EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

TOXICOLOGY & PEST CONTROL

F

ISSN
2090-0791

WWW.EAJBS.EG.NET

Vol. 15 No. 1 (2023)

www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci.* (F. Toxicology& Pest control) *Vol.15(1) pp 139-156 (2023)*
DOI: 10.21608/EAJBSF.2023.300035

Egypt. Acad. J. Biology. Sci., 15(1):139-156(2023)

Egyptian Academic Journal of Biological Sciences
F. Toxicology & Pest Control
ISSN: 2090 - 0791
http://eajbsf.journals.ekb.eg/

Root-Knot Nematode and Root-Rot Disease Complex Management in Tomato Plants with Virkon® S and *Trichoderma viride*

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ARTICLE INFO
Article History
Received:23/2/2023
Accepted:10/5/2023
Available:16/5/2023

***Keywords*:**
Pythium debaryanum, *Fusarium solani*, *Rhizoctonia solani*, *Meloidogyne incognita*, *Trichoderma viride*, Virkon S, root-knot nematode, root-rot, disease complex.

ABSTRACT

Associated microorganisms significantly influence plant diseases regarding inhibition and acceleration of the infection process. The root-knot nematode (N), *Meloidogyne incognita* complex with the root-rot fungal disease is an example of a disease complex that requires the collaboration of multiple pathogens to induce epidemic infection [(*Pythium debaryanum* (P), *Fusarium solani* (F) and *Rhizoctonia solani* (R)]. The disease complex is a significant problem due to the various interwoven diseases and the difficulties of using conventional approaches to control. Therefore, the study intended to introduce Virkon S as a nontraditional, non-selective, and non-persistent chemical sterilant agent for concurrent application with the multifunctional *Trichoderma viride* (T) bioagent. Based LC_{50} values tested by the poisonous medium technique after three days of incubation showed T bioagent had the highest tolerance to Virkon S (0.491 % w/v), while the R pathogen was the most sensitive to Virkon S (0.385 % w/v), followed by F (0.457 % w/v), and finally P (0.487 % w/v). Diseases caused by P, F, and R are ranked from most incidence to most severe on tomatoes. Applying T bioagent decreased disease significantly varied with pathogen accompanied by enhancing tomato growth parameters infected with whether nematode or pathogenic fungi. Conversely, the bioagent T successfully reduced fungal disease incidence and severity and the final nematode population in tomato roots and soils resulting from the complex infection of the nematode and fungi and varied considerably according to tested fungi with the least efficiency in PNT treatment. Moreover, applying Virkon S with T bioagent against the nematode-fungi disease complex enhanced plant growth parameters enhancement exceeded healthy plants to reach a maximum increase with NTR treatment supplemented with 1.5 % (w/v) Virkon S. While 1.5 and 3 % (w/v) Virkon S concentrations completely suppressed nematode parameters except for PNT treatment.

Our findings indicate that combining the Virkon S with *T. viride* results in preliminary control of the root-knot nematode-root rot fungi complex. Virkon S effectively suppresses the nematode population. Then, *T. viride* bioagent completes the march after the Virkon S's success in its mission and degrades in the soil. So that the *Trichoderma* parasite on the root rot fungi can colonize the rhizosphere region, enhancing tomato growth parameters significantly. This combination represents a new strategy against root-knot nematodes and the soil-borne fungus disease complex.

INTRODUCTION

Tomato plants are economic crops infected by several soil-borne fungal pathogens such as *Fusarium* spp., *Rhizoctonia solani*, *Pythium debaryanum*, and *Sclerotium rolfsii*, which cause serious diseases such as root rots and wilt and finally, reducing crop yield and quality (El-Mohamedy *et al.*, 2014; Kipngeno *et al.*, 2015). Root-knot nematodes (RKN) are economically significant polyphagous pests of obligatory plant parasites (Moens *et al.*, 2009). Also, RKN is considered a soil-borne disease; after removing the damaged plants, the soil retains inoculum for several years built-up population owing to phytonematode survival in deep soil layers (McKenry, 1999). More than 3000 plants are vulnerable to RKN infection; *Meloidogyne* spp. are responsible for a significant portion of the 100 billion dollar losses yearly related to nematode damage among the various genera of the nematode with some economic effect (Ralmi *et al.*, 2016). Based on production numbers and pricing from 2011-2012, it was estimated that Egypt lost L.E.15.85 (= $2.30) billion yearly owing to crop losses caused by the nematodes on 80 crops, 15 of which are life-sustaining (Abd-Elgawad, 2014). Because of the fast reproduction rate of the RKN, *Meloidogyne incognita*, vast amounts of eggs accumulate in the soil, significantly limiting vegetable output (Ali *et al*., 2022). It seems reasonable to expect that infection by one pathogen might alter the host's response to subsequent infections (Zavaleta-Mejia, 2002).

Root rot disease symptoms include browning and weakening of root tips, root lesions of varying size and color (reddish, brown, and black), yellowing and wilting of leaves, slowed plant development, diminished yield, and crop loss. The multiplication of RKN and root rot pathogens is facilitated by moderate to high soil moisture, poor drainage conditions, soil compaction, the appropriate temperature for pathogen development, monoculture, and other plant-stress-inducing variables (Ralmi *et al.*, 2016; Williamson-Benavides and Dhingra, 2021). Plant diseases involving multiple pathogens in the infection process are usually called "complex" because their diagnosis and subsequent control are more complex (Lamichhane *et al.*, 2017; Lamichhane and Venturi, 2015). Combined infection with soil-borne microorganisms often exacerbates the onset of symptoms in plants and can affect growth, development, and crop production (Porto *et al.*, 2020). These plant disease complexes are difficult to control and thus cause huge plant yield losses in many countries worldwide.

Farmers struggle to control such complex diseases of phytopathogens to produce high-yield and high-quality crops. Many Egyptian farmers use chemical control for diseases, although chemical pesticides harm the agroecosystem, humans, and animals. Only licensed fungicides or nematicides may not be accessible to handle root rot and the root-knot nematode disease complex in tomato plants (Abd-Elgawad and Askary, 2018). Thus, different disease control measures have been researched worldwide. Also, biological control agents and other sustainable management methods must be investigated.

Biological control is a complex process that involves distinct phases. The modes of action are well-known for some fungi (Vinale *et al.*, 2006). Antibiosis synthesizes substances with antibiotic activity, mycoparasitism or hyperparasitism, competition, and enzymes with cell wall-lytic activity. Besides, the induction of systemic resistance is among the mechanisms of microbial biocontrol agents (Junaid e*t al.*, 2013). *Trichoderma* spp. is an essential bioagent for managing different diseases in several crop plants (Kubicek *et al.*, 2001). Harman *et al.* (2004) described the *Trichoderma* as an active soil inhabitant and root system colonizer, a plant symbiosis, and parasitic to some pathogenic fungi. *T. viride* could inhibit soil-borne pathogenic fungi *in vitro*. The average inhibitory coefficient against *F. oxysporum*, *F. solani*, and *R. solani* was between 70 - 80% and was attributed to either toxin production or mycoparasitism (Howell, 2003).

Virkon® S, a broad-spectrum disinfectant, sanitizes medical equipment and surfaces. Virkon S is a disinfectant that kills bacteria, viruses, fungi, and spores using peroxygenic acid (50% potassium monoperoxysulphate, potassium hydrogen sulfate, and potassium sulfate) at 1% for 10 minutes (Chan and Bakar, 2005), as well as being a promising nematicide (Ali *et al.,* 2023). Although there is little documented evidence, Virkon S kills fungi and spores (Rogawansamy *et al*., 2015).

Trichoderma spp. not only highly tolerant and compatible with chemical pesticides of different chemical groups (Ali *et al.,* 2012; Ali and Ramadan, 2019) but also can decompose and remediate pollutants (Cocaign *et al*., 2013; Piyaviriyakul *et al.,* 2021). Therefore, the short life of Virkon S bioactivity and the *Trichoderma* fungus is considered ideal for preserving sustainability, eliminating environmental issues caused by fungicide residues, improving crop quality and productivity, and preventing infections resistant to fungi.

The study aimed to use alternative strategies to manage complex diseases that can reduce the impact of the disease and be eco-friendly at the same time to achieve clean agriculture. So, the direct effect of Virkon® S on fungi evaluated in the laboratory was explored. The study also sought to evaluate the combination of Virkon S and *T. viride* as an alternate treatment for managing the root rot fungus- *M. incognita* disease complexes. *Trichoderma* fungus was utilized to eliminate any remaining Virkon S, colonize the roots, and provide the plant with essential protection for the most prolonged period after Virkon S lost its efficacy during soil drenching.

MATERIALS AND METHODS

Preparation of Nematode and Fungal Inocula:

The RKN, *Meloidogyne incognita* inoculum was maintained in the screen house on susceptible eggplant cultivar planted in autoclaved soil infected with one egg mass to make a pure culture. The nematode was identified using the perineal pattern region of adult females and second infective-stage juveniles (IJs), as Eisenback and Triantaphyllou (1991) described. The RKN eggs were extracted from the roots of a pure culture of *M. incognita* on a tomato using the 0.5% NaOCl procedure (Hussey and Barker, 1973) and purified using two sieves of differing sizes (60 and 500 mesh). The extracted eggs were gently rinsed with distilled water to eliminate sodium hypochlorite and then incubated in Petri dishes at 25±2°C until hatching. Freshly hatched juveniles were collected using a micropipette.

Root rot tomato and soil samples collected from greenhouses in El-sharqia Governorate showed root rot signs. The fungi were isolated from roots and roots surrounding soil samples (El-Deriny *et al*., 2022; El-Mohamedy *et al*., 2014). Based on morphological characteristics (Beales, 2012; Sunpapao *et al*., 2022) determined with a microscope, the isolated fungi were three plant-pathogenic fungi: *Pythium debaryanum*, *Rhizoctonia solani*, and *Fusarium solani*, respectively, and an isolate of the biological control fungus *Trichoderma viride*.

Fungal Pathogenicity Test:

The three fungal plant-pathogenic was grown on sorghum medium to prepare inoculums. Glass bottles (0.5-liter size), each containing 100 g of clean sorghum seeds and enough water to cover the sorghum were autoclaved at 121 ºC for 20 minutes. Autoclaved sorghum medium was inoculated from PDA growth disks (7mm) of isolated fungi and incubated at 27±3 ºC for 18 days. Sandy loam soil was sterilized using a 5% formalin solution and closely covered with a plastic sheet for two weeks, then left for two weeks to eliminate formalin toxicity. Plastic pots (25cm in diameter) were sterilized by immersing them in a 5% formalin solution for 15 minutes and leaving them to dry.

Two kilograms of sterilized soil were used for each pot. Pots were infested with the fungal-inoculated sorghum medium at a rate of 5% of soil weight (w/w). The infested soil was watered and mixed thoroughly for two weeks to ensure an even distribution of fungi. In the case of the control treatment, the soil was mixed with the same amount of fungal-free sterilized sorghum mixture alone. Tomato seedlings (cv. Alisa) were used for planting in pots. Five replicates were used in each treatment. The root rot was recorded after 45 days of treatment.

Fungicidal Activity of Virkon S Against Isolated Fungi *in vitro*:

The formulation of Virkon® S (Antec International, Sudbury, Suffolk, United Kingdom) is a broad-spectrum disinfectant manufactured as pink granules. This formulation was subject to testing the fungicidal effect on the isolated fungi.

Based on preliminary experiments, the most appropriate range of concentrations was determined to evaluate the effectiveness of Virkon S on isolated fungi, which were 0.4, 0.6, 0.8, 1.0, 3.0, and 5.0 % (w/v). Each concentration was prepared separately in 0.5 L of autoclaved PDA medium, left to cool partially, and then supplemented with the specified Virkon S amount per concentration. The medium was shaken well to ensure homogeneous distribution of the Virkon S.

The amended medium was poured into sterilized Petri dishes (15 ml /plate 9 cm diameter) and allowed to solidify. The control treatment was free of Virkon S. Each plate was inoculated in the center with a 7 mm disc of fungal growth taken from the periphery of 7 days old cultures. Inoculated plates were incubated inverted at 28±2ºC eight days later, depending on the fungus growth rate. Linear growth was determined periodically until the mycelial covered the control treatment plates by measuring the mean of three diameters of the colony. Vincent's equation was used to determine the proportion of development inhibition of mycelia (Vincent, 1947).

$$\text{Inhibition (\%)} = \frac{\text{Control linear growth} - \text{Treatment linear growth}}{\text{Control linear growth}} \times 100$$

Virkon S and *Trichoderma viride* Effectively Manage Root Rot Fungi and RKN Disease Complex in The Screen House:

For soil infestation, fungal inocula were prepared using sorghum medium. Glass bottles (0.5 L) containing 100 g sorghum seeds were covered with water and autoclaved at 121°C for 20 minutes. Autoclaved bottles containing sorghum medium were inoculated separately with a fungal disc of mycelial growth (7 mm) of isolated fungi (*P. debaryanum, R. solani, F. solani,* and *T. viride*) and then incubated at 27 ± 3ºC for 18 days. Sandy loam soil and plastic pots (15 cm diameter) were sterilized using a 5 % formalin solution. Formalinised soil was closely covered using a plastic sheet for two weeks, then left for two weeks to eliminate formalin toxicity. At the same time, sterilized pots were conducted by immersing them in formalin solution for 15 minutes, then left to dry. Two kilograms of sterilized soil was used for each pot infested with the fungal-inoculated sorghum medium at 3% soil weight (w/w) and mixed well in a plastic bag to ensure the even distribution uniform of fungi. The infested soil was watered to guarantee fungus survival.

Tomato seedlings *Lycopersicon esculentum* Mill., cv. Alisa was grown in autoclaved peat moss, and one seedling per pot was transplanted. Immediately after planting, each pot was infested with 1,000 newly hatched infective juveniles (IJs) of *M. incognita*. The nematode inoculum was diluted in 10 ml water and pipetted into four holes surrounding the root system to infect the root of the second-stage juveniles. At the same time, the soil was drenched with Virkon S concentrations which were applied directly after nematode inoculation. Two concentrations of Virkon S compound (1.5 and 3%) were implemented at 10 ml/pot without adding water after treatment. Simultaneously, control pots were given 10 ml of distilled water. The experimental treatments transactions were as follows:

Healthy plants represent negative control without any additions. Similarly, positive control treatments included healthy plants that received Virkon S 1.5 % (V1.5%) or 3% (V3%); also, plants inoculated with an RKN nematode (N), *P. debaryanum* (P), *F. solani* (F), *R. solani* (R) and *T. viride* (T). Also, interactive factor treatments comprised NT, PT, FT, RT, NTP, NTP+V1.5%, NTP+V3%, NTF, NTF+V1.5%, NTF+V3%, NTR, NTR+V1.5%, and NTR+V3%. The experiment was conducted in the screen house at a temperature of 28±4 °C. Each treatment consisted of five pots. All treatments were given identical horticultural care.

Inoculated tomato seedlings were observed for disease assessment and growth parameters. The determined pathogenic parameters, such as root rot disease incidence and severity and nematode reproduction, were evaluated 45 days post-planting. Plants were carefully removed from their pots by soaking for one hour in water to remove clinging soil and maintain egg masses on the root surface. Data on plant growth, e.g., fresh root and shoot weight (g) and shoot and root length (cm), were recorded.

Roots were protected from drying out by being covered in tissue paper, and the number of galls and egg masses was enumerated per root system. In addition, nematode extraction from 100 g soil samples was accomplished by combining sieving and the Baermann trays method (Hooper *et al.*, 2005). Reproduction factor =Final population/Initial population. Root gall index was quantified as follows: 0: No galling; 1: 1-2 galls; 2: 3-10 galls; 3: 11- 30 galls; 4: 31- 100 galls; and 5: greater than 100 galls (Taylor and Sasser, 1978). Each eggmass number is designated an egg mass index based on a rating of 0 = no egg mass; 1=1-2; 2=3-10; 3=11-30; 4 = 31-100; and 5 = more than 100 egg masses/root system (Taylor and Sasser, 1978).

The number of root rot-affected plants was used to calculate disease incidence. While disease severity was rated using a modified (Rowe, 1980) scale where: 0 = healthy root, 1 = absence of internal browning, separate superficial lesions on tap root or stem base, and root lesions at emergence points of lateral roots, 2 = a brown tap root with a hint of interior browning at the tap root's tip., 3 = moderate browning on the inside of the whole tap root, 4 = severe browning inside, all the way from the tap root to the lower stem above the collar, and 5 = dead plants. Percentages of disease incidence (DI) and disease severity (DS) derived from the following formulas:

$$\text{DI (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of inspected plants}} \times 100$$

$$\text{DS (\%)} = \frac{\sum(\text{n. v})}{\text{N. V}} \times 100$$

Where: v is the value of the disease index scale, n is the number of plants assigned to the disease index scale, and N is the total number of plants, and V represents the maximum disease index scale value.

Statistical Analysis:

The complete randomized design was far more thorough than the laboratory study. Using Analystsoft Biostat Pro V 5.8.4.3 software, statistical evaluation of the dose-response relationship curve to derive the median lethal concentration (LC_{50}) values for the Virkon S was accomplished by the procedure described by Finney (1971). Conversely, the experimental units in the screen house study were arranged using a randomized complete block design. ANOVA at $P \leq 0.05$ and Duncan's multiple range test (DMRT) for mean comparison were performed on the data using MSTAT V4.

RESULTS

Moreover, fungi of *P. debaryanum*, *F. solani*, and *R. solani* varied greatly in their sensitivity to Virkon S according to their taxonomic groups [(*Pythium* is an oomycete,

Fusarium belonging to the phytopathogenic ascomycetes whereas *Trichoderma* spp. (Hypocreales, Ascomycota)]. Current results show the toxicity of six concentrations of Virkon S (0.4, 0.6, 0.8, 1, 3, and 5% (w/v)) against four fungi isolates, *P. debaryanum*, *F. solani*, *R. solani*, and *T. viride* was illustrated in Table 1. Results articulated that inhibition percentage was measured on the tested fungi thrice during incubation.

Results of linear growth inhibition percentages of current fungi indicated the correlation between concentrations of Virkon S and the obtained toxicity. As well as the fungicidal effect reached its maximum at concentrations 3 and 5 of Virkon S**.**

At low tests of Virkon S, 0.4 and 0.6 % (w/v) cause radial growth reduction. *P. debaryanum* (44.45 and 44.45 %) was the most tolerant fungus, followed by *F. solani* (40.00 and 50.00 %), *T. viride* (44.45 and 55.56 %), and *R. solani* (56.25 and 60.00%) was the most sensitive tested fungus to the deleterious effect of Virkon S with colony diameter after three days post application, respectively.

Table 1 *In vitro* fungicidal effect of Virkon S concentrations on linear growth of the tested fungi.

Fungi	Incubation period (day)	Fungal colony diameter (cm) exposed to Virkon S concentrations (%(w/v))						LC_{50} (%, w/v)
		0.4	0.6	0.8	1	3	5	
Pythium debaryanum*	3	5.00 (44.45)	5.00 (44.45)	2.50 (72.22)	2.00 (77.78)	2.00 (77.78)	1.00 (88.89)	0.487
	6	9.00 (-)	9.00 (-)	9.00 (-)	4.00 (55.55)	2.00 (77.77)	1.00 (88.89)	-
	8	9.00 (-)	9.00 (-)	9.00 (-)	2.00 (77.76)	2.00 (77.76)	1.00 (88,89)	-
Fusarium solani	3	3.00 (40.00)	1.50 (50.00)	0.80 (84.00)	0.40 (92.00)	00 (100)	00 (100)	0.457
	6	5.00 (37.50)	1.50 (81.25)	1.00 (87.50)	0.50 (93.75)	00 (100)	00 (100)	0.466
	8	7.50 (16.67)	3.00 (66.67)	1.00 (88,89)	0.50 (94.44)	00 (100)	00 (100)	-
Rhizoctonia solani	3	3.50 (56.25)	3.20 (60.00)	1.50 (81.25)	1.00 (87.50)	0.80 (90.00)	00 (100)	0.385
	6	6.50 (27.78)	6.00 (33.34)	3.00 (66.67)	2.00 (77.78)	1.00 (88.89)	00 (100)	0.735
	8	8.00 (11.12)	7.00 (22.23)	7.00 (22,23)	3.00 (66.67)	1.30 (85.55)	00 (100)	-
Trichoderma viride	3	5.00 (44.45)	4.00 (55.56)	3.00 (66.67)	0.40 (95.55)	0.20 (97.77)	00 (100)	0.491
	6	5.00 (44.45)	5.00 (44.45)	3.00 (66.67)	0.80 (91.11)	0.30 (96.67)	0.10 (98,89)	-
	8	7.00 (22.23)	7.00 (22.23)	5.00 (44.45)	1.00 (88,89)	0.50 (94.45)	0.30 (96.67)	-

Figures in parentheses () are inhibition percentages; - refers to the cessation of observation due to complete colony growth in the control dishes.

* Reduction percentage in sixth and eighth-day observation attributed to maximum growth in control (9 cm), although the control treatment growth is already complete.

On the other hand, after 6- and 8-days post-application, the sensitivity of fungus to concentrations of 0.4 and 0.6, and 0.8 % (w/v) of Virkon S illustrated that *P. debaryanum* was the most tolerant fungus and reached maximum colony diameter (9 cm) followed by *R. solani* (6.5 & 8.00), 6.00 & 7.00 and 3.00 & 7.00), *T. viride* (5.00 &7.00, 5.00 & 7.00 and 3.00 & 5.00), and *F. solani* (5.00 & 7.50, 1.50 & 3.00 and 1.00 & 1.00), respectively. At Virkon S concentrations of 3 and 5 % (w/v), with all tested times, *P. debaryanum* was the most tolerant fungus, where the colony diameter reached 2.00 and 1.00 cm after eight days post-treatment, followed by *T. viride* (0.50 and 0.30) and *R. solani* (1.30 and 0.00), whereas *F. solani* was the most sensitive one (0.00 and 0.00). The results indicated the potential of using the highest Virkon S concentrations in inhibition of linear growth of current fungi, and

LC_{50} (%) exhibited that *T. viride* recorded the highest value, 0.491 after three days post-treatment, followed by; *P. debaryanum* (0.487), *F. solani* (0.457), and *R. solani* (0.385) (Fig.1).

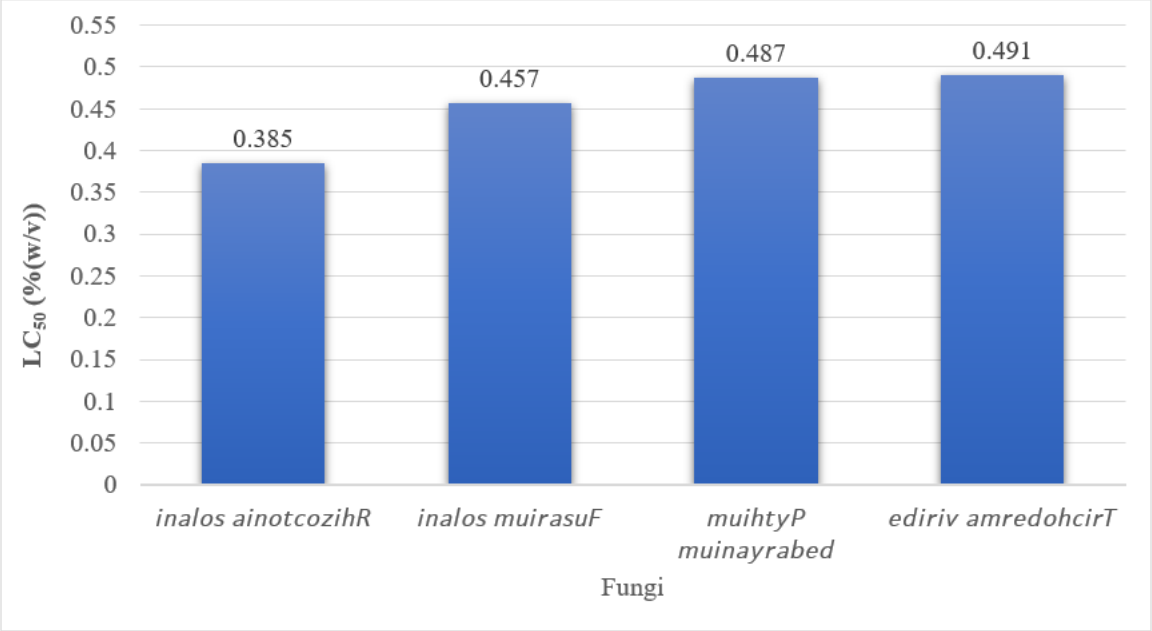

Fig. 1: Median lethal concentration (LC_{50}; %(w/v)) for Virkon S assessed using the solid poisonous media (PDA) technique after three days.

The results indicated that bio-agent *T. viride* could tolerate the low and high tested concentrations of Virkon S at short and long-time applications. Furthermore, the following evaluations should be made under field conditions to illustrate the combined effect of bioagents and Virkon S against soil-borne plant pathogens.

Assay for Disease Assuagement and Plant Growth Parameters of *Pythium debaryanum, Fusarium solani,* and *Rhizoctonia solani:*

The screen house experiment was conducted in a commercial screen house to screen disease assuagement and plant growth parameters of *P. debaryanum, F. solani,* and *R. solani* on tomato seedlings. Fungi exhibited severe damage to tomato seedlings. Tomato plants were cultivated in infected pots with pathogenic fungi to determine the overall effects of the pathogenic fungi on plant growth and disease assessment. The disease assessment and plant growth characteristics of the tomato-infected plants are summarized in Table 2. According to exhibited disease assessment, *P. debaryanum* caused the highest disease incidence (72.05 %) and disease severity (75.51%), whereas *R. solani* showed minor disease incidence (54.92 %) and disease severity (61.47 %). There was a significant decrease ($P \geq 0.05$) in root weight, shoot weight, root length, and shoot height in infected plants compared with healthy tomato plants. Plants of *P. debaryanum* displayed a maximum decrease in tested plant growth parameters with minimum root (1.27) and shoot (6.19) weight, root length (1.5) and shoot height (28.00), followed by *F. solani* with 1.32, 4.66, 1.50 and 29.00 with root weight, shoot weight, root length, and shoot height, respectively. The slightest decrease in plant growth parameters was found in tomato plants infected with *R. solani*.

In general, the mentioned disease incidence and disease severity is in agreement with linear fungus growth and colony diameter (cm), which reflect on tomato plant growth parameters, root weight (g), shoot weight (g), root length (cm), and shoot height (cm). Moreover, infected plants of tomatoes with pathogenic fungi, *P. debaryanum, F. solani,* and

R. solani, exhibited deleterious effects on plant growth parameters, and *P. debaryanum* was the most harmful fungi under screen house conditions.

Table 2: Disease assessment and plant growth parameter of the tested plant pathogenic fungi infested soil transplanted with tomato seedlings.

Treatment	Disease assessment		Plant growth parameter			
	Disease incidence (%)	Disease severity (%)	Root weight (g)	Shoot weight (g)	Root length (cm)	Shoot height (cm)
Healthy plant	-	-	15.53[a]	37.43[a]	18.63[a]	46.76[a]
Pythium debaryanum	72.05[a]	75.51[a]	1.27[c]	6.19[bc]	1.50[c]	28.00[b]
Fusarium solani	60.29[b]	73.08[b]	1.32[c]	4.66[c]	1.50[c]	29.00[b]
Rhizoctonia solani	54.92[c]	61.47[c]	2.07[b]	7.87[b]	1.30[b]	29.00[b]

*****According to DMRT, different letters in the same column indicate significant differences ($P \leq 0.05$).

Effects of *T. viride* on Disease Assessment and Plant Growth Parameters of Tomato Plants Infected with *M. Incognita* and Pathogenic Fungi Under Screen House Experiment:

In the present investigation, the role of three pathogenic fungi, *P. debaryanum*, *F. solani*, and *R. solani* and /or root-knot nematode, *M. incognita*, infecting tomato potentiality against disease assessment and plant growth parameters of tomato plants were evaluated. The results obtained revealed that the combination between *P. debaryanum* + *T. viride* (PT) showed a minor disease incidence (6.91 %) and disease severity (13.69 %), followed by *R. solani* + *T. viride* (RT) with disease incidence (7.02 %) and disease severity (12.08 %) and the highest disease incidence (7.29 %) and disease severity (15.37 %) exhibited with *F. solani* + *T. viride* (FT). A significant difference ($P \geq 0.05$) in disease severity reduction resulted from the interaction between bio-agent, *T. viride*, and pathogenic fungi, *P. debaryanum*, *F. solani*, and *R. solani*. Results in Table 3 illustrate the interaction between *M. incognita* + *T. viride* (NT), *P. debaryanum* + *T. viride* (PT), *F. solani* + *T. viride* (FT) and *R. solani* + *T. viride* (RT), and these combinations exhibited that disease severity reduction was 100, 86.31, 84.63 and 87.92 %, respectively.

Inoculation of tomato plants with *T. viride* revealed the maximum increase in plant growth parameters compared to uninoculated tomato plants (check plants). For example, root weight (g), shoot weight (g), root length (cm), and shoot height (cm) were 20.52, 53.44, 30.85, and 57.18 in plants inoculated with *T. viride* compared with 15.53, 37.43, 18.63 and 46.76 in healthy (uninoculated) plants, respectively.

Hight decrease in plant growth parameters resulted from the interaction between *M. incognita* + *T. viride* (NT) followed by *P. debaryanum* + *T. viride* (PT), whereas the slightest reduction in plant growth parameters exhibited from the interaction between *F. solani* + *T. viride* (FT) and *R. solani* + *T. viride* (RT).

Current results show that inoculation with *T. viride* decreased the disease assessment and disease assessment reduction % in tomato plants inoculated with pathogenic fungi, *P. debaryanum*, *F. solani*, and *R. solani*. On the other hand, most minor plant growth parameters were achieved in tomato plants infected with *M. incognita* when compared with infected tomato plants with pathogenic fungi and inoculated with *T. viride*.

Table 3: *Trichoderma viride* bioefficiency against soil-borne fungi and *Meloidogyne incognita* infested soil transplanted with tomato seedlings.

Treatment	Disease assessment			Plant growth parameter			
	DI (%)	DS (%)	DS Reduction (%)	Root weight (g)	Shoot weight (g)	Root length (cm)	Shoot height (cm)
Healthy (uninoculated) plant	-	-	-	15.53^{b}	37.43^{b}	18.63^{d}	46.76^{c}
T. viride (T)	00^{a}	00^{d}	100^{a}	20.52^{a}	53.44^{a}	30.85^{ab}	57.18^{a}
M. incognita + *T. viride* (NT)	00^{a}	00^{d}	100^{a}	5.88^{d}	13.26^{e}	29.00^{a}	30.00^{d}
P. debaryanum + *T. viride* (PT)	6.91^{a}	13.69^{b}	86.31^{c}	7.78^{c}	32.25^{d}	25.00^{c}	45.00^{c}
F. solani + *T. viride* (FT)	7.29^{a}	15.37^{a}	84.63^{d}	5.87^{d}	34.44^{c}	30.00^{ab}	52.00^{b}
R. solani + *T. viride* (RT)	7.02^{a}	12.08^{c}	87.92^{b}	5.80^{d}	34.28^{c}	31.00^{a}	46.00^{c}

DI: Disease incidence; DS. Disease severity
Different letters in the same column indicate significant differences ($P \leq 0.05$) according to DMRT.

In current results, the bio-efficiency of *T. viride* against a disease complex caused by RKN and soil-borne fungi, *P. debaryanum, F. solani*, and *R. solani* (disease assessment, tomato plant growth parameters, and *M. incognita* survival, reproduction (number of egg masses), galls numbers and RKN population (IJs / 100 g soil) in pot soil were illustrated in Table 4. Disease incidence (%) and disease severity (%) resulted from the interaction of soil fungi + nematode with *T. viride* significantly ($P \geq 0.05$) varied in reduction of disease severity according to soil-borne fungi and least efficiency resulted from NPT (41.62 %). On the other hand, the highest disease severity reduction (%) was obtained from NRT interaction, followed by NFT.

The disease assessments showed a deleterious effect on root weight (g) treated with soil-borne fungi, *P. debaryanum, F. solani*, and *R. solani*. The least root weight was exhibited with NRT, followed by NPT and NFT, respectively.

A solitary application with *M. incognita* showed the maximum reduction in tomato plant growth vs. the maximum improvement in tomato growth resulting from the application of *T. viride*. Moreover, the interaction between nematode and soil-borne fungi treated with *T. viride* exhibited a minor efficacy of *T. viride* against *R. solani* and more efficacy against *P. debaryanum* reflected on shoot weight, root length, and shoot high in applied tomato plants.

In the same trend, the nematicidal efficacy of *T. viride* against soil-borne fungi and *M. incognita* reproduced the least of nematode parameters such as gall index (GI), egg masses, and the number of J2/100 soil and reproduction factor (RF) of RKN. Conversely, the only destructive effect exhibited from the interaction between nematode and soil-borne fungus, *P. debaryanum*, treated with bio-agent *T. viride* whereas showed maximum efficacy with the interaction between *M. incognita* and *R. solani* and *F. solani.*

Generally, the efficacy of bio-agent *T. viride* in reducing disease assessment resulted from the complex of RKN and soil-borne fungi varied significantly according to tested fungi and reduced RF of nematode in pot soils.

Table 4*:* Bio efficiency of *Trichoderma viride* against disease complex of root-knot nematode and soil-borne fungi on tomato plant growth**.**

Treatment	Disease assessment			Plant growth parameter				Nematode parameter				
	DI (%)	DS (%)	DS Reduction (%)	Root weight (g)	Shoot weight (g)	Root length (cm)	Shoot height (cm)	GI	Egg masses No. /root	EI	IJs No. /100 g	RF
Healthy plant	-	-	-	15.53^{b}	37.43^{c}	18.63^{c}	46.76^{b}	-	-	-	-	-
T. viride (T)	00^{c}	00^{d}	100^{a}	20.52^{a}	53.44^{a}	30.85^{a}	52.18^{a}	-	-	-	-	-
M. incognita (N)	00^{c}	00^{d}	100^{a}	11.06^{d}	31.60^{e}	13.43^{e}	38.56^{c}	4.00^{a}	77.33^{a}	4.00^{a}	90.33^{a}	1.53^{a}
P. debaryanum+N+T (NPT)	38.91^{b}	41.62^{c}	58.38^{b}	13.30^{c}	34.73^{d}	15.36^{d}	39.60^{c}	2.00^{b}	5.00^{b}	2.00^{b}	7.66^{b}	0.13^{b}
F. solani+N+T (NFT)	41.38^{a}	45.01^{b}	54.99^{c}	19.033^{a}	50.06^{b}	26.30^{b}	49.30^{ab}	0.67^{c}	0.33^{c}	0.33^{c}	0.33^{b}	0.00^{b}
R. solani +N+T (NRT)	42.09^{a}	46.92^{a}	53.08^{cd}	11.53^{d}	32.43^{e}	14.40^{e}	38.73^{c}	0.33^{cd}	0.00^{c}	0.00^{c}	0.33^{b}	0.00 ^{b}

Disease incidence (DI), Disease severity (DS), Gall index (GI), Egg masses index (EI), and Reproduction factor (RF).
Different letters in the same column indicate significant differences ($P \leq 0.05$) according to DMRT.

Effects of Virkon S Concentrations on Disease Complex of *Meloidogyne incognita* and Soil-Borne Fungi Under Screen House Conditions:

Results in Table 5 spotted the combined effect of Virkon S and *T. viride* against the disease complex of root-knot nematode and soil-borne fungi on tomato plant growth. The study measured disease incidence, severity percentage, and some plant growth parameters and nematode reproduction (RF). Both the tomato growth parameters and nematode parameters showed direct.

Effectiveness of Virkon S and bioagent, *T. viride* application on the tomato growth parameters and nematode parameters showed highly significant effects ($P \geq 0.05$) of RF in roots and pot soils and directly proportional to the tomato plant growth parameters.

Table 5: The combined effect of Virkon S with *Trichoderma viride* against disease complex of root-knot nematode and soil-borne fungi on tomato plant growth.

Treatments	Disease assessment			Plant growth parameter				Nematode parameter				
	DI (%)	DS (%)	DS Reduction (%)	Root weight (g)	Shoot weight (g)	Root length (cm)	Shoot height (cm)	GI	Egg masses /root	EI	No IJs/100 g	RF
Healthy plants	-	-	-	15.53^{cd}	37.43^{d}	18.63^{c}	46.76^{b}	-	-	-	-	-
NTP+V 1.5%	29.64^{b}	36.48^{b}	63.52^{d}	18.70^{b}	41.86^{b}	19.20^{c}	46.40^{b}	1.00^{a}	0.67^{a}	0.00^{a}	0.33^{a}	0.07^{a}
NTF+V 1.5%	26.17^{c}	30.09^{c}	69.91cd	19.93^{b}	53.06^{a}	28.97^{b}	55.63^{a}	0.33^{b}	0.33^{ab}	0.33^{a}	0.00^{a}	0.00^{a}
NTR+V 1.5%	23.57^{d}	28.73^{c}	71.27^{c}	22.60^{a}	55.83^{a}	32.30^{a}	58.03^{a}	0.00^{b}	0.00^{b}	0.00^{a}	0.00^{a}	0.00^{a}
NTP+V 3%	21.36^{e}	25.07^{d}	74.93^{a}	16.46^{c}	41.00^{bc}	18.40^{c}	46.40^{b}	0.33^{b}	0.00^{b}	0.00^{a}	0.33^{a}	0.07^{a}
NTF+V 3%	37.15^{a}	42.61^{a}	57.39^{f}	11.06^{e}	32.86^{e}	14.00^{d}	39.43^{c}	0.00^{b}	0.00^{b}	0.00^{a}	0.00^{a}	0.00^{a}
NTR+V 3%	20.82^{e}	25.97^{d}	74.03^{b}	14.66^{d}	38.16^{c}	17.60^{cd}	47.03^{b}	0.00^{b}	0.00^{b}	0.00^{a}	0.00^{a}	0.00^{a}

RKN nematode (N), *P. debaryanum* (P), *F. solani* (F), *R. solani* (R) and *T. viride* (T), Virkon S (V), Disease incidence (DI), Disease severity (DS), Gall index (GI), Egg masses index (EI), Reproduction factor (RF).
Different letters in the same column indicate significant differences ($P \leq 0.05$) according to DMRT.

Comparison between disease incidence (DI) and disease severity (DS) in roots of tomato plants treated with combinations of nematode (N), *P. debaryanum* (P), *F. solani* (F), *R. solani* (R) and *T. viride* (T) and two concentrations of Virkon S varied greatly. Applying 1.5 % Virkon S decreased incidence (DI) and disease severity (DS) gradually from 29.64 & 36.48, 26.17 & 30.09, and 23.57 & 28.73 % with NTP, NTF, and NTR, respectively. The highest DI and DS recorded with NTF applied with Virkon S 3% (37.15 and 42.61%). The same trend was observed with tomato plant growth tested parameters. For instance, the most significant root weight, shoot weight, root length, and shoot high were observed with NTR+V 1.5%, followed by NTF+V 1.5% and NTP+V 1.5%, respectively. Conversely, NTF+V 3% exhibited minor tomato growth parameters, followed by NTR+V 3% and NTP+V 3% with insignificant differences ($P \geq 0.05$).

Concerning nematode parameters, application of V 1.5% and V 3% exhibited a maximum effect on nematode parameters (GI, egg masses/root, number of J2/100 soil and reproduction factors, RF) to reach 0.00 values with NTF and NTR.

It is essential to mention that the combined effect of Virkon S with *T. viride* against the disease complex of RKN and soil-borne fungi under screen house conditions showed enhancement in tomato plant growth parameters exceeded healthy plants to reach a maximum increase with NTR treated with V 1.5%. as well as two Virkon S concentrations suppressed nematode parameters except with combinations of NTP.

DISCUSSION

Microbial communities play a crucial role in their functionality, influencing the physiology and growth of plants. Even though many members of the rhizospheric microbiome are advantageous to plant development, plant pathogenic microbes also colonize the rhizosphere to breach the protective microbial barrier and defeat the innate plant defense systems to cause disease (Mendes *et al.,* 2013). The establishment of soil-borne illnesses is preceded by several interactions between microbes around the plant and the plant defense, ending in favor of the plant pathogen before the infection becomes endemic and disease signs arise on the plant. The agricultural soil is home to various dangerous and valuable creatures. Among the most significant detrimental organisms are nematodes and fungi that cause damping off, root rot, wilting, and plant death. Saprophytic fungal plant pathogenic species caused root diseases, including species related to *Fusarium*, *Macrophomina*, *Pythium*, *Rhizoctonia*, and *Sclerotium*. The most prevalent fungi may change with biotic soil structure (Baird *et al.,* 2004).

RKN is an obligatory, polyphagous, parasitic organisms-infected plant. Older plants that are heavily infected show wilt and perish too soon. All root-knot galls harm the vascular tissues of roots, impeding the normal circulation of water and nutrients through the plant. They also enhance the root system's sensitivity to disease-causing fungi and bacteria. In addition, sick plants may exhibit signs of nitrogen, potassium, or phosphorus deficiency despite appropriate amounts of these elements in the soil. Infected plants wilt throughout the day and then recover at night. Moreover, the roots are shorter and bushier than on healthy plants (Ralmi *et al.,* 2016).

The interaction between root-knot nematodes and pathogenic root rot fungi has been studied and referenced since the 1970s of the previous century (Mai and Abawi, 1987; Powell, 1971). Related organisms influence several plant diseases to a greater extent than we previously believed in terms of inhibition and acceleration. Many diseases require the presence of multiple organisms and cannot be caused by the infection of a single organism. *Meloidogyne incognita*-soil-borne disease complex showed a synergistic effect causing catastrophic losses (Akhtar *et al.,* 2005). Despite the antiquity of the problem, it still needs to be resolved, as it is difficult to control (Wolfgang *et al.,* 2019), and researchers are currently looking for an efficient and long-lasting solution to eradicate soil infections. So, the study introduced Virkon S to evaluate its effect against soil-borne fungi and *T. viride* tolerance to investigate the possibilities of combining them to control disease complexes.

Ali *et al.* (2023) elucidated that Virkon S is safe in soil applications without phytotoxic effects for plants and showed a considerable nematicidal effect reducing nematode reproduction. Also, Virkon S's effectiveness in nematode showed *in vitro* a high ability to dissolve gelatinous matrix surrounded nematode egg masses and disintegrate of egg structure, stopping egg hatching. In contrast, nematode juveniles showed hyperactivity followed by death within a few hours of exposure period and nematicidal potency accompanied by vigor plant vegetative growth after a single application with Virkon S,

besides enhancing plant defense systems measured by enzymatic and non-enzymatic oxidative stress parameters. These positive influences may pave the way for testing Virkon S against phytopathogens and *Trichoderma* spp. which have shown the ability to control soil-borne fungi and nematodes and improve yields (Nafady *et al.,* 2022; Tyśkiewicz *et al.*, 2022) to combine the effective and rapid effect of Virkon S with the extended and sustainable effect of the *Trichoderma* fungus, thus reducing the need for intervention with chemical pesticides later.

The biocide tested in this study is Virkon S, a complex formulation primarily composed of a peroxygen compound called potassium peroxymonosulfate (KMPS; 21.41%). This formulation also contains sodium chloride (1.5%) and other elements (77.09%), such as sulfamic acid (SA) to produce a low pH, sodium dodecylbenzene sulfonate (SDBS) as anionic surfactants to combine cleaning and disinfection, and inorganic buffers as sodium hexametaphosphate to stabilize these acid conditions (Gabbert *et al*., 2018; Geraldes *et al.,* 2021; Wu *et al.,* 2017). Virkon S is known for its broad-spectrum activity, non-corrosiveness to stainless steel, low ecotoxicity, high biodegradability, and low toxicity (McCormick and Maheshwari, 2004).

Sodium chloride (NaCl) increases percent mortality with an increase in exposure period (Khan and Khan, 1990). Also, SDBS plays a vital role in manipulating water's physical properties, causing the difficulty of nematode coexistence with the surrounding aquatic medium leading to death after a long period of exposure to the surfactant (El-Ashry *et al.,* 2019) in addition to Virkon S's permanent and renewable ability to generate hypochlorous acid (HOCl) as a potent biocide (Jones and Joshi, 2021). HOCl is responsible for Virkon S's broad-spectrum efficacy against numerous microorganisms, while its multi-component, non-selective nature (De Lorenzi *et al.,* 2008; Geraldes *et al.,* 2021). These factors may interact together to enhance Virkon S nematicidal effect. Virkon S fungicidal effect was limited and varied greatly *in vitro* against tested fungi. The exposed fungal colonies could tolerate a high concentration and completed their growth after the experimental endpoint. These tested concentrations (3-5% w/v) may be inapplicable in the field. However, *T. viride* showed the highest tolerance against Virkon S, which refutes the suspicion that there are any permanent adverse effects of the simultaneous application of Virkon S with *T. viride.* Due to the remarkable tolerance of the *Trichoderma* fungus to the toxicity of Virkon S, the control agent preserves its life after application to initiate two fundamental activities. The first is to eliminate the Virkon S's residual impact, and the second is to colonize the roots' perimeter and attack the existing fungal phytopathogens in the rhizosphere (Tripathi *et al.,* 2013; Vurukonda *et al.,* 2018; Zaidi and Singh, 2017).

Root colonization by *Trichoderma* after inoculation is postulated (Ali *et al.,* 2022). *Trichoderma* bioagent exhibits antagonistic behavior via various mechanisms, such as antibiotics and nutrition competition. To date, the primary mechanism of biological control by *Trichoderma* species involves two processes. Firstly, parasitism involves the recognition and invasion of phytopathogenic species through cell wall disruption and the uptake of released nutrients (Zin and Badaluddin, 2020). Mycoparasitism by secreting lytic enzymes such as cellulases, chitinases, β-1,3-glucanases, and proteases, *Trichoderma* mycoparasitism directly kills plant pathogens (Parmar *et al.,* 2015; Vejan *et al.,* 2016). Secondly, through the induction of plant resistance to disease by causing structural changes in roots during interactions with pathogens (Kumar *et al*., 2019). During *Trichoderma* spp. penetration, the formed hook-like structures penetrate the host cell wall, as described by (Ozbay and Newman, 2004). At the same time, *Trichoderma* species spread along the host hyphae and secrete cell wall-degrading enzymes during the osmotic process. (Omann *et al.,* 2012) suggest that this process involves the production of several biologically active compounds,

including cell wall degrading enzymes and secondary metabolites, which can kill target pathogens.

Also, positive effects on plant growth and health are associated with siderophore production, the production of the plant hormone indole-3-acetic acid, or bioagent metabolites. Also, facilitate the induction of systemic resistance (ISR). Many plants utilize both ISR and systemic acquired resistance (SAR) to respond to local pathogen attacks in both the plant's initially attacked plant organ and distant, unaffected parts, unlike SAR, induced by pathogenic factors, while ISR induced by non-pathogenic bacterial or fungal determinants (such as lipopolysaccharides, siderophores, and salicylic acid) and changes in plant cell wall composition, as well as production of pathogenesis-related proteins and phytoalexins (Levy *et al*., 2018).

Conclusion

T. viride tolerated Virkon S well in lab tests on toxic solid mediums. Virkon S also inhibited the fungi *R. solani*, *P. debaryanum*, and *F. solani*. As the *T. viride* showed considerable efficiency in reducing the incidence of the tested diseases and improved the plant growth traits, but the efficiency of *T. viride* decreased significantly nematodes introduced to pathogenic fungi in the soil, whether on the occurrence of disease or reproduction of nematodes and the characteristics of vegetative growth of tomato plant. When Virkon S was added after applying *T. viride* to soil contaminated with nematodes and pathogenic fungi, Virkon S provided the best support to *T. viride* efficiency, which was evident with increasing Virkon S concentration showed a significant improvement in reducing nematodes and fungi infection, accompanied with significant improvement in plant growth. Ultimately, combining Virkon S and *T. viride* offers promising management techniques for disease complex control using nontraditional, non-selective chemical sterilants and multifunction bioagents (*T. viride*).

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ARABIC SUMMARY

إدارة المعقد المرضي لنيماتودا تعقد الجذور وأمراض أعفان الجذور على نباتات الطماطم باستخدام الفيركون إس والترايكوديرما فيردي

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تؤثر الكائنات الحية الدقيقة المصاحبة للنبات بشكل كبير على العديد من الأمراض النباتية بتثبيطها أو وتسريع عملية العدوى، وكمثال المعقد المرضي لنيماتودا تعقد الجذور *Meloidogyne incognita* مع أمراض أعفان الجذور والذي يتطلب تعاون العديد من مسببات الأمراض الفطرية كـ(*Pythium debaryanum* و *Fusarium solani* و *Rhizoctonia solani*) لإنتاج الإصابة الوبائية. يعتبر المعقد المرضي مشكلة عويصة بسبب الأمراض المتداخلة والصعبة السيطرة عليها بالأساليب التقليدية. لذلك هدفت الدراسة لتقديم عامل التعقيم الكيميائي الفيركون إس Virkon® S غير التقليدي وغير متخصص وغير الثابت للتطبيق المتزامن مع عامل المكافحة الحيوية *Trichoderma viride*. وبناءً على قيم التركيز نصف المميت التي تم اختبارها باستخدام تقنية البيئة المسممة بعد ثلاثة أيام من التحضين، أظهر فطر الترايكوديرما أعلى درجة التحمل للفيركون إس (0.491 ٪ وزن\حجم)، بينما كان فطر الرايزوكتنيا الأكثر حساسية للفيركون إس (0.385 ٪ وزن\حجم)، يليه فطر الفيوزاريم (0.457٪ وزن\حجم)، وأخيراً فطر البيثيم (0.487 ٪ وزن\حجم). وبإجراء اختبار القدرة المرضية للفطريات الممرضة كان فطر البثيم الأشد انتشار والأشد ضراوة على نباتات الطماطم يليه الفيوزاريم ثم الرايزوكتنيا.

كما أدي تطبيق التريكودرما لتقليل الأمراض الفطرية مع وجود اختلاف معنوي لفاعليته باختلاف الفطر، كما صاحبه تحسن معايير النمو الخضري للطماطم سواء المصابة بالنيماتودا أو الفطريات الممرضة. بالمقابل نجح الترايكوديرما في تقليل انتشار الأمراض الفطرية وشدتها وتكاثر النيماتودا في جذور الطماطم والتربة في المعقد المرضي للنيماتودا مع الفطريات الممرضة والتي اختلفت بشكل كبير باختلاف للفطر الممرض، وسُجلت أقل كفاءة مكافحة للتريكودرما في معاملة النيماتودا وفطر البثيم والترايكوديرما. علاوة على ذلك أظهر تطبيق الفيركون إس مع التريكودرما ضد معقد النيماتودا- فطريات أعفان الجذور تحسن في نمو النبات قد تجاوز النباتات السليمة (بدون معاملة)، وقد تم الحصول على أقصى زيادة في النمو الخضري لنباتات الطماطم في معاملة النيماتودا والرايزوكتنيا والتريكودرما المضاف لها الفيركون إس 1.5 ٪ (وزن\حجم). في حين قمعت تركيزات الفيركون إس 1.5 و3 % (وزن\حجم) تمامًا مقاييس النيماتودا باستثناء المعاملة النيماتودا والبثيم والتريكودرما.

تظهر النتائج المتحصل عليها أن الجمع بين الفيركون إس والتريكودرما فيردي يؤدي مبدئيا لمكافحة المعقد المرضي للنيماتودا والفطريات الممرضة، حيث يقوم الفيركون إس بقمع تعداد النيماتودا بشكل فعال ثم يكمل التريكودرما المسيرة بعد نجاح الفيركون إس في مهمته وتحطمه بالتربة، فيتطفل التريكودرما على فطريات أعفان الجذور الموجود في منطقة ريزوسفير ويستعمرها معززا نمو الطماطم بشكل كبير. لتمثل تلك التركيبة استراتيجية جديدة ضد المعقد المرضي لنيماتودا تعقد الجذور والفطريات القاطنة بالتربة.