathogenic isolate being challenged. The evaluation primarily focused on qualitative measurements of antagonistic activity, such as the observation of growth inhibition zones between *Trichoderma* and pathogenic isolates. For example, on *Fusarium* (F81), isolates of *Trichoderma* were effective in decreasing the growth of pathogens and T14 was the most effective *Trichoderma* isolate as it decreased growth to 1.92 cm compared to control (9 cm). In addition, all isolates of *Trichoderma* were effective in decreasing the growth on *Pythium* (Py97), and T23 was the most effective *Trichoderma* isolate as it decreased growth to 1.17 cm compared to control (9 cm) *i.e*. it inhibited growth by 87%.

In vitro, bacterial strains showed high level of antagonism against the fungal pathogens (Ashour *et al.* 2004). All the *Trichoderma* sp. showed its ability to inhibit the pathogen (Gomathi and Ambikapathy 2011). Genus *Trichoderma* treatment showed the rapid growth which colonized medium as well as reduced the mycelia growth of the pathogenic fungi (Mir *et al.* (2011). Successful antagonism was showed with *Trichoderma* species and interaction with pathogenic fungi (Hussein *et al.* 2018).

Soil analysis

The soil sample was obtained from the Agricultural Research Center, Giza, Egypt. The results of the soil analysis according to Jackson (1973) showed that the soil was clay, which was the predominant type, representing 60.74% of the sample, in addition chemical parameters were presented in Table (6).

Table 6. Physico-chemical properties of soil used during this study

Soil properties	Value
Physical properties	
Sand %	39.26
Clay %	60.74
Texture	Loam
Chemical properties	
pH	7.79
EC (dS/m)	3.09
Soluble cations(meq/100g soil)	
Mg^{++}	0.91
Ca^{++}	1.93
Na^{+}	6.30
K^{+}	1.10
Soluble anions(meq/100g soil)	
Cl^{-}	5.20
$HCO3^{-}$	0.28
$CO3^{-}$	ND
$CO4^{-}$	4.77

ND: Not Detected (below detection limit)

Under glasshouse conditions, efficiency of *Trichoderma* isolates on controlling damping off of flax seedlings

In order to evaluate the efficiency of *Trichoderma* isolates in controlling damping off disease in flax seedlings under glasshouse conditions. *Trichoderma* isolates were tested for their ability to suppress the incidence and severity of damping off caused by fungal pathogens. The specific *Trichoderma* isolates used in this study were selected based on their known antagonistic activity against damping off pathogens. The *Trichoderma* isolates included T12, T14, T20, T21, T23, and T34.

Table (7) represents efficiency of *Trichoderma* isolates on controlling damping off, plant height, and dry weight of flax seedlings grown in soil infested with *R. solani* (Rs68) under glasshouse conditions. Three *Trichoderma* isolates (T14, T23, and T34) were effective in controlling the disease. The maximum efficiency in controlling disease (0.00% damping-off) showed with two isolates of *Trichoderma* (T14 and T23). Species of *Trichoderma* are very effective biocontrol agents and should be further biocontrol applications (Afify *et al.* 2017). They were considered either ineffective in controlling flax seedling blight and improving agronomic traits (Zayed *et al.*, 2019).

Table 7. Effect of *Trichoderma* isolates on plant height, and dry weight of flax seedlings grown in soil infested with *R. solani* (Rs68) under glasshouse conditions

Trichoderma isolates	Damping-Off % % Transformed[a]		Plant height (cm)	Dry weight (mg)
T12	100.00	10.03	0.00	70.00
T14	0.00	0.71	24.48	20.00
T20	91.00	9.54	9.96	13.20
T21	92.00	9.61	19.92	0.00
T23	0.00	0.71	20.36	14.40
T34	34.00	5.36	22.16	6.00
infested soil [b]	88.00	9.39	14.30	62.00
autoclaved soil [c]	0.00	0.71	18.76	52.00
LSD(P< 0.05)		1.34	10.34	24.65

[a]The percentage values were converted to √x+0.5 in order to standardize the data and stabilize variances across the whole data range Autoclaved soil infested with *R. solani* (Rs68) Autoclaved soil without fungal pathogen

Table (8) represents efficiency of *Trichoderma* isolates on controlling damping off, plant height, and dry weight of flax seedlings grown in soil infested with *Fusarium* sp. (F81) under glasshouse conditions. Three *Trichoderma* isolates (T14, T23, and T34) were effective in controlling the disease compared to control and T23 showed the maximum efficiency (1.00% damping-off). There were no significant difference between all treatments and infested control with plant height and dry weight.

Table (8) aimed to assess the efficiency of three *Trichoderma* isolates (T14, T23, and T34) in controlling damping off, as well as their impact on plant height and dry weight in flax seedlings grown in soil infested with *Fusarium* sp. (F81) under glasshouse conditions. Table (8) represents the results of the evaluation. *Trichoderma* isolate T23 demonstrated the maximum efficiency in controlling damping off disease, with an incidence of 1.00%. This indicates that the application of T23 effectively reduced the occurrence of damping off in the flax seedlings.

Table 8. Effect of *Trichoderma* isolates on plant height, and dry weight of flax seedlings grown in soil infested with *Fusarium* sp. (F81) under glasshouse conditions

Trichoderma isolates	Damping-Off % % Transformed[a]		Plant height (cm)	Dry weight (mg)
T12	56.00	7.29	25.12	6.00
T14	3.00	1.22	22.24	12.00
T20	78.00	8.83	21.94	10.00
T21	84.00	9.16	13.02	10.00
T23	1.00	1.04	25.12	14.00
T34	9.00	3.06	22.04	16.00
infested soil [b]	75.00	8.66	22.44	24.00
autoclaved soil [c]	0.00	0.71	23.00	38.00
LSD(P<0.05)		1.69	6.03	14.11

[a]The percentage values were converted to √x+0.5 in order to standardize the data and stabilize variances across the whole data rangeAutoclaved soil infested with *Fusarium* sp. (F81). (c) Autoclaved soil

Table (9) represents efficiency of *Trichoderma* isolates on controlling damping off, plant height, and dry weight of flax seedlings grown in soil infested with *Penicillium* sp. (Pen58) under glasshouse conditions. All *Trichoderma* isolates were effective in controlling the disease. The maximum efficiency in controlling disease (3.00% damping-off) showed with soil contained T14. In case of plant height, only T12 and T23 increased plant height significantly compared to infested control.

Trichoderma isolates T14, T12, and T23 were evaluated as treatments, while an infested control group was included. *Trichoderma* isolate T14 demonstrated the maximum efficiency in controlling damping off disease, with an incidence of 3.00%. This finding indicates that the application of T14 effectively reduced the occurrence of damping off in the flax seedlings

Table 9. Effect of *Trichoderma* isolates on plant height, and dry weight of flax seedlings grown in soil infested with *Penicillium* sp. (Pen58) under glasshouse conditions

Trichoderma isolates	Damping-Off %	% Transformed[a]	Plant height (cm)	Dry weight (mg)
T12	6.00	1.98	23.36	60.00
T14	3.00	1.54	22.04	40.80
T20	7.00	2.37	21.04	58.00
T21	4.00	1.87	23.08	54.00
T23	4.00	1.72	25.60	66.00
T34	11.00	3.13	22.52	54.00
infested soil [b]	45.0	6.66	19.32	62.00
autoclaved soil [c]	0.00	0.71	19.52	8.40

[a]The percentage values were converted to $\sqrt{x+0.5}$ in order to standardize the data and stabilize variances across the whole data range Autoclaved soil infested with *Penicillium* sp. (Pen58) Autoclaved soil

Table (10) represents efficiency of *Trichoderma* isolates on controlling damping off, plant height, and dry weight of flax seedlings grown in soil infested with *Pythium* (P97) under glasshouse conditions. Four *Trichoderma* isolates (T14, T20, T21, and T23) were effective in controlling the disease compared to the infested control. Soil contained T34 mixed with *Pythium* sp. (P97) showed increase in damping-off percentage compared to the infested control. T14 and T23 showed the maximum efficiency in controlling disease (0.00% damping-off). In case of plant height, there was no significant difference between all treatments and infested control. In case of dry weight, the maximum value was represented by T34 (26mg).

Table 10. Effect of *Trichoderma* isolates on plant height, and dry weight of flax seedlings grown in soil infested with *Pythium* sp. (Py97) under glasshouse conditions

Trichoderma isolates	Damping-Off %	% Transformed[a]	Plant height (cm)	Dry weight (mg)
T12	50.00	6.78	21.84	18.00
T14	0.00	0.71	22.08	14.00
T20	39.0	5.97	21.00	18.00
T21	12.00	3.52	21.00	12.00
T23	0.00	0.71	23.64	18.00
T34	86.00	9.27	13.24	26.00
infested soil [b]	53.00	7.28	23.80	3.60
autoclaved soil [c]	5.00	2.05	14.84	1.60
LSD (P<0.05)		1.69	6.62	17.01

[a]The percentage values were converted to $\sqrt{x+0.5}$ in order to standardize the data and stabilize variances across the whole data range Autoclaved soil infested with *Pythium* sp.(P97) Autoclaved soil

Our study found that when T34 was mixed with *Pythium* sp. (P97) in the soil, there was an increase in damping-off percentage compared to the infested control. This unexpected result suggests a potential interaction between T34 and *Pythium* (P97) that may have contributed to the higher damping-off percentage. *Trichoderma* isolates T14 and T23 demonstrated the maximum efficiency in controlling damping off disease, with a damping-off percentage of 0.00%. This indicates that the application of T14 and T23 effectively suppressed the occurrence of damping off in the flax seedlings infested with *Pythium* (P97). The analysis of plant height did not reveal any significant differences between the *Trichoderma* treatments and the infested control group. This suggests that the *Trichoderma* treatments did not have a noticeable impact on plant height in the flax seedlings infested with *Pythium* (P97) in this study.

Our study found that only *Trichoderma* isolates T12 and T23 significantly increased plant height compared to the infested control. This suggests that these specific isolates have growth-promoting effects on flax seedlings under the influence of *Penicillium* sp. (Pen58) infestation. The mechanisms underlying the plant growth-promoting effects of T12 and T23 will be further explored and discussed. Similar to previous results on *Fusarium* sp., the analysis of dry weight there are any significant differences between the *Trichoderma* treatments and the infested control group. This indicates that the *Trichoderma* treatments did not have a noticeable impact on the dry weight of the flax seedlings under the influence of *Penicillium* sp. (Pen58) infestation. However, it is important to note that our study primarily focused on evaluating the efficacy of *Trichoderma* isolates on plant height and in controlling damping off disease.

In the current assay, we observed variations between *Trichoderma* isolates in the antagonistic activity against different pathogenic isolates. Degree of inhibition varied with the specific *Trichoderma* isolate and the pathogenic isolate being challenged. Some *Trichoderma* isolates showed stronger antagonistic activity against certain pathogenic isolates, while others were less effective. The differences in the efficiency of antagonistic activity among *Trichoderma* spp. against certain pathogenic fungi are affected by several factors depending on natural of each species (Ashour *et al.* 2021 & Muzakir *et al.* 2022). Further investigations may be required to explore the potential effects of *Trichoderma* treatments on plant growth parameters. Microorganisms have the capability to suppress plant diseases (Afify and Ashour 2024).

Identification of potential *Trichoderma* species

From soil samples, six Tichoderma isolates were obtained. On differeniate media were used to evaluate the growth performance of *Trichoderma* spp. *In vitro.* In order to compare the effect of we used four from culture media as Nutrient agar (NA), Sabouraud Dextrose Agar (SDA), Potato Dextrose Agar (PDA) and Carrot agar (CA) to enhance the growth of six *Trichoderma* isolates, T12, T14, T20, T21 ,T23 and T34. Fig. (5) showed that Carrot agar (CA) culture media was the best media that enhanced the growth of all tested *Trichoderma* isolates.

Only the best *Trichoderma* isolates as three species were identified by morphological methods under light microscope (Fig. 6). The growth of these colonies are speed, pigment secretion, conidiospore color were all studied on the PDA medium.

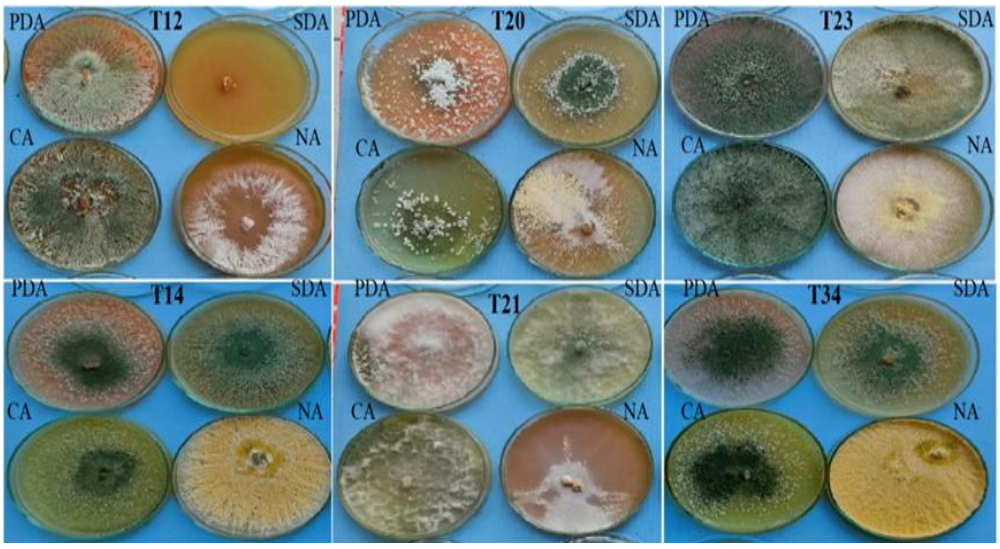

Fig. 5. Growing six isolates of *Trichoderma* on different media: (PDA), (SDA), (CA) and (NA).

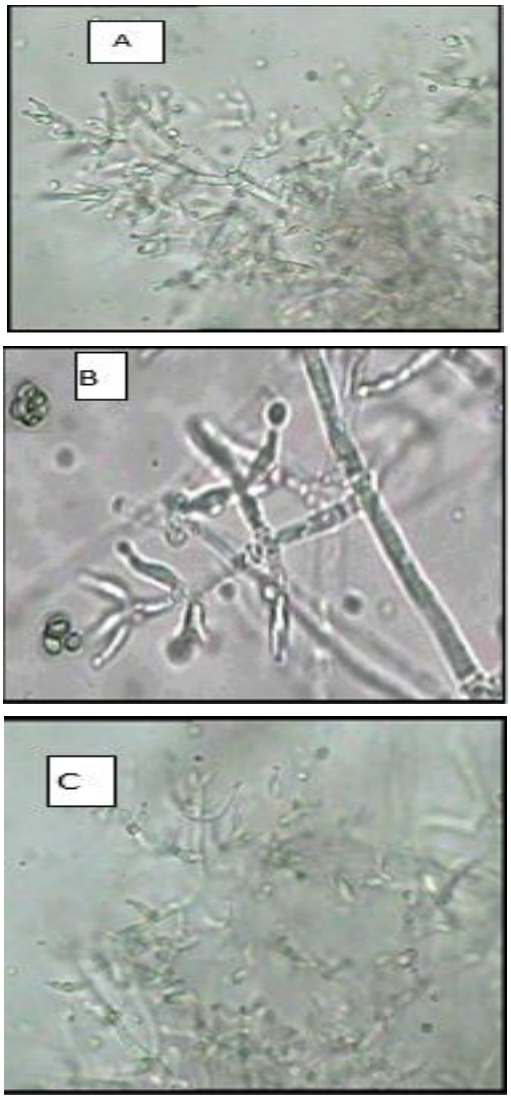

Fig. 6. Microscopic observation of *Trichoderma* spp. (A) *Trichoderma harzianum* T12, (B) *Trichoderma longibrachiatum* T14, and (C) *Trichoderma viride* T23.

The fungal isolate T12 was belonging to *Trichoderma harzianum*. In ten days at 28°C, the characters of colonies are fast growing and diameter reaching about 5-7 cm. While, on malt medium, colonies are white with bright to dull green. Phialides are in whorls of 3-5 flask shaped, 6.0X3µm. Conidia are subglobose and mass with green in 3.5X2.0 µm. Chlamydospores are abuntand terminal or intracalary.

T14 was belonging to *Trichoderma longibrachiatum*. Colonies are fast growing and reaching 5-7 cm. On Malt medium, colonies are whitish and greyish to green color. Phialides are in whorls of 2-4 flask shaped, 9.5X2.5 µm. Mass with green 5.2X2.4 µm and ellipsoidal conidia.

T23 was belonging to *Trichoderma viride*. Colonies are fast growing and reaching 5-7 cm. On Malt medium, colonies are whitish green color. Phialides are in whorls of 2-4 flask shaped, 11.8X2.8 µm. Conidia are spherical to sub spherical and green in mass 3.2µm (Fig.6).

Members of the Trichoderma genus are known as imperfect fungi, fast growing in culture and produce numerous green spores. These occur worldwide and generally there are with root, soil and plant (Howell 2003). All the three *Trichoderma* species were relationship each other between them in the mycelial growth rate, colony appearance, shape of conidia and conidiophores and branching pattern of phialides (Shah *et al.* 2012). Species of genus Trichoderma belong to the subdivision Deuteromycetes, members of which most strains are have an asexual life cycle and do not have a determinate sexual state (Harman 2004). Furthermore, in all types of agricultural soils there are mainly asexual fungi are present (Naher *et al.* 2014). The *Trichoderma* isolates T12, T14 and T23 were identified based on morphological (via microscopic examination) and cultural characteristics.

CONCLUSION

This study analyzed 102 fungal isolates from seedlings infected with damping-off symptoms in Giza, Dakahlia, Gharbia, and Kafr El-Sheikh. In a glasshouse setting, the isolates' pathogenicity was assessed using the flax cultivar Giza 12. The most effective fungal isolates were F81, RS68, PY97, and Pen58. The most effective *Trichoderma* isolate was *T. Longibrachiatum*, which decreased growth by 78.67%. The study concluded that the use of Tirchoderma-based is safe for the farmers and good for the environment. Therefore, following work to be done apply could be easy to produce as formulatios.

REFERENCES

Afify, Aida H. and Ashour, A.Z.A. (2024). Controlling damping-off disease on cotton seedlings caused by *Rhizoctonia solani* and *Fusarium oxysporum* via Plant Growth-Promoting Rhizobacteria (PGPR). J. Agric. Chem. and Biotechn., Mansoura Univ. 15(10): 119-123.

Afify, Aida H.; Bayoumy, Samia M.; El-Sayed, A.B. and Elshal, S.A. (2017). Biocontrol of sugar beet pathogens *Fusarium* wilt by *Trichoderma viride*. J. Agric. Chem. and Biotechn., Mansoura Univ., 8(11): 271-275.

Aly, A. A.; Hussein, E. M.; Asran, A. A.; El-HAwary, O. G.; Mosa, A. A. and Mostafa, M. H. (2013). Non-differential interaction between isolates of *Rhizoctonia solani* and flax cultivars. *Icelandic Agricultural Sci.*, 26: 37-44.

Aly, A. A.; Hussein, E. M.; EL-Awamri, A. A.; Mansour, M. T. and Habeb, M. M. (2017). Identification and pathogenicity of *Fusarium* spp. isolated from flax roots Agric. Res. (Egypt), 95(1): 25-51.

Aly, A. A.; Hussein, E. M.; Mostafa, M. A. and Ismail, A. I. (1996). Distribution, identification, and pathogenicity of *Fusarium* spp. isolated from some Egyptian cotton varieties. Menofiya J. of Agric. Res. (Egypt), 21: 819–836.

Ashour, A.Z.A. and Afify, Aida H. (2025). Suppression of seeds mycoflora involved on cotton seedling damping-off disease by chitinolytic Actinomycetes. J. Agric. Chem. and Biotechn., Mansoura Univ.,16(2): 15-19.

Ashour, A.Z.A.; Afify, Aida H. and Kineber, M.E.A. (2004). Biological control of flax seedling blight disease. J. Agric. Sci. Mansoura Univ., 29(9): 4865-4874.

Ashour, A.Z.A.; El-Sawah, A.M. and Afify, Aida H. (2021). Use of *Trichoderma* spp. for biological control of sugar beet damping-off caused by *Fusarium* spp. J. Agric. Chem. and Biotechn., Mansoura Univ.,12(4): 75-77.

Barnett, H. L. and Hunter B. B. (1972). Illustrated genera of imperfect fungi. 3rd edition. Burgess Minneapolis Minnesota. The American Phytopathological society, p 241.

Cariou-Pham, E. and Bonnan, S. (2006). Biological control of flax scorch using *Glomus intraradices* and *Trichoderma atroviride*. Modern Multidisciplinary Applied Microbiology: Exploiting Microbes and Their Interactions Modern Multidisciplinary Applied Microbiology, 380-384.

Cumagun, C. J. R. (2012). Managing plant diseases and promoting sustainability and productivity with *Trichoderma* : The philippine experience (review articles) Agric. Sci. Technol., 14: 699–714.

Devika, C.; Singhalage, I. D. and Seneviratne, G. (2021). Modification of nutrient agar medium to culture yet-unculturable bacteria living in unsanitary landfills. Ceylon J.of Sci., 50(4): 505-512.

Gilmen, J. C. (1966). A Manual of soil fungi (2ndEd). The Iowa State College. Press, Iowa, p 450.

Gomathi, S. and Ambikapathy, V. (2011). Antagonistic activity of fungi against *Pythium debaryanum* (Hesse) isolated from Chilli field soil. Adv. in Appl. Sci. Res., 2(4): 291-297.

Gordon, W. L. (1956). The occurrence of *Fusarium* species in Canada. V. Taxonomy and geographic distribution of *Fusarium* species in soil. Canad. J. of Botany, 34(6): 833-846.

Gruzdevienė, E.; Brazauskienė, I.; Repečkienė, J. and Lugauskas, A. (2008). The occurrence of pathogenic fungi during flax growing season in Central Lithuania. J. of Plant Prot. Res. 48(2): 255-265.

Harman, G. E.; Howell, C.R.; Viterbo, A.; Chet, I. and Lortio, M. (2004). *Trichoderma* species-opportunistic, avirulent plant symbionts. Nature Rev. Micribiol., 2: 43-56.

Howell, C. R. (2003). Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. Plant disease, 87(1): 4-10.

Hussein, E.M.; Aly, A.A.; El-Awamri, A.A. and Habeb, Marian M. (2018). Use of electrophoretic patterns and isozymes to characterize *Trichoderma* isolates from cotton roots. J. Agric. Chem. and Biotechn., Mansoura Univ. Vol. 9(10): 219-222.

Irina, D. and Christian, P.K. (2004). Species and biodiversity in Trichoderma and Hypocera: from aggregate species to species clusters. J. of Zhejiang Uni. Sci., 6: 100-112.

Jackson, M. L. (1973). Soil chemical analysis, pentice hall of India Pvt. Ltd., New Delhi, India, 498: 151-154.

Jahan, N.; Sultana, S.; Adhikary, S. K.; Rahman, S. and Yasmin, S. (2013). Evaluation of the growth performance of *Trichoderma harzianum* (Rifai.) on different culture media. J. Agri. Vet. Sci., 3: 44-50.

Meyer, J. A. (1967). Recherches sur les Fusarioses. II. Ecologie et pathogénie du «*Fusarium oxysporum*». Annual Epiphytes, 18(2): 241-247.

Mir, G.H.; Devi, L.S.; Ahmad, S.; Kumar, V.M. and Williams, P. (2011). Antagonistic potential of native isolates of *Trichoderma viride* on corn rot pathogen complex of saffron *(Crocus sativus)* in Kashmir. Plant Pathol. J., 10: 73-78.

Moyse, J.; Lecomte, S.; Marcou, S.; Mongelard, G.; Gutierrez, L. and Höfte, M. (2023). Overview and management of the most common eukaryotic diseases of flax (*Linum usitatissimum* L.). Plants, 12(15): 2811.

Mustafa, A.; Khan, M. A.; Inam-ul-Haq, M.; Pervez, M. A. and Umar, U. (2009). Usefulness of different culture media for *In vitro* evaluation of *Trichoderma* spp. against seed borne fungi of economic importance. Pak. J. Phytopathol., 21(1): 83-88.

Muzakir, Hifnalisa; Jauharlina, J. and Sriwati, R. (2022). Antagonistic screening of *Trichoderma* spp. isolated from patchouli rhizosphere. In IOP Conf. Series: Earth and Environmental Sci., 951(1): 12021.

Naher, L.; Yusuf, U.; Ismail, A. and Hossain, K. (2014). *Trichoderma* spp.: A biocontrol agent for sustainable management of plant diseases. Pak. J. Bot., 46(4): 1489-1493.

Nofal, A.M.; Abdel-Ghany, T.M. and Abd-El-Hamed, W.F.M. (2021). Significance of local *Trichoderma* isolates in controlling *Pythium ultimum* and *Rhizoctonia solani* on bean in Egypt. Egyptian J. of Phytopathol. 49(2): 131-140.

Papavizas, G. C. (1985). Annual Rev. of Phytopathol., 23: 23-54.

Papavizas, G. C. and Lewis, J. A. (1981). Introduction and augmentation of microbial antagonists for the control of soilborne plant pathogens. Biological control in crop production, pp 461.

Perryman, S.A.M. and Fitt B.D.L. (2000). Effects of diseases on the growth and yield of spring linseed (*Linum usitatissimum* L.). Annals of Appl. Biol., 136: 197–207.

Piper, C. S. (1945). Soil and plant analysis. (New York) Soil Sci., 59(3): 263.

Rahman, M.; Borah, S. M.; Borah, P. K.; Bora, P.; Sarmah, B. K.; Lal, M. K.; Tiwari, R. K. and Kumar, R. (2023). Deciphering the antimicrobial activity of multifaceted rhizospheric biocontrol agents of solanaceous crops viz., *Trichoderma harzianum* MC2, and *Trichoderma harzianum* NBG. Frontiers in Plant Sci., 14: 1141506.

Samuels, G. J. and Hebbar, P. K. (2015). *Trichoderma* : identification and agricultural applications (Vol. 602). St. Paul, MN: APS Press.

Scognamiglio, T.; Zinchuk, R.; Gumpeni, P. and Larone, D. H. (2010). Comparison of inhibitory mold agar to Sabouraud dextrose agar as a primary medium for isolation of fungi. J.of Clinical Microbiol., 48(5): 1924-1925.

Shah, Shaiesta; Nasreen, Sahera and Sheikh, P.A. (2012). Cultural and morphological characterization of *Trichoderma* spp. associated with green mold disease of *Pleurotus* spp. in Kashmir. Res. J. of Microbiol., 7(2): 139-144.

Skidmore, A. M. and Dickinson, C. H. (1976). Colony interactions and hyphal interference between septoria nodorum and phylloplane fungi. Transactions of the British Mycological Soc., 66(1): 57-64.

Sneh, B.; Burpee, L. and Ogoshi, A. (1991). Identification of *Rhizoctonia* species. APS press.

Steyaert, J. M.; Weld, R. J.; Mendoza-Mendoza, A. and Stewart, A. (2010). Reproduction without sex: conidiation in the filamentous fungus *Trichoderma* . Microbiol., 156(10): 2887-2900.

Zayed, S.; Aly, A.; Asran, A. and Mansour, M. (2019). Field Evaluation of Beneficial and Deleterious Effects of *Trichoderma* spp. on the Incidence of Flax Seedling Blight and on some Agronomic Traits. Agric.l Chem. and Biotechnol., 10(4): 83-87.

فاعلية أنواع التريكودرما فى المقاومة الحيوية لمرض موت بادرات الكتان

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الملخص

في هذه الدراسة جمعت عينات من بادرت الكتان المصابة من مختلف محافظات مصر (الجيزة والدقهلية والغربية وكفر الشيخ). وقد تم الحصول على 102 عزلة فطرية ممرضة من هذه العينات .تم تعريف العزلات على أنها ريزوكتونيا سولاني ,(9,8%) فيوزاريوم ,(29,4%) بيثيوم ,(7,84%) بينسيليوم ,(8,82%) ألترناريا ,(20,59%) تريكودرما ,(2,94%) أسبرجيلس ,(9,8%) كيتوميوم ,(0,98%) سفيروسوريوم ,(0,98%) وعزلات اخري مجهولة .(8,824%) داخل الصوبة الزجاجية , تم تقييم القدرة المرضية لجميع العزلات الفطرية البالغ عددها 102 عزلة على بادرات الكتان صنف جيزة 12 وكانت أكثر الفطريات الممرضة هي فيوزاريوم (F81) وريزوكتونيا سولانى (RS68) وبيسيوم (Py97) و بينيسيليوم (Pen58). تم إختبار التضاد الفطري لستة عزلات من فطر التريكودرما معمليا ضد الفطريات الممرضة المختارة. وكانت عزلة (T20) هي الأكثر فعالية حيث قللت النمو الطولي لفطر F81بنسبة 78.67% ، كما أنها قللت النمو الطولي لفطر RS68 بنسبة 88.89% ، و قللت أيضاً النمو الطولي لفطر و Pen58 بنسبة .37% في حين أن عزلة T23 قللت النمو الطولي لفطر Py97 بنسبة87 ٪ .كما أظهرت النتائج في الصوبة الزجاجية عن فاعلية عزلات التريكودرما في مكافحة مرض موت بادرات الكتان بواسطة العزلات الممرضة ,F81, RS68 Pen58 . Py97, أما بالنسبة لنتائج التجارب داخل الصوبة الزجاجية ,فقد أدي إستخدام المبيد الحيوي T14 و T23 إلي تقليل نسبة الإصابة بفطر RS68 إلي صفر٪ .اما بالنسبة لفطر F81أدي إستخدام T23 إلي تقليل نسبة الإصابة إلي 1 ٪ . بالنسبة لفطر Py97 أدي إستخدام المبيد الحيوي T14 و T23 إلي تقليل نسبة الإصابة إلي صفر٪ . تم تعريف عزلات التريكودرما ذات التأثير المعنوى فى مقاومة مرض موت بادرات الكتان على أنها تريكودرما هارزيانم (T12) و تريكودرما لونجيبركاتيم (T14) و تريكودرما فيريدى (T23) وذلك بتنمية عزلات التريكودرما على البيئات المختلفة ثم بالفحص الميكروسكوبى لثلاثة عزلات من التريكودرما الأكثر تأثيرا.وأخيرا، فإن إستخدام عوامل المكافحة الحيوية مثل التريكودرما يمكن أن يكون إستراتيجية آمنة لمكافحة الأمراض الفطرية في الكتان.