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Potentiality of *Trichoderma* Species against *Fusarium oxysporum* f. sp. *cucumerinum* and *Meloidogyne javanica* Disease Complex in Cucumber Plants

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ABSTRACT

Biological control is considered an important approach in the recent years for controlling many plant diseases. *Trichoderma* spp. is a unique genus with a wide range of activity against most important plant pathogens. In this investigation, we have evaluated antagonism of four species of *Trichoderma* (*T. album, T. asperellum, T. hamatum* and *T. koningii*) against root-knot nematode (*Meloidogyne javanica*) and *Fusarium* wilt disease (*Fusarium oxysporum* f. sp. *cucumerinum*) on cucumber plant at two locations (Giza and Sakha) under greenhouse conditions. Effect of *Trichoderma* on disease severity of *F. oxysporum* f. sp. *cucumerinum* and mortality of *M. javanica* (J2) were examined under laboratory conditions. Results indicate that all tested *Trichderma* spp. inhibited the mycelial growth of *F. oxysporum* f. sp. *cucumerinum* and gave high mortality % of *M. javanica* juveniles *in vitro*. The best antifungal activity was shown by *T. album* and *T. hamatum* against cucumber wilt pathogen. After 24 hours, *T. hamatum* and *T. album* had almost 100% nematode mortality. Under greenhouse conditions, a significant enhancement was found in growth parameters of cucumber treated with *T. album* and *T. hamatum* at two locations. The application of *T. hamatum* to soil infected with *F. oxysporum* and *M. javanica* resulted in the highest antagonistic effect at Giza location. All treatments caused a reduction in final population density of *M. javanica* whether in the presence of *Fusarium* or not. At Giza location, the treatment utilizing *T. album* were shown to be the most effective in lowering the total number of galls and egg masses on cucumber roots infected with nematodes. However, in the presence of *Fusarium* fungus + nematodes, *T. hamatum* was the best treatment. At Sakha location, no significant differences were observed among *Trichoderma* spp. when nematodes and fungus were used together. *T. koningii* with nematode only resulted in a lower number of egg masses. Most treatments were more effective in reducing *M. javanica* reproduction factor (RF) when the nematode was present alone. In both sites, the highest increase in chitinase activity was recorded with treatment by *T. hamatum* and *Fusarium*.

***Keywords*:** *Fusarium oxysporum* f. sp. *cucumerinum; Meloidogyne javanica*; Cucumber; *Trichoderma* spp., Biological control.

INTRODUCTION

Cucumber (*Cucumis sativus* L), one of the most significant and commercial crops, is grown both in open fields and in greenhouses. Cucumber is openly produced in Egypt in about 10,776 acres, yielding about 99,781 tonnes of fruit, whereas cucumber is cultivated in greenhouses in about 36,356 acres, yielding about 193,372 tonnes of fruit (FAOSTAT, 2018). Root-knot nematodes, *Meloidogyne* spp., are a costly pest that threatens all types of economic crops around the world (Hussain et al., 2011). These

nematode species frequently cause damage to tropical and subtropical vegetable crops (Mukhtar et al., 2013). *Meloidogyne arenaria, M. javanica, M. incognita* and *M. hapla* are the most frequent root-knot nematode species. They can bring about major economic losses in a number of agricultural crops (Khalil, 2013; Sulaiman and Shireen, 2020). Cucumber is susceptible to a variety of injuries, primarily caused by root-knot nematodes (*Meloidgyne* spp.) and a variety of fungal infections, at all stages of development, resulting in a significant reduction in the number of cucumber plants per area and/or yield per plant. The wilt disease caused by the fungal pathogen *Fusarium oxysporum* (Schlechtend.:Fr.) f. sp. *cucumerinum* (Owen) Snyder & Hansen (FOC) is listed as the most aggressive soil-borne diseases of cucumber (Armstrong and Armstrong, 1978), causes a very harmful and severe economic damage on cucumber crops in various regions around the world, including Spain, Canada, Thailand, China, Japan, England and Greece (Martinez et al., 2003; Vakalounakis et al., 2004 and Afifi, et al.,2017). Phytonematodes can be interacting with other pathogen in soil. As a result for interaction mode, synergistic and disease severity or antagonism can be caused. Root-knot nematode and the *Fusarium* disease complex are the most damaging or well-known pests in horticultural crops, causing significant losses all over the world (Akhtar et al., 2005 and Hadian et al., 2011). Synergistic interaction between *Fusarium* spp. and *Meloidogyne* spp. on cucumber increases disease severity (Patil et al., 2018 and Vijayashanthi et al., 2020).

Therefore, controlling plant-parasitic nematodes and soil-borne diseases is a major global concern. Managing root-knot nematodes and soil-borne fungi is a major challenge for playhouse growers all over the world. Several agricultural techniques have been tested against *Meloidogyne* spp. (Collange et al., 2011) and *Fusarium oxysporum* (Armstrong and Armstrong, 1978). However, these procedures are not reliable, economical, safe, and harmless to the non-targets. As a result, management strategies should target the *Fusarium* wilt disease as well as the root-knot nematodes. Controlling root-knot nematodes with nematicides is a practical strategy (Giacometti et al., 2010 and Nicolopoulou-Stamati et al., 2016). *Meloidogyne* spp. has been chemically regulated to an economic threshold level. However, due to the environmental contamination created by the usage of nematicides and the resulting hazards, attempts have been made to develop alternate nematode management strategies (Pest Control Products Board. 2017). One feasible alternative to the use of nematicides is the use of biological control agents (Dutta and Thakur, 2017; Patil et al., 2021).

Trichoderma is the most extensively grown fungus, which can be found in any soil (Harman et al., 2004a). Among the plant growth-promoting fungi, *Trichoderma* species are probably the most commonly used microorganisms for agricultural crop promotion (Abdelrahman et al., 2016 and Jogaiah et al., 2013). These species are well-known for their capacity to inhibit a variety of soil-borne fungal and nematode diseases, whether in the greenhouse or the open field. *Trichoderma* spp. is extensively used to combat plant diseases, particularly nematodes. According to Windham et al. (1989), egg production of root-knot nematode was reduced after soil treatment with a *Trichoderma* spp. conidial solution. *Trichoderma* spp. can colonize root surfaces and cortex, as well as promote plant growth (Sharon et al., 2001). *Trichoderma harzianum* confers systemic resistance to root-knot nematode invasion (Naserinasab et al., 2011) and plant diseases (Harman et al., 2008). In mycoparasitism, *Trichoderma* spp. prevented root-knot nematode eggs from hatching by releasing fungal metabolites, but *T. viride* and *T. harzianum* removed gravid females by producing hydrolytic enzymes

(Jegathambigai et al., 2011). *Trichoderma harzianum* was antagonistic to *Phytophthora infestans* in dual culture (Kerroum et al., 2015), inhibited *Macrophomina phaseolina* and *F. oxysporum f. sp. lycopersici* (Javaid et al., 2014), and reduced *F. graminearum* mycelial growth (Foroutan, 2013). It also inhibited the growth of *F. oxysporum* f. sp. *lycopersici in vitro* and effectively repressed it in a greenhouse setting (Selvakumar et al., 2014). *Trichoderma* species can use a variety of actions to achieve their goals (Ibrahim et al., 2020), including parasitism, metabolite production (antifungal compounds and antibiotics), and the creation of polymers and protein degrading enzymes (glucanases, chitinases, and proteases). Furthermore, these fungi can boost plant growth (through phytohormone production) and induce systemic disease resistance, making their application in agricultural settings even more intriguing (Monte et al., 2019).

The purpose of this study was to compare the effects of four *Trichoderma* spp. in reducing *M. javanica* and *F. oxysporum* f. sp. *cucumerinum*, which infect cucumber plants, as well as their effects on plant growth under greenhouse conditions at two different locations.

MATERIALS AND METHODS

Plant materials

In this investigation, cucumber cv. Merag was employed. Seeds were procured from Egypt's Agricultural Research Centre, Horticultural Research Institute.

Source of nematode inoculum

Eggs of the root-knot nematode (*M. javanica*) were extracted from heavily galled tomato roots cv. Super Strain taken from a tomato-cultured greenhouse. After being cleaned with tap water, galled roots were sliced into 1 cm pieces. Sodium hypochlorite (NaOCl) was employed to separate eggs from their gelatinous matrix, according to Hussey and Barker (1973). After being agitated violently for 3 minutes, the suspension was completely washed with tap water through a sieve combination (60 and 400 mesh) to eliminate the NaOCl. Eggs were collected on a 400 mesh sieve, rinsed in tap water, and incubated for three days in a beaker. The newly hatched juveniles (J2s) were collected. The number of juveniles per milliliter was calculated.

Source of cucumber wilt pathogen, *F. oxysporum* f. sp. *cucumerinum* (FOC) and inoculum preparation

The cucumber wilt pathogen *F. oxysporum* f. sp. *cucumerinum* (FOC) isolate utilized in this study was sourced from the Vegetable Diseases Research Department, Plant Pathology Research Institute, Agricultural Research Centre, Giza, Egypt. (GenBank accession No. KT461496). For greenhouse experiments, FOC spore suspension was produced according to Markakis *et al*. (2016). Conidia were created by culturing the pathogen in potato dextrose broth (PDB) for 7 days at 160 rpm and 25°C in the dark in a rotary incubator. The conidia were then, extracted by filtration through three layers of cheesecloth and centrifuged for 10 minutes at 3,000 g. Spores were re-suspended in sterile distilled water, with a concentration of $1x10^7$conidia/ml.

Inoculum preparation of *Trichoderma* species:

Four different species of *Trichoderma* were used: *T. album, T. asperellum, T. hamatum* and *T. koningii* which were all procured from the Vegetable Diseases

Research Department, Plant Pathology Research Institute, Agricultural Research Centre, Giza, Egypt. All species were grown and kept at 25°C in an incubator on a PDA medium. Mycelial plugs of any investigated species of *Trichoderma* growing on PDA were collected four days after incubation using a cork borer (0.5-cm diameter) and transferred to Czapix's Dox broth (Allen, 1950) in a flask 500 ml. The flasks were incubated for 7-10 days at 25°C in a shaking incubator.

In vitro evaluation of *Trichoderma* species against:

A- Root-knot nematode, *M. javanica.*

At three concentrations ($1x10^6$, $2x10^6$, and $4x10^6$) of the used spore suspension, four species of *Trichoderma* were evaluated against root-knot nematode, *M. javanica*. One ml of each strain was transferred to one hundred freshly hatched *M. javanica* juveniles (J2s) in vials. Each treatment was repeated five times. Five vials of nematodes only were left without treatment as a control, following Demeure and Freckman's approach (1981). The fatality percentages for each concentration were calculated after 24, 48, and 72 hours at 25±2°C. The following formula was used to compute the revised mortality percentages (Schneider and Orelli1947)

$$\text{Nematode mortality } \% = \left(\frac{\text{Number of dead juveniles in treatment} - \text{ Number of dead juveniles in control}}{100 - \text{Number of dead juveniles in control}}\right) x100$$

B –*Fusarium* wilt disease, *F. oxysporum* f. sp. *cucumerinum*

This experiment employed pure cultures of *F. oxysporum* f. sp. *cucumerinum* and four *Trichoderma* species that were seven days-old. Five mm discs were taken from each species and placed in fresh Petri plates (9-cm in diameter) with PDA medium and grown in the dark at 25±2°C for the *in vitro* test. To measure linear growth of the studied *Trichoderma* spp. against *F. oxysporum* f. sp. *cucumerinum*, the confronting method was utilized. Two discs, one from the pathogen and the other from the bio-agent, were placed 7 cm apart in each Petri dish. The following formula was used to compute pathogen reduction (inhibition) percentages:

$$\text{Fungal inhibition } \% = \left(\frac{\text{The linear growth in control treatment} - \text{The linear growth of treated fungus}}{\text{The linear growth in control treatment}}\right) x100$$

In vivo evaluation of *Trichoderma* species in greenhouse

Spore suspension concentration ($4X10^6$) of four *Trichoderma* species was prepared to measure their effect on *F. oxysporum. f. sp. cucumerinum* (FOC) and *M. javanica*-second stage juveniles (J_2s) parasitizing cucumber plant under greenhouse conditions during spring season 2021 in two different locations, Giza and Sakha. Three seedlings of cucumber cv. Merag were planted in 20- cm diameter plastic pots containing a 3 kg mixture of clay and sandy soils (1:1 w/w). After two weeks, the cucumber seedlings were inoculated with 1000 newly hatched juveniles (J_2s) of *M. javanica* for each 1kg soil, so each pot contained 3000 newly hatched *M. javanica* (J_2) and FOC spores suspension was added as in Table (1) by drenching pots with 20-ml of 1.0×10^7 conidia/ml.

A week after inoculation with nematode and *Fusarium*, *Trichoderma* spp. were inoculated. Each *Trichoderma* species was tested against only fungus, only nematode, and fungus + nematode inoculation at the same time. The nematicide Oxamyl was applied at rate of 0.5 g/pot in five pots (replicates) with nematode only. Uniform (fungicide) was applied at a recommended dose (2 ml/l) in five pots with *Fusarium*

only. Another five pots were treated with both Oxamyl and Uniform as well as were inoculated with nematode and *Fusarium*. Five inoculated pots by root-knot nematodes were left without adding any material (*Trichoderma* spp.). Also, five inoculated pots by *Fusarium* were left without adding *Trichoderma* spp. in addition to other five replicates of inoculated pots by *Fusarium* and nematode together were remained without *Trichoderma* spp. Five pots with healthy seedlings (without inoculation with nematodes, *Fusarium* or any treatments) were served as control. The trial was repeated twice at Giza governorate (25±4°C) and Sakha county (22±4°C).

Table 1: Various treatments used in the current study

1	*F. oxysporum* + *T. album*	**2**	*M. javanica* + *T. album*
3	*F. oxysporum* + *M. javanica* + *T. album*	**4**	*F. oxysporum* + *T. asperellum*
5	*M. javanica* + *T. asperellum*	**6**	*F. oxysporum* + *M. javanica* + *T. asperellum*
7	*F. oxysporum* + *T. hamatum*	**8**	*M. javanica* + *T. hamatum*
9	*F. oxysporum* + *M. javanica* + *T. hamatum*	**10**	*F. oxysporum* + *T. koningii*
11	*F. oxysporum* + *T. koningii*	**12**	*F. oxysporum* + *M. javanica* + *T. koningii*
13	*F. oxysporum*	**14**	*M. javanica*
15	*F. oxysporum* + *M. javanica*	**16**	Oxamyl + *M. javanica*
17	Uniform+ *F. oxysporum*	**18**	Oxamyl and Uniform + *F. oxysporum* + *M. javanica*
19	Untreated plant (uninoculated)		

All pots were arranged in the greenhouse in a randomized block design. After sixty days of inoculation by nematode and *Fusarium*, the plants were harvested. Juveniles of *M. javanica* were extracted from one kg soil per pot by sieving and modified Baermann techniques (Goodey, 1963). According to Byrd et al. (1983), roots were dyed with acid fuchsine in lactic acid and microscopically inspected for counting nematode developmental stages and egg masses. Root galls and egg masses were also recorded using the root gall and egg mass index scales for 0-5 where 0=no galls, 1=1:2, 2=3:10, 3=11:30, 4=31:100 and 5>100 per root system (Taylor and Sasser, 1978). In addition, the following formula to compute disease incidence and severity were used:

$$\%\ \text{Disease incidence} = \left(\frac{\text{Number of infected plants}}{\text{Total number of plants}}\right) \text{x100}$$

According to Liu et al. (1995), disease severity of infected plants was assessed using a scale rating from 0 to 5 based on leaf wilt symptoms: 0 indicates no symptoms; 1 indicates plants with up to 25% of leaves with symptoms; 2 indicates plants with >25-50% of leaves with symptoms; 3 indicates plants with >50-75% of leaves with symptoms; 4 indicates plants with >75-100% of leaves with symptoms; and 5 indicates plants with complete death. The following formula was used to calculate percentage disease severity:

$$\%\text{Disease severity} = \left(\frac{\Sigma nr}{5N}\right) \text{x100}$$

Where n is the number of plants in each category, r represents the rating category, N represents the total number of plants, and 5 represents the highest rating category.

Determination of soil chitinase activity

Ten grams of soil around roots in each pot for each treatment were collected and mixed well (each treatment alone), Twenty grams were taken as a sample from each treatment in two different locations, Giza and Sakha at the end of experiment, soil samples allowed to dry at 25°C and stored in the dark at 4°C until used. The chitinase enzyme activity in soil samples was assayed according to the method described by Rodriguez-Kabana et al. (1983).

Analytical Statistics

Each experiment was examined separately. Duncan's Multiple Range Test (DMRT) was used to compare the treatment means (Gomez and Gomez 1984). WASP1 was employed for the analysis, with the crucial difference set at $P \leq 0.05$ and the results were interpreted.

RESULTS

Trichoderma species efficacy (*in vitro*):

A-Fusarium oxysporum* f. sp. *cucumerinum

The findings (Table 2 and Fig. 1) clearly demonstrated that *T. album*, followed by *T. hamatum*, and *T. koningii*, were the best in terms of influencing the linear growth development of *F. oxysporum* f. sp. *cucumerinum*. Growth of the fungal pathogen was completely filled the Petri dishes (9- cm diameter) at seven days after inoculation in check treatment. Petri dishes were left 15 days after inoculation and linear growths were measured where the same trend of data appeared. For the increase percentage of liner growth between seven and fifteen days, *T. hamatum* gave the lowest percentage of increase (25.75%) followed by *T. koningii* and *T. album* (35.65 and 36.86%, respectively) whereas, *T. asperellum* gave the highest percentage of increase (42.81%).

Table 2: Effect of four species of *Trichoderma* on linear growth of *F. oxysporum* f. sp. *cucumerinum* after 7 and 15 days of exposure *in vitro*.

Bioagents	Linear growth of *Fusarium* after				Increase percentage of linear growth
	7 days		15 days		
	Linear growth	Inhibition	Linear growth	Inhibition	
T. album	2.57[a]	71.44	4.07[a]	54.78	36.86
T. asperellum	3.30[c]	63.33	5.77[c]	35.89	42.81
T. hamatum	2.97[b]	67.00	4.00[a]	48.11	25.75
T. koningii	3.03[b]	66.33	4.67[b]	55.56	35.65
Control	9.00[d]		9.00[d]		
LSD at 0.05	0.060		0.248		

According to Duncan's multiple range test, the means in each column followed by the same letter(s) did not significantly differ at $P \leq 0.05$.

$$\text{Increase of linear growth\%} = \left(\frac{\text{Linear growth at 15 days} - \text{linear growth at 7 days}}{\text{Linear growth at 15 days}}\right) \text{x100}$$

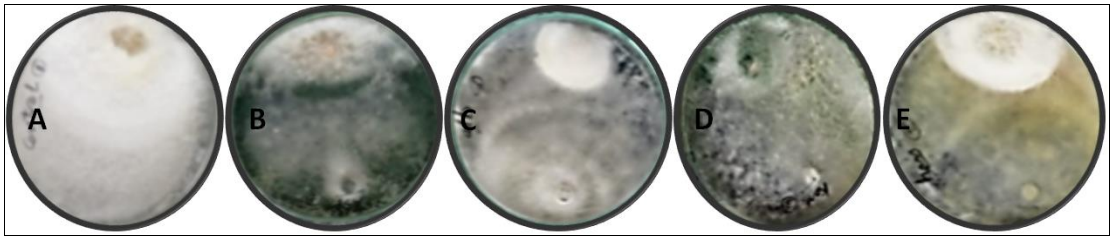

Figure 1: Inhibition of *F. oxysporum* f. sp. *cucumerinum* growth by biological control agents: (A) *F. oxysporum* f. sp. *cucumerinum* alone, (B) *Trichoderma koningii*, (C) *T. album*, (D) *T. asperellum* and (E) *T. hamatum*.

B- *Meloidogyne javanica*

As shown in Table (3) and Fig (2), the inhibitory effect of the four *Trichoderma* species on the mortality or activity of nematode juveniles was dependent on concentration i.e. the nematode's mortality increase as the spore suspension concentration and exposure time increased. *T. album* and *T. hamatum* (100%) had the highest proportion of juvenile death after 24 hours of exposure time. On the other hand, both *T. koningii* and *T. asperellum* had a high mortality rate after 72 hours of exposure, with percentages of 99.6 and 92.6%, respectively. After treatment with *Trichoderma* spp., *M. javanica* juveniles were observed to be lifeless with stiff and straight bodies, but the J2s in the untreated control were actively moving (Fig. 2). In the intestinal tracts of nematodes treated with *Trichoderma*, several unusual patch-like formations were visible, which were freshly produced vacuoles following the destruction of internal organs by *Trichoderma's* nematicidal activity.

Table 3: Effect of four species of *Trichoderma* on the mortality percentages of second-stage juveniles of *Meloidogyne javanica* after 24, 48, and 72 hours of exposure.

Treatments	Concentration of Spore suspension	Nematode mortality %		
		24h	48h	72h
T. album	$1X10^6$	12.5	28.5	40.4
	$2X10^6$	36.6	57.0	71.3
	$4\ X10^6$	100.0	100.0	100.0
T. asperellum	$1X10^6$	25.9	41.8	56.5
	$2X10^6$	39.2	55.7	73.1
	$4\ X10^6$	80.3	83.7	92.6
T. hamatum	$1X10^6$	42.1	51.2	64.6
	$2X10^6$	57.5	76.7	94.8
	$4\ X10^6$	100.0	100.0	100.0
T. koningii	$1X10^6$	25.7	36.0	80.7
	$2X10^6$	37.2	51.8	60.1
	$4\ X10^6$	90.3	97.4	99.6
Control		--	--	---

$$\text{Nematode mortality } \% = \left(\frac{\text{Number of dead juveniles in treatment} - \text{Number of dead juveniles in control}}{100 - \text{Number of dead juveniles in control}}\right) x100$$

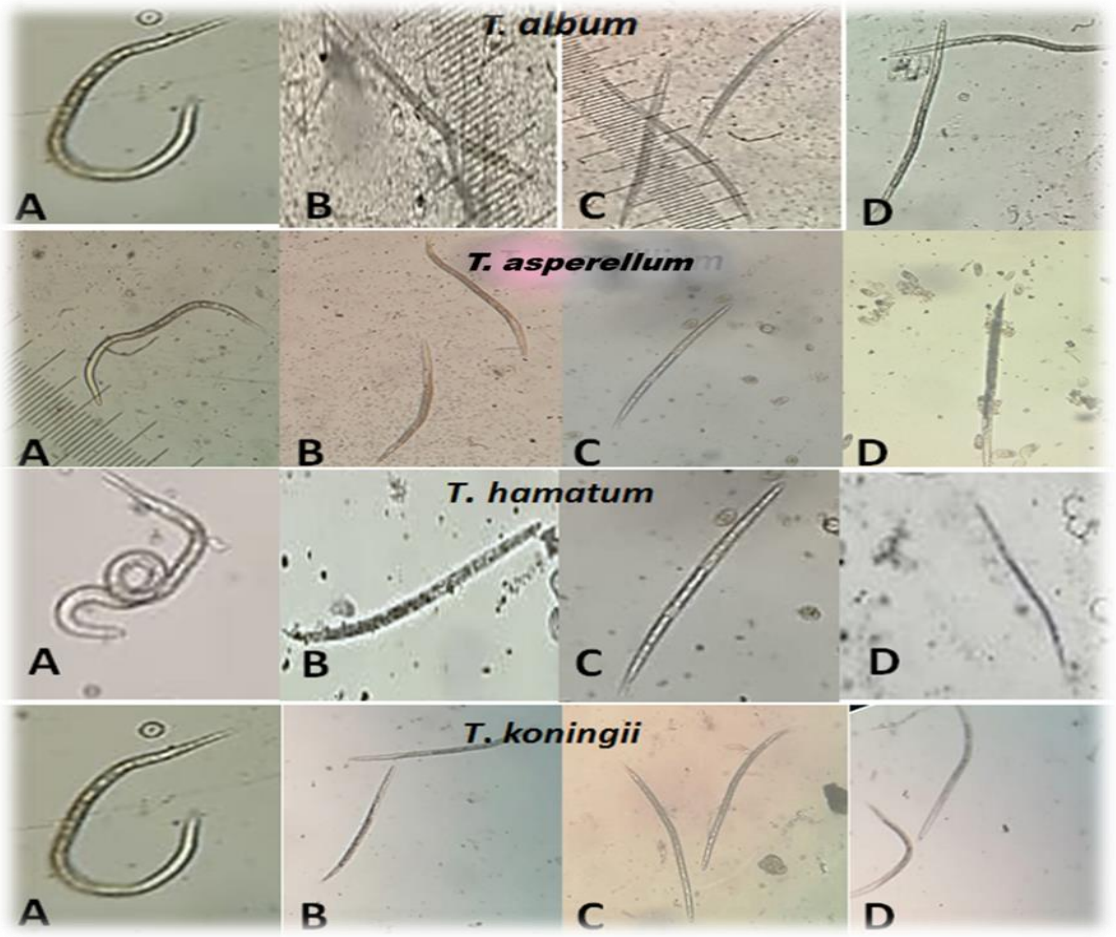

Figure 2. Effects of *Trichoderma* spp. on second-stage juveniles of *Meloidogyne javanica* where: A= Control (sterilized distilled water), B= *Trichoderma* spp. at a concentration of $1x10^6$, C= *Trichoderma* spp. at a concentration of $2x10^6$ and D= *Trichoderma* spp. at a concentration of $4x10^6$.

Evaluation of different species of *Trichoderma* against *F. oxysporum* f. sp. *cucumerinum* and *M. javanica* at two different locations under greenhouse conditions:

A - The effects of *Trichoderma* spp. on the growth variables of infected cucumber plants in Giza governorate

At the Giza site, the effect of *Trichoderma* species (*T. album*, *T. asperellum*, *T. hamatum*, and *T. koningii*) on cucumber plant growth development response infected with *M. javanica* or/and *F. oxysporum* f. sp. *cucumerinum* is shown (Tables 4&5). All of the treatments enhanced plant growth metrics, with variable degrees. Among the *Trichoderma* treatments, *T. hamatum* and *T. koningii* demonstrated the largest improvement in shoot length, whether with nematode, fungus, or both. *T. koningii* and *T. album* also caused the greatest induction of root length in *M. javanica*-infected plants. Cucumber plants infected with *F. oxysporum* f. sp. *cucumerinum* and treated with *T. hamatum* had the longest roots. All *Trichoderma* treatments substantially increased the length of cucumber roots infected with *M. javanica* and *F. oxysporum* f. sp. *cucumerinum* as compared to untreated inoculated plants. On the other hand, treatment with *T. album* and *T. hamatum* resulted in the highest reduction in shoot weight in nematode-treated plants (Table 5). *T. album* and *T. koningii* also performed

the best and considerably enhanced the shoot weight of pathogenic fungus-infected cucumber plants. *T. album* also reached on top when it boosted the shoot weight of plants treated with both *M. javanica* and *F. oxysporum* f. sp. *cucumerinum*.

Table 4: Effect of *Trichoderma* spp. on plant length of cucumber infected with *Meloidogyne javanica* or/and *Fusarium oxysporum* f. sp. *cucumerinum* at Giza governorate under greenhouse conditions.

Treatments	Shoot length (cm)			Root length (cm)		
	Nematode	Fungus	Nematode + Fungus	Nematode	Fungus	Nematode + Fungus
T. album	39.0^{c}	44.8^{c}	41.3^{c}	10.3^{ab}	11.0^{bc}	8.7^{bc}
T. asperellum	32.0^{d}	38.0^{d}	33.7^{d}	7.3^{b}	9.7^{cd}	7.7^{c}
T. hamatum	54.0^{a}	63.0^{a}	44.7^{bc}	8.0^{b}	18.0^{a}	10.7^{b}
T. koningii	50.7^{ab}	54.0^{b}	42.7^{bc}	13.7^{a}	7.7^{de}	11.0^{b}
Oxamyl or/and uniform	48.3^{b}	46.5^{c}	47.2^{b}	9.7^{ab}	6.7^{de}	10.7^{b}
Untreated plant (inoculated)	29.1^{d}	31.6^{e}	28.5^{e}	8.0^{b}	5.7^{e}	4.5^{d}
Untreated plant(uninoculated)	54.0^{a}	54.0^{b}	54.0^{a}	13.7^{a}	13.7^{b}	13.7^{a}
LSD	4.49	4.26	4.91	4.27	3.02	2.36

According to Duncan's multiple range test, the means in each column followed by the same letter(s) did not differ at $P \leq 0.05$.

Trichoderma album and *T. koningii* also performed the best and considerably enhanced the shoot weight of pathogenic fungus-infected cucumber plants (Table 5). *Trichoderma album* also reached on top when it boosted the shoot weight of plants treated with both *M. javanica* and *F. oxysporum* f. sp. *cucumerinum*. Also, it showed a similar tendency, where it had the highest results in boosting root weight in plants treated with fungus, nematodes, or both. In terms of shoot dry weight, most *Trichoderma* species showed a considerable rise in the dry weight of plants that were infected with the nematode only. Treatment with *T. album* and *T. koningii* was shown to be the most effective in raising the plant's shoot dry weight when plants were exclusively treated with fungus. Treatments with *T. album*, *T. asperellum*, and *T. koningii*, on the other hand, increased the dry weight of cucumber shoots in plants infected with nematodes and fungus. It was found that using Oxamyl or/and Uniform resulted in a significant increase in cucumber shoot dry weight (Table 5).

Table 5: Impact of *Trichoderma* spp. on fresh and dry weights of cucumber plants infected with *Meloidogyne javanica* or *Fusarium oxysporum* f. sp. *cucumerinum* or both at Giza governorate under greenhouse conditions.

Treatments	Shoot weight (g)			Root weight (g)			Shoot dry weight (g)		
	Nematode	Fungus	Nematode + Fungus	Nematode	Fungus	Nematode + Fungus	Nematode	Fungus	Nematode + Fungus
T. album	25.5^{a}	25.8^{a}	18.8^{ab}	7.47^{a}	5.63^{a}	6.2^{a}	3.97^{a}	4.4^{a}	2.6^{b}
T. asperellum	19.4^{cd}	19.5^{c}	16.4^{c}	5.83^{d}	4.7^{b}	5.7^{b}	3.4^{a}	3.03^{bc}	2.6^{b}
T. hamatum	23.6^{ab}	22.0^{b}	15.8^{cd}	6.46^{b}	3.37^{d}	5.8^{b}	3.37^{a}	2.7^{c}	1.4^{c}
T. koningii	21.9^{bc}	25.0^{a}	18.2^{b}	3.73^{g}	5.57^{a}	2.8^{d}	1.8^{bc}	3.77^{ab}	2.7^{b}
Oxamyl or/and Uniform	23.5^{ab}	18.8^{c}	14.9^{de}	4.13^{f}	4.03^{c}	2.9^{d}	2.93^{ab}	4.1^{a}	4.1^{a}
Untreated plant (inoculated)	16.8^{d}	19.5^{c}	14.8^{e}	6.1^{c}	3.53^{d}	5.6^{b}	1.53^{c}	1.2^{d}	1.0^{c}
Untreated plant (uninoculated)	19.4^{cd}	19.4^{c}	19.4^{a}	4.83^{e}	4.83^{b}	4.8^{c}	3.8^{a}	3.8^{ab}	3.8^{a}
LSD	2.9	2.5	0.99	0.175	0.23	0.27	1.26	0.96	0.95

According to Duncan's multiple range test, the means in each column followed by the same letter(s) did not differ at P≤ 0.05.

B-The effect of *Trichoderma* spp. on the growth of variables of infected cucumber plants in Sakha county.

The effect of the same previous species of *Trichoderma* on cucumber growth parameters infection with *M. javanica* or/and *F. oxysporum* f. sp. *cucumerinum* at Sakha county location is shown in Tables (6&7). The results showed that *T. hamatum* was the most effective at lengthening the shoots of cucumber plants infected with nematodes, fungus, or both. The root length of cucumber plants infected with *M. javanica* was significantly improved by *T. hamatum* and *T. koningii*. Also, *T. koningii* reached on top with regard to extending the root length of plants that had been harmed by fungus. *Trichoderma hamatum*, on the other hand, improved the root length of plants treated with fungus and nematode. In comparison to untreated inoculated plants, conventional Oxamyl increased the shoot weight of cucumber infected with *M. javanica* (Table 7). Treatment with *T. asperellum*, on the other hand, resulted in the smallest percentage rise in shoot weight of nematode-infected plants. *Trichoderma album* was the first to increase shoot weight in plants infected with pathogenic fungus *F. oxysporum* f. sp. *cucumerinum*. *Trichoderma album* was the first to increase shoot weight in plants infected with pathogenic fungus *F. oxysporum* f. sp. *cucumerinum*. All treatments of *Trichoderma* spp. substantially enhanced the shoot weight of cucumber infected with *M. javanica* and *F. oxysporum* f. sp. *cucumerinum* ($P \leq 0.05$) compared to the untreated inoculated plant.

Table 6: Influence of *Trichoderma* spp. on plant length of cucumber plant infected with *Meloidogyne javanica* or/and *Fusarium oxysporum* f. sp. *cucumerinum* at Sakha County under greenhouse conditions.

Treatments	Shoot length (cm)			Root length (cm)		
	Nematode	Fungus	Nematode + Fungus	Nematode	Fungus	Nematode + Fungus
T. album	51.2^{c}	58.2^{c}	51.7^{c}	8.6^{c}	11.7^{c}	8.4^{e}
T. asperellum	43.5^{d}	48.0^{d}	48.2^{d}	8.5^{c}	9.7^{d}	8.7^{e}
T. hamatum	67.4^{a}	75.2^{a}	56.3^{b}	14.2^{a}	6.7^{ef}	12.5^{b}
T. koningii	61.3^{b}	63.4^{b}	52.1^{c}	11.5^{a}	17.1^{a}	11.5^{c}
Oxamyl or/and Uniform	60.3^{b}	56.1^{c}	59.1^{b}	10.8^{b}	7.7^{e}	10.3^{d}
Untreated plant (inoculated)	36.3^{e}	31.4^{e}	29.3^{e}	4.5^{d}	5.6^{f}	3.5^{f}
Untreated plant (uninoculated)	66.0^{a}	66.0^{b}	66.0^{a}	15.2^{a}	15.2^{b}	15.2^{a}
LSD	4.49	4.26	4.91	4.27	3.02	2.36

According to Duncan's multiple range test, the means in each column followed by the same letter(s) did not significantly differ at $P \leq 0.05$.

Trichoderma album showed the highest root weight of plants infected with *M. javanica* or *F. oxysporum* f. sp. *cucumerinum*. *Trichoderma album*, as well as *T. asperellum* and *T. hamatum*, were found to increase root weight in cucumber plants infected with root-knot nematode and *Fusarium* wilt was the most effective at lengthening the shoots of cucumber plants infected with nematodes, fungus, or both. The root length of cucumber plants infected with *M. javanica* was significantly improved by *T. hamatum* and *T. koningii*. Also, *T. koningii* reached on top with regard to extending the root length of plants that had been harmed by fungus. *Trichoderma hamatum*, on the other hand, improved the root length of plants treated with fungus and nematode. In comparison to untreated inoculated plants, conventional Oxamyl increased the shoot weight of cucumber infected with *M. javanica* (Table 7). Treatment with *T. asperellum*, on the other hand, was resulted in the smallest percentage rise in shoot weight of nematode-infected plants. *Trichoderma album* was the first to increase shoot weight in plants infected with pathogenic fungus *F. oxysporum* f. sp. *cucumerinum*. *Trichoderma album* was the first to increase shoot weight in plants infected with pathogenic fungus *F. oxysporum* f. sp. *cucumerinum*. All treatments of *Trichoderma* spp. substantially enhanced the shoot weight of cucumber infected with *M. javanica* and *F. oxysporum* f. sp. *cucumerinum* ($P \leq 0.05$) compared to the untreated inoculated plant. *Trichoderma album* showed the highest root weight of plants infected with *M. javanica* or *F. oxysporum* f. sp. *cucumerinum*. *Trichoderma album*, as well as *T. asperellum* and *T. hamatum*, were found to increase root weight in cucumber plants infected with root-knot nematode and *Fusarium* wilt disease. In terms of plant dry weight, *T. album* was observed to enhance the dry weight of plants infected with nematode or fungus. There was no significant difference among the different *Trichoderma* species in raising the dry weight of cucumber plants in those infected with both pathogens.

C-Greenhouse evaluations of *Trichoderma* spp. against *Fusarium* wilt of cucumber at Giza governorate and Sakha county.

This experiment looked at the impact of four *Trichoderma* species on the occurrence and severity of wilt disease on cucumber plants inoculated with *F. oxysporum* f. sp. *cucumerinum* and *M. javanica* at Giza and Sakha localities under greenhouse conditions. Table (8) revealed that treating *F. oxysporum* f. sp. *cucumerinum* with root-knot nematode increased the incidence of wilt disease as compared to infecting the plant with the fungal pathogen alone, with or without *Trichoderma* inoculation. The application of *T. hamatum* to soil infected with *F. oxysporum* and soil infected with *F. oxysporum* and *M. javanica* resulted in the highest antagonistic effect, with 26.7 and 33.3% disease incidences and low disease severities reaching 4.6 and 8.3%, respectively, with significant differences among all treatments. *Trichoderma koningii* recorded 33.3 and 40% illness incidence with 15.7 and 18.4% percent disease severities when the soil was infested by *F. oxysporum* and *M. javanica*, respectively.

At the Giza location, high disease incidences were found when plants were inoculated with *F. oxysporum* and *M. javanica* or *F. oxysporum* only, with 86.7 and 80.0%, with high disease severity percentages of 52.6 and 45.2% respectively. Whereas, *T. hamatum* and *T. koningii* induced the same lowest disease incidences. percentages of 20 and 33%, respectively when the soil was infested by *F. oxysporum* alone or with *M. javanica* at the Sakha location. Disease severities differed with *T. hamatum* treatment recording 5.8 and 10.1%, when the soil was infected by *F.oxysporum* alone or with *M. javanica*, respectively. Untreated inoculated treatment with either *F. oxysporum* alone or with *M. javanica*, on the other hand, had the highest

Table 7: Impact of *Trichoderma* spp. on fresh and dry weights of cucumber plants infected with *Meloidogyne javanica*, *Fusarium oxysporum* f. sp. *cucumerinum* or both at Sakha county under greenhouse conditions.

Treatments	Shoot weight (g)			Root weight (g)			Shoot dry weight (g)		
	Nematode	Fungus	Nematode + Fungus	Nematode	Fungus	Nematode + Fungus	Nematode	Fungus	Nematode + Fungus
T. album	33.3^{ab}	36.3^{a}	29.2^{b}	7.4^{ab}	7.9^{a}	6.7^{a}	5.3^{a}	6.06^{a}	2.9^{b}
T. asperellum	28.9^{cd}	29.5^{cd}	27.3^{cd}	5.7^{cd}	5.7^{bc}	6.4^{a}	4.4^{bc}	4.03^{c}	2.5^{bcd}
T. hamatum	33.1^{ab}	30.6^{c}	26.8^{d}	5.5^{d}	5.9^{bc}	6.3^{a}	3.9^{c}	3.4^{cd}	2.2^{bcd}
T. koningii	32^{abc}	34.2^{b}	27.9^{c}	3.7^{e}	6.6^{b}	2.9^{c}	2.6^{d}	5.17^{b}	2.83^{bc}
Oxamyl or/and Uniform	34.9^{a}	26.8^{e}	27.7^{cd}	8.1^{a}	3.5^{d}	4.7^{b}	3.9^{c}	4.13^{c}	2.13^{cd}
Untreated plant (inoculated)	26.2^{d}	28.8^{d}	24.8^{e}	6.5^{bc}	5.2^{c}	5.2^{b}	2.8^{d}	2.7^{d}	2.1^{d}
Untreated plant (uninoculated)	30.4^{bc}	30.4^{c}	30.4^{a}	6.4^{c}	6.4^{b}	6.4^{a}	5.2^{ab}	5.2^{b}	5.2^{a}
LSD	3.7	1.2	1.13	0.9	0.9	0.8	0.8	0.78	0.727

According to Duncan's multiple range test, the means in each column followed by the same letter(s) did not significantly differ at $P \leq 0.05$.

Table 8: Effect of four *Trichoderma* species on wilt disease incidence and severity percentage on cucumber plants inoculated with *Fusarium oxysporum* f. sp. *cucumerinum* (FOC) and nematode under greenhouse conditions at Giza governorate.

Bioagents	Disease Incidence %		Disease Severity %	
	FOC	FOC+Nematode	FOC	FOC+Nematode
T. album	33.3^{bc}	46.7^{c}	10.8^{c}	16.4^{b}
T. asperellum	40.0^{b}	60.0^{b}	12.3^{c}	17.1^{b}
T. hamatum	26.7^{c}	33.3^{d}	4.6^{d}	8.3^{c}
T. koningii	33.3^{bc}	40.0^{cd}	15.7^{b}	18.4^{b}
Uniform	6.7^{d}	13.3^{e}	0.34^{e}	1.42^{d}
Untreated plant(inoculated)	80.0^{a}	86.7^{a}	45.2^{a}	52.6^{a}
Untreated plant (uninoculated)	0.0^{e}	0.0^{f}	0.0^{f}	0.0^{e}
LSD	6.62	7.38	1.71	1.78

According to Duncan's multiple range test, the means in each column followed by the same letter(s) did not significantly differ at $P \leq 0.05$.

Table 9: Effect of four *Trichoderma* species on wilt disease incidence and severity percentage on cucumber plants inoculated with *Fusarium oxysporum* f. sp. *cucumerinum* (FOC) and *Meloidogyne javanica* under greenhouse conditions at Sakha county.

Bioagents	Disease Incidence %		Disease Severity %	
	FOC	FOC+Nematode	FOC	FOC+Nematode
T. album	33.3^{b}	40.0^{bc}	9.8^{c}	19.3^{b}
T. asperellum	26.7^{bc}	53.3^{b}	10.3^{c}	15.7^{c}
T. hamatum	20.0^{c}	33.3^{c}	5.8^{d}	10.1^{d}
T. koningii	20.0^{c}	33.3^{c}	12.5^{b}	20.4^{b}
Uniform	6.7^{d}	6.7^{d}	1.12^{e}	2.31^{e}
Untreated plant(inoculated)	86.7^{a}	93.3^{a}	48.9^{a}	55.8^{a}
Untreated plant(uninoculated)	0.0^{e}	0.0^{e}	0.0^{f}	0.0^{f}
LSD at 0.05	7.50	9.19	1.53	1.31

According to Duncan's multiple range test, the means in each column followed by the same letter(s) did not significantly differ at $P \leq 0.05$.

percentages of 20 and 33%, respectively when the soil was infested by *F. oxysporum* alone or with *M. javanica* at the Sakha location. Disease severities differed with *T. hamatum* treatment recording 5.8 and 10.1%, when the soil was infected by *F.oxysporum* alone or with *M. javanica*, respectively. Untreated inoculated treatment with either *F. oxysporum* alone or with *M. javanica*, on the other hand, had the highest disease incidences and severities, with 86.7 and 93.3% disease incidence and 48.9 and 55.8% disease severity, respectively (Table 9).

D – Effect of *Trichoderma* spp. on population density of *M. javanica* at Giza governorate and Sakha county.

With or without *Fusarium*, all treatments utilizing different species of *Trichoderma* resulted in a reduction in the total number of nematodes infecting cucumber at Giza location (Table 10). The *T. album* treatment resulted in the greatest reduction in the total number of nematodes. There were no substantial differences in the presence of the fungus with nematodes among the various *Trichoderma* species. The treatments utilizing *T. album* and *T. koningii* were shown to be the most effective in lowering the total number of galls (67.8 and 66.6 galls /plant, respectively) on cucumber roots infected with nematodes. However, in the presence of *Fusarium* fungus and nematodes, *T. hamatum* which induced 81.8 galls per plant with a root gall index (4.0) was the best treatment. In the case of nematode alone with egg mass index (4.0), the treatment using *T album*, *T. asperellum* and *T. koningii* produced the best results in reducing the number of egg masses (38.4, 45 and 36.8 egg masses/plant, respectively), but there were no significant differences between *Trichoderma* species in reducing the number of egg masses in case of nematodes with fungus.

Table 10: Efficiency of *Trichoderma* spp. on population density of *Meloidogyne javanica* alone or interacted with *Fusarium* wilt disease at Giza governorate under greenhouse conditions.

Treatments	Final nematode population (soil and roots)		Galls (RGI)		Egg masses (EI)	
	Nematode	Nematode + fungus	Nematode	Nematode + fungus	Nematode	Nematode + fungus
T. album	1306.8^{d}	2515.6^{b}	67.8^{c} (4.0)	102.4^{b} (5.0)	38.4^{c} (4.0)	54.6^{b} (4.0)
T. asperellum	3461^{b}	2546.8^{b}	98.8^{b} (4.0)	93.2^{bc} (4.0)	45^{c} (4.0)	50.4^{b} (4.0)
T. hamatum	2393.6^{c}	2489^{b}	90.2^{b} (4.0)	81.8^{c} (4.0)	71.4^{b} (4.0)	42.6^{b} (4.0)
T. koningii	2373.6^{c}	2592.4^{b}	66.6^{c} (4.0)	86.8^{bc} (4.0)	36.8^{c} (4.0)	46.4^{b} (4.0)
Oxamyl	337.4^{e}	426.8^{c}	22.4^{d} (3.0)	32.4^{d} (4.0)	12^{d} (3.0)	16.8^{c} (3.0)
Control	6492.6^{a}	6647.8^{a}	229.6^{a} (5.0)	201^{a} (5.0)	165^{a} (5.0)	133.2^{a} (5.0)
LSD	96.4	425.6	18.8	17.5	11.6	15.9

Each value is the mean of five replicates. Final population of nematodes = No.nematodes in 1kg soil + No.nematodes in 1g plant root.According to Duncan's multiple range test, the means in each column followed by the same letter(s) did not significantly differ at $P \leq 0.05$. Numbers between parentheses represent the root galls and egg masses indices where 0= no galls or egg masses, 1= 1:2, 2= 3:10, 3= 11:30, 4= 31:100 and 5= more than 100 galls or egg masses / root system.

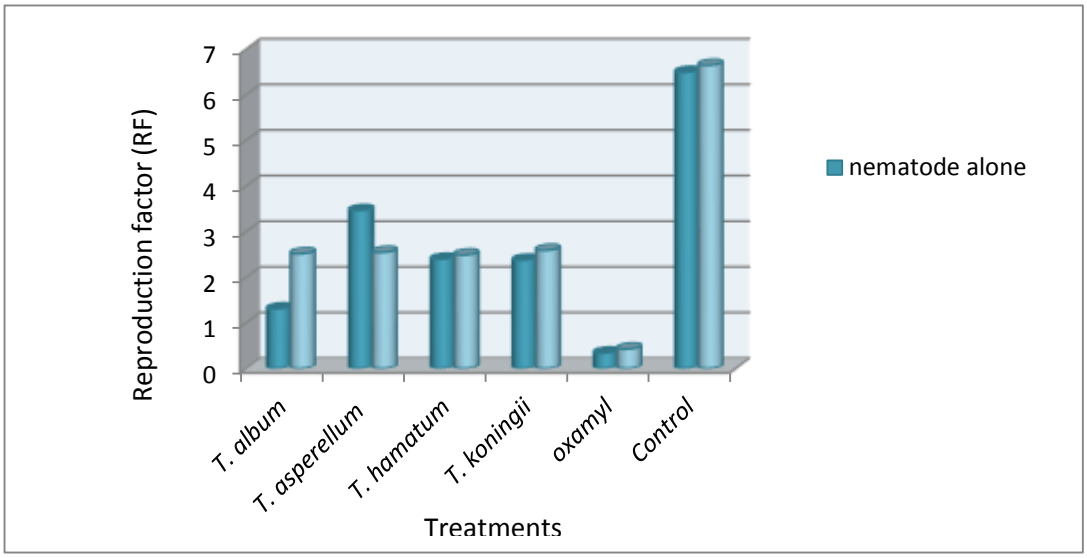

Figure 3. Effect of *Trichoderma* spp. on reproduction factor of *M. javanica* alone or with *Fusarium oxysporum* f. sp. *cucumerinum* infecting cucumber plants under greenhouse conditions at Giza governorate.

Figure (3) showed the effect of treatments on reducing the reproduction factor of nematodes. A decrease in the reproduction factor was observed in the case of nematode alone using *Trichoderma* spp. except for treatment with *T. asperellum*, where a lower reproduction factor (RF= 2.5) was found in the presence of fungus with nematodes than nematode alone (RF= 3.4).

At Sakha location, all employed treatments greatly reduced the number of nematodes in the soil and roots (Table 11). However, *T. asperellum* treatment had the least effect in reducing the number of nematodes in the soil and roots when plant infected with *M. javanica* alone, but there were no significant differences among *Trichoderma* spp., when nematodes and fungus were used together. Furthermore, with pots infected just with *M. javanica*, no significant variations ($P \leq 0.05$) in the number of galls were found among different species of *Trichoderma.* Only *T. koningii* with nematode resulted in a lower number of egg masses (31.8 egg masses/ plant). However, in the case of *Fusarium* with nematode, *T. asperellum*, *T. hamatum*, and *T. koningii* had the lowest number of egg masses index (4.0). It is clear that all *Trichoderma* treatments lowered the *M. javanica*-reproduction factor when compared to untreated inoculated plants (Fig. 4). Moreover, most treatments were more effective when *M. javanica* was present alone rather than when *M. javanica* was present with *F. oxysporum* f. sp. *cucumerinum*.

E- Chitinase activity in soil at Giza governorate and Sakha county

Enzyme which acts in soil (Chitinase activity) was studied during screening of *T. album*, *T. asperellum*, *T. hamatum*, and *T. koningii* against *Fusarium* wilt and *M. javanica* in the rhizosphere of cucumber plants at two locations (Giza and Sakha) under greenhouse conditions. Data presented in Figure (5) showed that all treatments have different chitinase activities in soil. In all treatments, chitinase enzyme activity in soil was greater in Sakha than in Giza. Treatment by *T. hamatum* with *F. oxysporum* f. sp. *cucumerinum* had the greatest activity of chitinase enzyme in soil (9.1 and 8.7 N-acetyl-β–D-glucosamine (NAGA) mg/g soil/hour) in both areas (Sakha and Giza), respectively followed by the comparative treatment, fungus combined with nematode (6.4 and 5.8 NAGAmg/g soil/hour). While, chitinase activity in soil treated

by *T. koningii* with *Fusarium* and nematode united together gave 4.5 and 3.9 NAGAmg/g soil/hour.

Table 11: Influence of *Trichoderma* spp. on population density of *Meloidogyne javanica* alone or interacted with *Fusarium oxysporum* f. sp. *cucumerinum* infecting cucumber plants under greenhouse conditions at Sakha county.

Treatments	Final nematode population (soil and roots)		No. of Galls (RGI)		No. of Egg masses (EI)	
	Nematode	Nematode + fungus	Nematode	Nematode + fungus	Nematode	Nematode + fungus
T. album	2435.4^{c}	2802.8^{b}	82.6^{b} (4.0)	96^{b} (4.0)	50.4^{bc} (4.0)	63^{b} (4.0)
T. asperellum	3468.4^{b}	2633.4^{b}	86^{b} (4)	76.8^{c} (4)	52.2^{b} (4)	41^{c} (4)
T. hamatum	2525.2^{c}	2818.8^{b}	70.6^{b} (4)	81.4^{bc} (4)	33^{cd} (4)	33.4^{c} (4)
T. koningii	2717.8^{c}	2691.8^{b}	68^{b} (4)	77.6^{c} (4)	31.8^{d} (4)	44.8^{c} (4)
Oxamyl	343.8^{d}	394.8^{c}	19.6^{c} (3)	23.8^{d} (3)	10.4^{e} (2)	14.2^{d} (3)
Control	6827^{a}	7119^{a}	222^{a} (5)	204^{a} (5)	149.2^{a} (5)	114.6^{a} (5)
LSD	463.03	424.1	26.1	17.9	17.7	15.3

Each value is the mean of five replicates. Final population of nematodes = Number of nematodes in 1kg soil + Number of nematodes in 1g plant root. According to Duncan's multiple range test, the means in each column followed by the same letter(s) did not significantly differ at P≤ 0.05. Numbers between parentheses represent the root galls (RGI) and egg masses index (EI) where 0= no galls or egg masses, 1= 1-2, 2= 3-10, 3= 11-30, 4= 31-100 and 5= more than 100 galls or egg masses / root system.

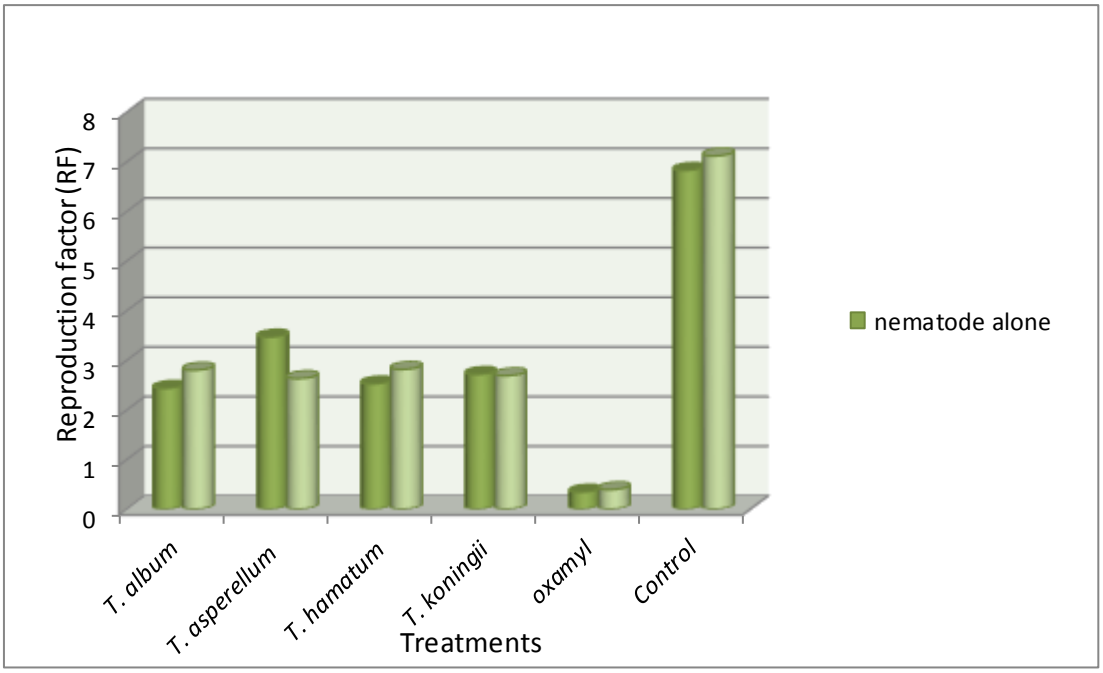

Figure 4: Effect of *Trichoderma* spp. on the reproduction factor of *M. javanica* alone or in combination with *F. oxysporum* f. sp. *cucumerinum* infecting cucumber under greenhouse conditions in Sakha county.

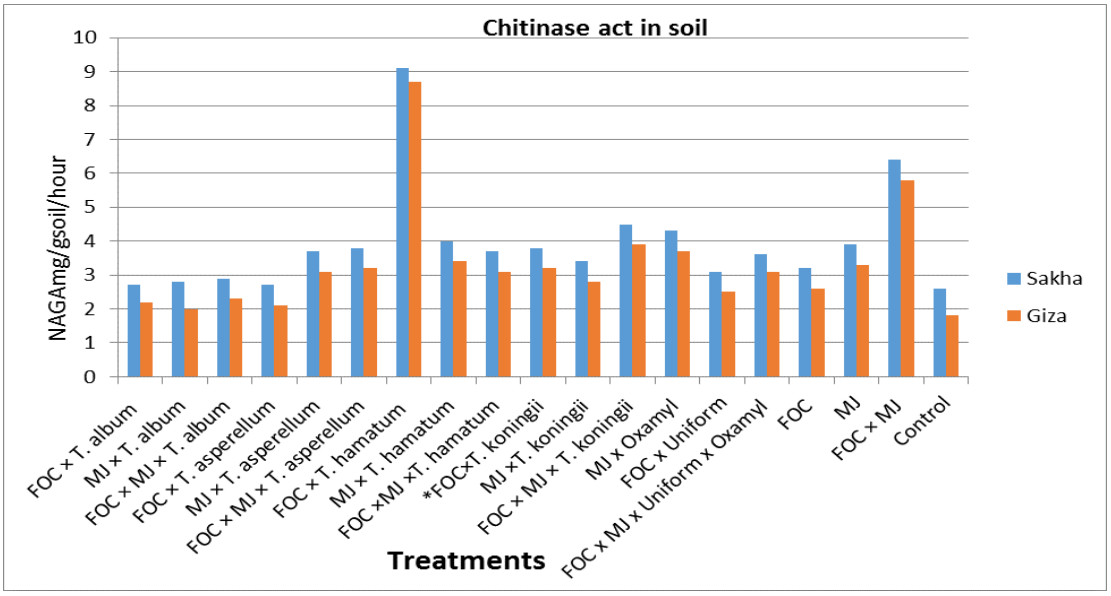

Figure 5: Chitinase enzyme activity in farmed soil infected with *F. oxysporum* f. sp. *cucumerinum* (FOC) and *Meloidogyne javanica* (MJ) alone or in combination, and treated with *Trichoderma* spp. in Giza governorate and Sakha county under greenhouse conditions.

With regard to treatments of nematode with different species of *Trichoderma*, it was noted that soil treated with *T. hamatam* showed an increment in chitinase enzyme in two locations Sakha and Giza (4 and 3.4 NAGAmg/g soil/hour, respectively). However, *T. koningii* was the most effective in boosting the rate of chitinase enzyme in soil in the conjugated treatment with this fungus and nematode (4.5 and 3.9 NAGAmg/g soil/hour) at Sakha and Giza locations, respectively.

DISCUSSION

It was noticed that all *Trichoderma* spp. inhibited the growth of the pathogenic fungus *F. oxysporum* f. sp. *cucumerinum*. Activation for *in vitro* effectiveness of *Trichoderma* species against *Fusarium* had been reported by Kamel et al. (2020). Saravanakumar et al. (2016) findings back up the present data on the fungicidal activity of 10 *Trichoderma* isolates, which showed an inhibition rate of more than 85% for *F. oxysporum* f. sp. *cucumerinum*. The competition for nutrients and space, mycoparasitism, and the synthesis of antibiotic compounds and hydrolytic enzymes, according to Harman et al. (2004b) are the key processes involved in *Trichoderma* antagonistic activity.

Higher concentrations of *Trichoderma* spp. appeared to have a great influence on nematode mortality. After 24 hours, *T. hamatum* and *T. album* caused almost 100% nematodes mortality. These findings were comparable with those of Migunova et al. (2018), who found that the three different *Trichoderma* species induced 100% juvenile mortality in *M. javanica* and 94.1 to 100% juvenile mortality in *M. incognita*. Costa et al. (2001) showed that all *Trichoderma* spp. filtrates were toxic to *M. incognita*, resulting in 98% immobilized and dead J2. Balardin et al. (2021), hypothesized that the mortality of *Meloidogyne* species-J_2 by various isolates of *Trichoderma* could be linked to the presence of enzymes called proteases and chitinases, which are involved in the degradation of the nematode cuticle, which is a strong covering consisting of proteins and chitin.

The incidence and severity percentages of illness were considerably greater in the treatment where *M. javanica* was inoculated with *F. oxysporum* f.sp. *cucumerinum* in the control (inoculated) and all treatments in the pot experiment. A similar result was reported in the protected cucumber culture, where the nematode *M. incognita* was infected 7 days prior to the fungus *F. oxysporum* f. sp. *cucumerinum*, which has a greater disease incidence, followed by simultaneous nematode and fungus inoculation than the control (Patil et al., 2018). On the other hand, regardless of whether nematode or pathogenic fungus was present or both, all treatments of *Trichoderma* spp. significantly improved plant growth metrics. This conclusion was in line with previous researches (Sahebani and Hadavi, 2008; Affokpon et al., 2011; Radwan et al., 2012; Jamshidnejad et al., 2013 and Elgorban et al., 2014), who found that different *Trichoderma* isolates boosted shoot weight, and reduced root knot galling and reproduction. According to Cutler et al. (1986), secondary metabolites generated by *Trichoderma* spp., such as koningin A (*T. koningii*) and 6-pentyl-alpha-pyrone (*T. harzianum*), are plant growth regulators. *Trichoderma* species were also reported to boost plant growth (through phytohormone synthesis) and induce systemic disease resistance, making them even more tempting for usage in agricultural settings (Monte et al., 2019).

All *Trichoderma* spp. utilized in our study lowered disease incidence and severity percentages compared to control (inoculated). *T. hamatum* and *T. koningii* caused the lowest symptom incidence and severity percentages. Cucumber farming is plagued by the fungal-nematode complex. It was a major threat in East China, where 67.6% of symptoms has been documented (Dong et al., 2004). *Trichoderma asperellum* was found to be effective against the cucumber vascular wilt pathogen, according to Saravanakumar et al. (2016). *Trichoderma koningii* was successful in suppressing *F. oxysporum*. f. sp. *Phaseoli*, according to Otadoh et al. (2010).

The quantity of nematodes in the soil and on the roots was considerably decreased by all of the treatments evaluated. Among the tested treatments, *T. album* at the Giza location reduced the total final nematode population. According to Radwan et al. (2012), *T. album* was shown to be beneficial in controlling *M. incognita* by lowering the quantity of galls on tomato roots and J_2 in the soil. When pathogenic fungus was found with nematode, *T. hamatum* reduced the number of egg masses. To protect a plant from disease damage, *T. hamatum* colonizes the roots and causes systemic alterations in plants (Alfano et al., 2007). *Trichoderma asperellum*, also, caused a significant reduction in the amount of egg masses on cucumber roots. According to Kiriga et al. (2018), *T. asperellum* M2RT4 was the most effective isolate for decreasing *M. javanica* galls, egg masses, and deposited eggs. *Trichoderma asperellum* has two unique genes that code for phenylalanine and hydroperoxide lyase, which promote systematic resistance and phytoalexin accumulation to give cucumber resistance against *Pseudomonas syringae* PV. *Lachrymans* (Yedidia et al. 2003). In comparison to other species at the Sakha site, *T. asperellum* treatment had the least effect on nematode counts in the soil and on the roots. This finding was supported by Hajji, et al. (2016), who found that *T. viride* and *T. harzianum* exhibited greater and similar nematicide effects than *T. asperellum*.

Trichoderma species manufacture chitinases in a variety of ways. In our study, *T. hamatum* exhibited the highest rate of enzyme activity, whether with nematode or fungus separately. According to Sayed et al. (2019), *T. asperelloides* and *T. hamatum* have strong chitinase activity. Elad and Henis (1982) and Haran et al. (1996) established that the biological control of the *Trichoderma* genus against plant pests and diseases is mostly due to the release of hydrolytic enzymes such as -1, 3-

glucanase, chitinase, protease, and lipase, which are responsible for antagonism. Furthermore, the gelatinous matrices of root-knot nematode egg masses have been found to stimulate the development of proteolytic and chitinolytic enzymes by the fungus (Sharon et al., 2007; González et al., 2012).

Depending on where they are present, *Trichoderma* species have different effects on plant, fungus, and nematode. Varying environmental variables, microorganisms on our planet may have unique adaptations to different environments, e.g., soil, climate, plant cover, etc. (Louzada et al., 2009). As a result, a *Trichoderma* species antifungal efficacy likely differed depending on the climate in which the fungus is used.

CONCLUSION

The *Fusarium* wilt-root knot nematode complex disease is one of the most well-known and economically significant maladies in the world, since they both influence root system functions (water and mineral uptake). The importance of applying biocontrol agents, *Trichoderma* spp., in minimizing the damage caused by this fungi-nematodes complex in order to manage the disease on commercial crops was proven in this study. Treatment with *Trichoderma* species decreased the fungal-nematode disease complex and enhanced plant growth parameters.

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الملخص العربى

فاعليه انواع من فطر التريكوديرما ضد مرض الذبول الفيوزاريومي ونيماتودا تعقد الجذور على نبات الخيار

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[١] قسم بحوث الأمراض النيماتودية - معهد بحوث أمراض النباتات – مركز البحوث الزراعية – الجيزه – مصر.

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تعتبر المكافحه البيولوجية احد الطرق الهامه في السنوات الاخيرة لمكافحة العديد من الامراض النباتية. وجنس *Trichoderma* هو جنس فريد من نوعه له نطاق واسع من النشاط ضد أهم مسببات الأمراض النباتية. في هذا البحث تم تقييم تأثير بعض أنواع من *Trichoderma* وهي , (*T. album, T. asperellum, T. hamatum* (*T. koningii*) ضد نيماتودا تعقد الجذور *Meloidogyne javanica* والذبول الفيوزاريومي *Fusarium oxysporum* f. sp. *cucumerinum* على نبات الخيار في موقعين (جيزه و سخا) تحت ظروف الصوبة. ايضا تم دراسة تأثير هذه الأنواع من *Trichoderma* على شده المرض لفطر *Fusarium* وموت يرقات النيماتودا تحت ظروف المعمل. اوضحت النتائج ان كل انواع *Trichoderma* المستخدمه ادت الى تثبيط نمو الميسليوم لفطر *Fusarium* كذلك اعطت نسبة موت عالية ليرقات النيماتودا *M. javanica* تحت ظروف المعمل. اظهرت المعاملات باستخدام *T. album* و*T. hamatum* افضل نشاط مضاد لفطر *Fusarium* كذلك اعطت نسبة موت ١٠٠% ليرقات النيماتودا بعد ٢٤ ساعة. وجد تحسن معنوي في نمو نباتات الخيار المعاملة ب *T. album* و*T. hamatum* في كلا الموقعين تحت ظروف الصوبة. في منطقة الجيزه، ادت المعاملة باستخدام *T. hamatum* الي اعلى تاثير مضاد في التربه المعاملة بالفطر والنيماتودا معا. تسببت جميع المعاملات الى خفض الكثافة العددية للنيماتودا سواء في وجود او عدم وجود فطر *Fusarium*. كانت المعاملة باستخدام *T. album* افضل المعاملات في خفض عدد العقد وكتل البيض على جذور الخيار المصابة بالنيماتودا فقط في منطقة الجيزه. ولكن في حالة وجود فطر *Fusarium* مع النيماتودا فكانت افضل المعاملات هي المعاملة باستخدام *T. hamatum*. في منطقة سخا لوحظ عدم وجود فروق معنوية بين انواع *Trichoderma* المختلفة في حالة وجود فطر *Fusarium* مع النيماتودا. لكن اعطت المعاملة باستخدام *T. koningii* افضل النتائج في خفض عدد كتل البيض. معظم المعاملات كانت افضل تأثيرا في خفض معامل التكاثر للنيماتودا في حاله وجود النيماتودا بمفردها. وفي كلا الموقعين، سجل زيادة النشاط الانزيمي للكيتينيز في التربة عند المعاملة باستخدام *T. hamatum* مع فطر *Fusarium*.