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ORIGINAL PAPER

Management of Damping-off and Root Rot Diseases on Bentgrass (*Agrostis stolonifera* L.) by Vermicomposting and Some other Agents with Special Reference to *Fusarium thapsinum* First Reported as Root Rot Pathogen in Egypt

Abdel-Wahed, G.A.*; Hassan, M.S.S.; Shehata, A.S.F. and Imara, D.A.

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ABSTRACT

Fusarium thapsinum was isolated for the first time from bentgrass in Egypt. Several other fungal species, including *Fusarium roseum*, *Fusarium semitectum*, *Fusarium thapsinum*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and fungal like *Mucor* sp. are widespread in Egypt and are responsible for significant losses to bentgrass. *Fusarium thapsinum* is a fungus causes bentgrass root rot. It was approved to be responsible partially for root rot based on its morphological traits, disease signs, and pathogenicity test. By sequencing the ribosomal RNA genes internal transcribed space (ITS region), identification of this species' was verified. During 2021 and 2022 growing seasons, *Fusarium thapsinum*, *R. solani*, and *S. sclerotiorum* achieved the greatest majority on isolation from bentgrass. *Rhizoctonia solani* was more pathogenic than the other fungi. In this study, disease severity and disease incidence were determined. The effects of Vermicomposting (VMC) either single or in combination with *Trichoderma harzianum*, *Trichoderma viride*, *Bacillus subtilis*, *Azotobacter vinelauvii*, *Azospirillum* sp. in addition to Potassium silicate and Vitavax 200 as a single treatment were evaluated under *in vitro* and greenhouse. *In vitro* treatments significantly inhibited the mycelial growth of the three tested fungi. These controlling agents showed variations in their detrimental effect against fungal growth. In greenhouse trials, Vitavax 200 and VMC with *Trichoderma harzianum* were the best treatments for controlling root-rot followed by VMC with *Trichoderma viride* and VMC with *Bacillus subtilis*, increased bentgrass growth parameters and pre- and post-emergence damping off were decreased, as shown by the maximum total chlorophyll. All the treatments significantly improved turf quality and growth parameters in the field experiments and decreased percentage of infected area also, increased total phenols and defense-related enzymes.

Keywords: Bentgrass, *Agrostis stolonifera*, Damping-off, Root Rot, *Fusarium thapsinum*, Vermicomposting, *Azotobacter vinelauvii*, *Trichoderma harzianum*, *Trichoderma viride*, *Azospirillum* sp., *Bacillus subtilis*

***Correspondence: Abdel-Wahed, G.A.**
E-mail: gomaaarafat@arc.sci.eg
Gomaa A. Abdel-Wahed
https://orcid.org/0000-0001-5539-8827
Mabrouk S.S. Hassan
https://orcid.org/0000-0002-3477-339X
Abou-Ghanima, S.F. Shehata
https://orcid.org/0000-0003-1698-7375
Doaa A. Imara
https://orcid.org/0000-0003-1439-2722
Plant Pathology Research Institute, Agricultural Research Center, 12619, Giza, Egypt.

INTRODUCTION

Bentgrass (*Agrostis stolonifera* L.) is an important and essential element from the environmental point of view as it works to reduce pollution and recreation (Stackhouse *et al*., 2020). The pathogen, *Fusarium thapsinum* can be found in both tropical and temperate climates. It causes root and stalk rots, seed rots, and seedling blights in a variety of crops. Some of the most prevalent diseases are root and stalk rots (Abd-Elsalam, 2009; Chala *et al*. 2019 and Zakaria, 2023). China has the first report of *Fusarium thapsinum*-related maize stalk rot by Zhang *et al*. (2021). Brown patch (*Rhizoctonia solani* Kuhn) can cause damage to creep bentgrass (*Agrostis stolonifera* L.), (Blazier and Conway, 2004). According to Ahmed *et al*. (2022), vermicomposting reduces the occurrence of tomato root rot. Traditional thermophile organic composts promote the growth of a limited number of microorganisms, whereas vermicomposting is home to an enormous diversity and activity of microbes. Many antagonistic microorganisms included in vermicomposting ensure effective biocontrol of soil borne fungi (Singh *et al*., 2008). Naraghi *et al*. (2013) recorded that using microbes for biological control might replace or lessen the need for fungicides. Naeimi and Zare (2013) mentioned that in biological control research, a variation of fungal antagonists, including *Trichoderma* spp., have been successfully used. *T. harzianum* was isolated from healthy pea

plants and was used in controlling root rot diseases on pea (Attia *et al*., 2022).

This study goal is to determine *Fusarium thapsinum* genetically, morphologically and to give a description for the characteristics of the disease. Evaluate the efficacy of vermicomposting and some antagonists in reducing diseases caused by fungi in bentgrass.

MATERIALS AND METHODS

1- Purification, identification, and frequency analysis of the isolated fungi

To obtain infected samples, soil particles were removed, and then infected bentgrass organs were sliced up and they received thorough washing under running water. These pieces were surface sterilized by soaking for two minutes in 2% sodium hypochlorite, followed by three washings by sterilized water, and drying between sterilized filter papers. Four pieces were aseptically placed onto a potato dextrose agar medium (PDA) in petri plates. For three to seven days, plates were incubated at 25±2 °C. The emerging fungi were purified using the single spore or hyphal tip technique suggested by Dhingra and Sinclair (1995). Mycology Research and Plant Disease Survey Department, Plant Pathology Research Institute, ARC, Giza, Egypt, have verified this identity. Pure cultures were stored at a low temperature (5°C) on PDA slants. The following formula was used to determine the frequency of each isolated fungus.

$$\textbf{Frequency \% of fungus} = \frac{\textbf{Number of each fungal colony}}{\textbf{Total number of isolated fungal colonies}} \times 100$$

2. Identification of *F. thapsinum*:

2.1. Morphological characteristics:

Cultural characters on PDA, the obtained monoconidial colonies were kept alive. They were then sub-cultured for studying cultural and morphological features (Klittich *et al*., 1997 and Leslie and Summerell, 2006).

2.2. Molecular studies:

In sterile Petri plates with autoclaved potato dextrose agar (PDA) medium, fungal isolates were incubated for 7 days at 25±2ºC. (Pitt and Hocking, 2009). Intron (Biotechnology Corporation, Korea) contributed the Patho-gene-spin DNA/RNA extraction kit, which was used to extract DNA from cultures sent to the (Molecular Biology Research Unit, Sigma). The ITS1 and ITS4 primers were used to directly sequence the PCR amplifications. 50 ng of genomic DNA were diluted. Oligonucleotide primers were used for PCR amplifications, DNA sequencing, alignment, and phylogenetic analysis as described by White *et al*. (1990).

3. Pathogenicity test:

Under the greenhouse environment, pathogenicity test and other greenhouse experiments were carried out at "Agric. Exp. Sta. Sids, Beni-Suef governorate". Inocula of the isolated fungi, *F. semitectum*, *F. thapsinum*, *M. phaseolina*, *F. roseum*, *R. solani*, *S. sclerotiorum* and the fungal like *Mucor* sp. were grown on maize-meal-sand medium incubated at 25±2°C while *S. sclerotiorum* was incubated at 18°C. Clay and sand soil (1:1, w/w) was autoclaved for 30 minutes at 121°C. Separate infestations by the tested fungi were at the rate of 1% (w/w), in sterilized pots (30-cm-diam) filled with aerated sterilized clay and sand soil (1:1, w/w). 100 bentgrass seeds totaling 0.05 g were sown for each treatment. Bentgrass seeds were previously thoroughly surface sterilized for 2 minutes with 2% sodium hypochlorite, before using the tested treatments; they were dried between layers of sterilized filter papers after being washed three times in distilled water. Four replicates were used for each treatment, according to the methods recorded by Abdel-Monaim and Atwa (2019) and Mahdy and Mahmoud (2022).

Percentages of pre- and post-emergence damping off, plant survival percentage and percentages of root rotted plants were recorded at 15, 45, and 60 days after planting, (Abdel-Wahed and Abdel-Rahman, 2022).

$$\textbf{Disease Incidence (\%)} = \frac{\textbf{The number of diseased plants}}{\textbf{Total number of seeds planted}} \times 100$$

Root rot disease severity was also measured at 60 days after planting using the developed scale (0–4), where 0 = healthy seedling, 1 = very little root rot, 2 = moderate root rot, 3 = severe root rot and 4 = complete root rot, as follows (Abd-Elmoity and Ali, 2016):

$$\textbf{Disease severity \%} = \frac{\textbf{Sum of (n} \times \textbf{v)}}{\textbf{5N}} \times 100$$

Where:

n = Number of infected roots in each category.

v = Numerical value of each category.

N = Total number of roots in the samples.

4 -Bentgrass diseases management:

4.1. *In vitro*:

4.1.1. Vermicomposting:

Preparation of Vermicomposting stock solutions:

In a plastic container, 1 kg of vermicomposting was soaked in five liters of water, after passing through two layers of cheesecloth, filter Melbourne 0, 45 µm was used to sterilize the vermicomposting filtrate. A concentration of 100, 200, and 300 ppm from

filtrated sterilized vermicomposting were created and added to the PDA before solidification.

4.1.2. Antagonistic bio-agents

The Department of Microbiology, Soil, Water and Environment Research Institute, ARC, Giza, kindly provided the authors with five species of antagonistic bio-agents. These bio-agents included *Trichoderma viride*, *Trichoderma harzianum*, *Bacillus subtilis*, *Azotobacter vinelauvii*, and *Azospirillum* sp. On PDA medium, *T. viride* and *T. harzianum* were cultured separately for 7 days at 25±2ºC.While *A. vinelauvii*, *B. subtilis*, and *Azospirillum* sp. were raised for three days at 28°C in 250-mL flasks of nutritional broth. Using a hemocytometer slide, the cell suspension of each organism was standardized to provide 10^9 CFU/mL (Hafez *et al*., 2018).

Checking antagonism

According to Brain and Hemming (1945), *Trichoderma* spp. were cultivated and incubated for 14 days on gliotoxin fermentation medium (GFM), and sterilization was made with filtrate Melbourne (0,45 µm). To create final concentrations of 100, 200, and 300 ppm, the determined amounts of each Trichoderma bioagent culture filtrate was mixed separately with PDA. Each of the 5 mm-diameter discs bearing the tested fungal (*F. thapsinum*, *R. solani*) growth was placed in the center of a PDA plate and incubated at 25±2ºC, while *S. sclerotiorum* was incubated at18±2°C. Concentrations of 100, 200, and 300 ppm from each of *A. vinelauvii*, *B. subtilis*, and *Azospirillum* sp. were each mixed separately with PDA medium. Twenty mL of the medium were placed into each Petri dish (Larkin *et al*., 1998). Five replication plates were used for each treatment. When control treatments covered the plates, an estimate of the average growth diameter was determined (Fokemma, 1973).

4.1.3. Potassium silicate (K_2SiO_3) and Vitavax 200:

Concentrations of 50, 100, and 200 ppm from potassium silicate and vitavax 200 were created and added to the PDA before solidification, five mm-diameter discs of the investigated fungi namely, *F. thapsinum*, *R. solani*, and *S. sclerotiorum*, were used to inoculate the media. A control sample of PDA without potassium silicate or Vitavax 200 sources was kept (check). For each treatment, five plates served as replicates. All plates were incubated at 25±2ºC while *S. sclerotiorum* was kept at 18±2°C. At the end of the experiment, an estimate of the average growth diameter was determined (Shen *et al*., 2010 and Derbalah *et al*., 2022). Biocontrol agents, composition, concentration used, and the producers are shown in Table (1).

Table (1): Controlling agents used, the composition, the concentrations used, and the Producers of the tested materials used in lab.

Controlling agent	Composition	Concentration used *in Vitro* ppm	The Producers
Vermicompost	*Bacillus megaterium*, *Bacillus pumillus*, *Pseudomonas oxalaticus*, *Rhizobium japonicum*, and earthworm (*Lumbricus Terrestris* lysing products) and other parameter properties	0.0,100, 200, and 300	(Central L ab. of Organic Agriculture (CLOA); ARC; Egypt)
Trichoderma viride	(*Trichoderma viride*) 3×10^7CFU/mL	0.0,100, 200, and 300	Department of Microbiology, Soil, Water and Environment Res. Inst., ARC, Giza
Trichoderma harzianum	*Trichoderma harzianum*, 3×10^7CFU/mL	0.0,100, 200, and 300	
Bacillus subtilis	*Bacillus subtilis*, 3×10^7 CFU/gm	0.0,100, 200, and 300	
Azotobacter vinelauvii	10^6-10^8 CFU/gm	0.0,100, 200, and 300	
Azospirillum sp	10^6-10^8 CFU/gm	0.0,100, 200, and 300	
Potassium silicate	Consists of 10% potassium oxide, 25% silicon oxide	0.0, 50, 100, 200	(Central Drug House(P) LTD Post Bon No. 7138, New Delhi-110002)
Vitavax 200 75 % WP	(37.5% carboxin (5,6 –dihydro-2 methyl – 1,4 – oxathin -3- carboxanilide) + 37.5% Thiram (tetramethyl thiuram disulfide))	0.0, 50, 100, 200	Cerexagri-Nisso LLC, Japan

4.2. Greenhouse Experiment

4.2.1. Treatments of bentgrass plants with vermicomposting and some other agents:

This study was conducted to assess the efficiency of the tested treatments, namely (VMC) and its combination with *T. harzianum*, *T. viride*, *B. subtilis*, *A. vinelauvii*, and *Azospirillum* sp. to control root rot disease caused by *F. semitectum*, *F. thapsinum*, *M. phaseolina*, *F. roseum*, *R. solani*, and *S. sclerotiorum* in bentgrass plants. Sterilized pots filled with sterilized soil infested with the tested fungus were seeded with bentgrass seeds of at the rate 0.05 gL pots of bentgrass seed containing 100 seeds for each treatment. All of the pots were kept in the greenhouse at 27± 2°C, 11±2 hours of light each day, and 61-63% relative humidity. Similar to the methods

described under "Pathogenicity test", soil infestation was conducted, according to Serag El-Din *et al*. (2020) and Abdel-Wahed and Abdel-Rahma (2022).

The following bio-agents were applied at the rate of 4 ml/kg seeds for each of VMC, VMC plus *T. harzianum*, VMC plus *T. viride*, VMC plus *B. subtilis*, VMC plus *A. vinelauvii*, VMC plus *Azospirillum* sp. 3g/ l water for Vitavax 200 and potassium silicate.

After 15, 30, and 60 days from planting, pre and post emergence damping-off, as well as plant survival were evaluated. Plant height (cm), fresh and dry weights, and estimated chlorophyll, were recorded at 60 days after planting (Abdel-Wahed and Abdel-Rahman, 2022).

4.2.2. Chlorophyll estimation:

A strip of the plant was placed in a test tube with 10 ml of 80% acetone and the tube was closed with a stopper to prevent the evaporation of acetone. The tube was kept for 15 minutes while shaking every 5 minutes. With the use of filter paper, the acetone was put through a Buchner funnel. With the addition of previously made 80% acetone (CH 3) 2 CO, the extracted acetone's total volume was increased to 100 mL Using an ultraviolet-visible spectrophotometer, the produced sample's absorbance was measured at two wavelengths, 654 and 663 nm, to determine its chlorophyll a (Chl a) content (Srichaikul *et al*., 2011).

4.3. Field experiments*:*

This trial was carried out in fields at Beni-Suef governorate Club in the years 2021 and 2022 that had previously been known to have bentgrass root rot fungi infestations during sampling.

4.3.1. Vermicomposting and some other agents application in fields during 2021 and 2022

Four replicated plots per each treatment, (10×5 m; 50 m^2/each) were used. In January, plots were sprayed with bio-agents at the rate of 4 ml/ l water for VMC either single or in combination with VMC plus each of alone *Trichoderma harzianum*, *Trichoderma viride*, *Bacillus subtilis*, *Azotobacter vinelauvii*, *Azospirillum* sp., 3g/ l water for Vitavax 200 and potassium silicate. Spraying was performed three times with 15 days between each spray. Percentage area of infection and turf quality were recorded in February. On a scale of 1 to 6, with 6 indicating no disease and 1 denoting the worst overall appearance. The data were collected 6, 7, and 8 weeks after planting (Kremer *et al*., 2000). Moreover, plant height (cm), dry weight (g), and fresh weight (g) per plant were measured.

4.3.2. Resistance-related biochemical alterations:

Using the Folin ciocalteau technique recommended by Lafka *et al*. (2007), the total phenol was measured. The Worthington enzyme manual approach was used to measure peroxidase activity (Worthington, 1971). Technique for measuring polyphenol oxidase activity was used (Esterbaner, 1977). At the Central Lab for Biotechnology, Plant Pathol. Res. Inst., ARC, enzyme analysis was done.

Statistic evaluation:

Four replicates and a completely randomized design were used to create this experiment. The L.S.D. test at 5% was used in this statistical study, which was carried out on a computer using Statistics Software version 8 (Snedecor and Cochran, 1989).

RESULTS

I- Purification, identification, and frequency of the isolated fungi:

Seven different fungi species were identified after isolation from naturally infected bentgrass showing symptoms of root rot collected from Beni-Suef governorate. Each species was identified using the morphological characters; *F. thapsinum* was identified according to cultural characteristics (Fig. 1) and molecular characters (Fig. 2). The isolated fungi were identified as *Fusarium roseum* L.K. emend. Snyd. & Hans. *Fusarium semitectum* Berk. & Rav., *F. thapsinum* GAAE, *Macrophomina phaseolina* (Tassi) Goid., *Rhizoctonia solani* Kühn, *Sclerotinia sclerotiorum* (Lib.) de Bary and the fungal like *Mucor* sp. Results (Table, 2) demonstrate that *R. solani* and *S. sclerotiorum* followed by *F. thapsinum* had the highest means of frequency (%). Frequency percentages of these isolates were 25.00, 21.87, and 18.75% respectively.

Table, (2): Frequency % of fungi isolated from bentgrass plants collected from Beni-Suef governorate, 2021.

Isolated fungi	Frequency (%)
Mucor sp.	6.25
Fusarium semitectum Berk. & Rav.	9.37
Fusarium thapsinum GAAE	18.75
Macrophomina phaseolina (Tassi)Goid.	12.50
Fusarium roseum L.K. emend. Snyd. & Hans.	14.25
Rhizoctonia solani Kühn	25.00
Sclerotinia sclerotiorum (Lib.) de Bary	21.87

2. Identification of *F. thapsinum:*

2.1. Morphological characteristics:

2.1.1. PDA characters:

Fusarium thapsinum produces a lot of white mycelium, which as it ages may turn darker (violet colors). Some isolates have the potential to develop dark-colored sclerotia. The agar's pigmentation varies quite a bit. The majority of strains develop a unique yellow pigment that serves as both a diagnostic marker and the inspiration for the species epithet, (Fig.1-A and B).

2.1.2. Macroconidia characteristics:

Size of macroconidia (µm) 29-52 × 3-4, Septa of macroconidia 4-6, (Fig.1-C). Slightly falcate or straight, thin walled, and rather slender. Curved and tapering shape of the apical cell. Foot form is relatively underdeveloped in basal cells.

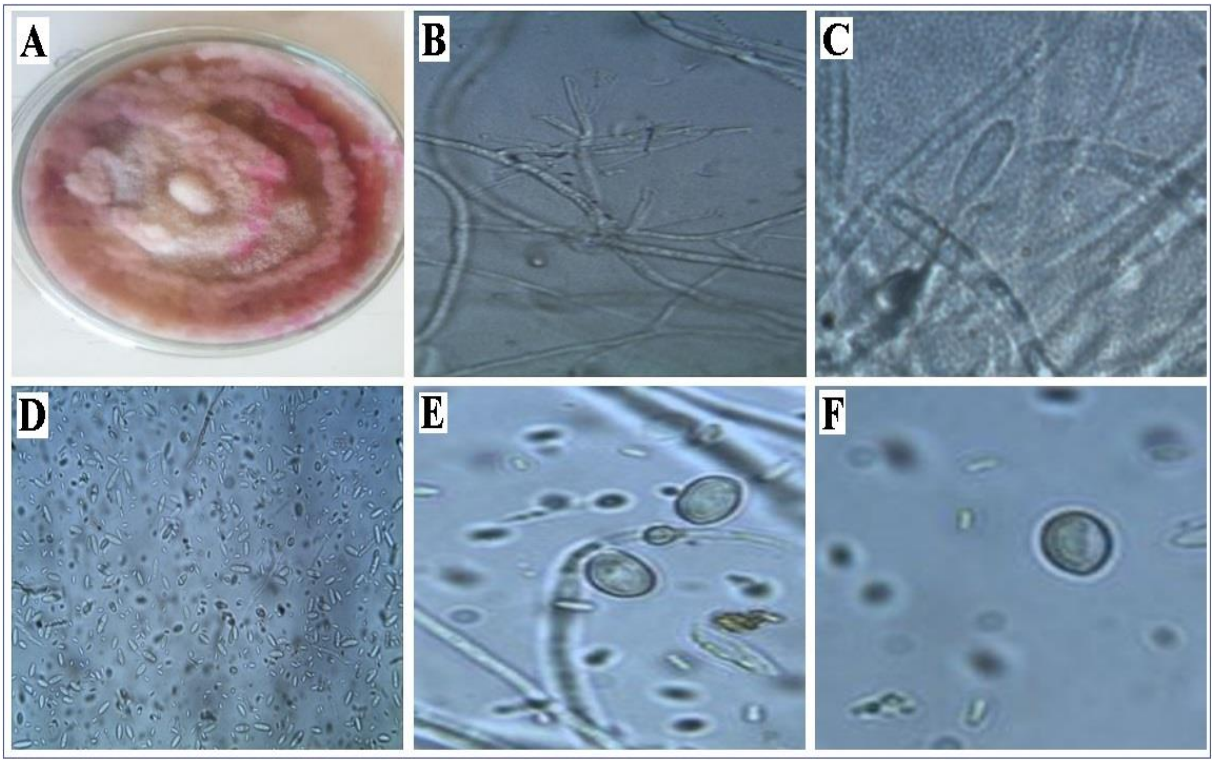

Fig. (1): Showing, A: PDA colony of *F. thapsinum*; B: Mycelium of *F. thapsinum*; C: Macroconidia; D: Microconidia; E-F: Napiform microconidia on PDA medium, microscopic examination= 400 x.

2.1.3. Microconidia characteristics:

Size of microconidia (µm) 5-19 × 2-4, Septa of microconidia 0-1, usually club-shaped with flate end base (Fig.1 E and F). Mycelium presentation in the air: long chains are typical, but smaller clusters of a few spores and false heads can also appear. Conidiogenous cells: Monophialides, which sporadically form in pairs and resemble "rabbit ears.,"

2.1.4. Chlamydospores. Absent.

2.2. Molecular study of *F. thapsinum:*

2.2.1. The DNA of *F. thapsinum* has been amplified:

According to agarose gel electrophoresis, rDNA region amplification using the primers ITS1 and ITS4 resulted in products with an estimated size of 600 bp (Fig. 2). Thereafter, similarity scores between each test sequence and the referred sequences received from the GenBank in the range of 90 to 100%.

Fig. (2): The ITS-1 and ITS-4 primers' banding patterns

2.2. 2. Sequences of *Fusarium thapsinum*:

Identification of *Fusarium thapsinum* isolated from bentgrass by molecular means was investigated. Quality sequence to identify the nearest cousin with the greatest similarity. As shown in Fig. (3) the tested strain displayed 100% identity and 100% coverage with numerous *F. thapsinum* acquired from the GenBank (OQ674102).

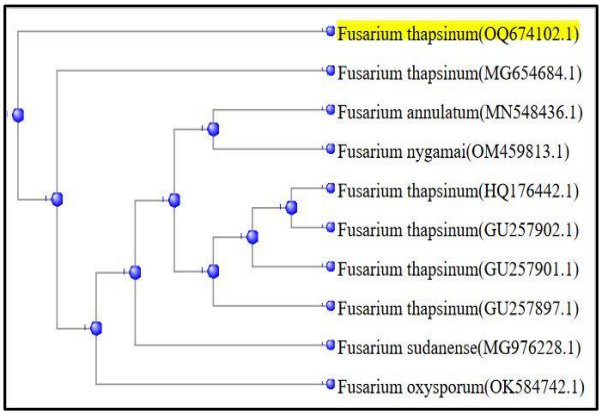

Fig. (3): Multiple Sequence Alignment by CLUSTALW and phylogenetic tree *Fusarium thapsinum* NCBI GenBank accession No.: OQ674102.

3 - Pathogenicity test

Pre- and post-emergence damping-off values were significantly higher when the seeds were planted in the infested soil. The fungi tested (Table, 3 and Fig. 4) could infect the seedlings of bentgrass. *R. solani* and *F. thapsinum* showed the highest pre, post, and root rot diseases. *R. solani* and *F. thapsinum* reported plant survival rates of 4 and 3%, respectively.

Data in Table, (3) demonstrate that, *R. solani* recorded the highest percentages of disease severity, being 67.0% on the average while, *Mucor* sp., *M. phaseolina* and *F. roseum* showed the least in this regard, being 5, 20 and 23 % disease severity without significant differences.

Table (3): Percentages of pre and post emergence damping-off, root rot, plant survivals and Disease severity (%) after sowing by 15, 30, and 60 days in infested soil under greenhouse conditions.

Fungi	Damping-off		Root-rot (%)	Plant Survivals (%)	Disease severity (%)
	Pre-emergence (%)	Post-emergence (%)			
Mucor sp.	4	8	8	80	5
F. semitectum	24	36	8	32	35
F. thapsinum	36	43	18	3	55
M. phaseolina	24	26	10	40	20
F. roseum	20	25	11	44	23
R. solani	44	42	10	4	67
S. sclerotiorum	36	37	20	7	45
Control	0.0	0.0	0.0	100	0.0
Mean	23.5	27.1	10.6	38.8	35
LSD at 0.05 %:	3.60	4.30	2.60	6.80	5.50

Fig. (4): Bentgrass infection by the most aggressive fungi (*Rhizoctonia solani*, *F. thapsinum* and *Sclerotinia sclerotiorum*) planted in artificially infested soil(s) under greenhouse conditions.

4- Disease control:

4.1. *In vitro*:

4.1.1. The effect of culture filtrates of two Trichoderma bio-agents on the linear growth of *F. thapsinum*, *R. solani*, and *S. sclerotiorum* after five days incubation:

Results in Table (4) show that culture filtrates of *T. viride* and *T. harzianum* significantly inhibited the linear growth of *F. thapsinum*, *R. solani*, and *S. sclerotiorum*, moreover the inhibitory effect was increased by increasing the concentration, being 71.9, 34.9, and 13.1 mm on the average, respectively at concentrations of 100, 200, and 300 ppm. Moreover, *T. viride* was the most effective bioagent in this regard followed by *T. harzianum*. Moreover, *T. viride* at 300 ppm completely inhibited the linear growth of the pathogens.

Table (4): Effect of culture filtrates of two *Trichoderma* species on the linear growth of *F. thapsinum*, *R. solani*, and *S. sclerotiorum* after five days incubation.

Con. ppm	Average linear growth (mm)								General mean
	Trichoderma harzianum				*Trichoderma viride*				
	Fusarium thapsinum	*Rhizoctonia solani*	*Sclerotinia sclerotiorum*	Mean	*Fusarium thapsinum*	*Rhizoctonia solani*	*Sclerotinia sclerotiorum*	Mean	
0.0	90	90	90	90	90	90	90	90	90
100	80.2	77.6	78.8	78.8	65.6	62.5	66.9	65	71.9
200	50.3	40.9	45.6	45.6	24.3	23.2	25.4	24.3	34.9
300	26.6	25.8	26.3	26.2	0.0	0.0	0.0	0.0	13.1
Mean	61.7	58.5	60.1	-	44.9	44.2	45.5	-	-

L.S.D.at 5% for: Fungi (F) = 0.12, Concentrations (C) = 5.20, Treatments (T) =3.30, F×C = 1.22, F×T = 2.40, C×T = 3.23, F×C×T= 2.30

4.1.2. Influence of culture filtrates of *A. vinelauvii, Azospirillum* sp. and *B. subtilis* on the linear growth of *F. thapsinum, R. solani* and *S. sclerotiorum*, five days after incubation at 25±2ºC while *S. sclerotiorum* was at 18±2°C.

The antagonistic activity of the three examined bioagents was tested against *F. thapsinum*, *R. solani*, and *S. sclerotiorum*. Data presented in Table (5) show that the mycelial growth of the three tested pathogenic fungi was significantly reduced by the filtrates of all the tested organisms. The average linear growth was 80.2, 54.7, and 22.4 mm at the concentrations of 100, 200, and 300 ppm, on the average respectively, when the incorporated concentration was steadily increased. Moreover, *B. subtilis*, followed by *A. vinelauvii*, were the most effective bioagent in this regard.

4.1.3. Influence of Potassium silicate and Vitavax 200 on linear growth of *F. thapsinum, R. solani* and *S. sclerotiorum*, 5 days after incubation at 25±2ºC, while for *S. sclerotiorum* was at 18±2°C:

Data in Table (6) indicate that both Vitavax 200 and potassium silicate significantly inhibited the linear growth of the pathogenic fungi. This inhibitory effect was gradually increased by increasing the tested concentration on PDA. The average linear growth at concentrations of 100, 200, and 400 ppm, recorded 72.8, 43.5 and 11.3 mm, respectively. Potassium silicate had a significant impact on *R. solani* than *F. thapsinum*, with average linear growth rates of 62.8 and 67.0 mm, respectively. Vitavax 200 was the most effective treatment in this regard. The linear growth of the three tested pathogens was also completely inhibited by Vitavax 200 at a rate of 400 ppm.

4.1.4. Influence of Vermicomposting on the linear growth of *F. thapsinum, R. solani* and *S. sclerotiorum*, 5 days after incubation.

Tabulated data (Table, 7), show that filtrates of vermicomposting significantly inhibited the linear growth of *F. thapsinum*, *R. solani*, and *S. sclerotiorum*. The average linear growth was 63.3, 22.8, and 0.0 mm on the average at the concentrations of 100, 200, and 300 ppm, respectively; the inhibition activity of the tested treatments was increased with increasing their concentration. Moreover, Vermicomposting at 300 ppm completely inhibited the linear growth of the tested pathogens.

Table (5): Influence of culture filtrates of *A. vinelauvii, Azospirillum* sp. and *B. subtilis* on linear growth of *F. thapsinum, R. solani* and *S. sclerotiorum*, 5 days after incubation.

Con. ppm	Average linear growth (mm)												General mean
	Azotobacter vinelauvii				*Azospirillum* sp.				*Bacillus subtilis*				
	Fusarium thapsinum	*Rhizoctonia solani*	*Sclerotinia sclerotiorum*	Mean	*Fusarium thapsinum*	*Rhizoctonia solani*	*Sclerotinia sclerotiorum*	Mean	*Fusarium thapsinum*	*Rhizoctonia solani*	*Sclerotinia sclerotiorum*	Mean	
0.0	90	90	90	90	90	90	90	90	90	90	90	90	90
100	85.2	82.5	80.6	82.7	83.2	82.0	86.4	83.8	70.4	74.3	77.6	74.1	80.2
200	65.8	55.7	50.5	57.3	67.6	60.6	63.4	63.8	40.6	42.8	45.6	43	54.7
300	33.4	30.9	30.0	31.4	31.3	32.0	35.2	32.8	10.8	13.2	15.5	13.1	22.4
Mean	68.6	64.7	62.7	-	68.0	66.1	68.7	-	52.9	55.0	57.1	-	-

L.S.D.at 5 % for: Fungi (F) = 0.21, Concentrations (C) = 6.50, Treatments (T) =4.10, F×C = 2.92, F×T = 3.50, C×T = 4.03, F×C×T= 3.10

Table (6): Influence of Potassium silicate and Vitavax 200 on the linear growth of *F. thapsinum, R. solani* and *S. sclerotiorum*, 5 days after incubation.

Con. ppm	Average linear growth (mm)								General mean
	Potassium silicate				Vitavax 200				
	Fusarium thapsinum	*Rhizoctonia solani*	*Sclerotinia sclerotiorum*	Mean	*Fusarium thapsinum*	*Rhizoctonia solani*	*Sclerotinia sclerotiorum*	Mean	
0.0	90	90	90	90	90	90	90	90	90
100	85.0	80.8	82.6	82.8	62.6	60.3	65.7	62.8	72.8
200	67.6	60.5	64.2	64.1	23.5	19.5	25.8	22.9	43.5
400	25.6	20.0	22.6	22.7	0.0	0.0	0.0	0.0	11.3
Mean	67.0	62.8	64.8		44.0	42.4	45.3		

L.S.D.at 5 % for: Fungi (F) = 3.01, Concentrations (C) = 7.20, Treatments (T) =9.10, F×C = 4.22, F×T = 3.40, C×T = 5.23, F×C×T= 2.90

Table (7): Influence of Vermicomposting filtrates on the linear growth of *F. thapsinum, R. solani* and *S. sclerotiorum*, 5 days after incubation at 25±2ºC while for *S. sclerotiorum* was at 18±2°C.

Con. ppm	Average linear growth (mm)			
	Fusarium thapsinum	*Rhizoctonia solani*	*Sclerotinia sclerotiorum*	Mean
0.0	90	90	90	90
100	63.4	62.3	64.4	63.3
200	24.2	20.2	24.2	22.8
300	0.0	0.0	0.0	0.0
Mean	44.4	43.1	44.6	
L.S.D.at 5%	for: Fungi (F) = 0.10, Concentrations (C) = 5.0, F×C = 1.22			

4.2. Greenhouse applications

4.2.1 Influence of the active treatments on bentgrass pre- and post-emergence damping-off caused by *F. thapsinum, R. solani, and S. sclerotiorum* 60 days after sowing under greenhouse conditions:

Presented data (Table, 8 and Fig., 5) indicate that treatments significantly decreased the percentages of pre- and post-emergence damping-off of bentgrass and increased the healthy survived seedlings, VMC plus *T. harzianum* and Vitavax 200 were much more effective.

4.2.2. Influence of control treatments on some growth parameters of bentgrass 60 days after sowing in infested soil under greenhouse conditions.

Data presented in Table (9) demonstrate that the combination of VMC with each of *T. harzianum*, *T. viride*, *B. subtilis*, *A. vinelauvii*, and *Azospirillum* sp. in addition, potassium silicate and Vitavax 200 significantly improved the growth parameters. VMC plus *T. harzianum* and Vitavax 200 fungicide treatments were much more effective.

Fig. (5): Influence of vermicomposting treatment on controlling root rot caused by *Rhizoctonia solani, F. thapsinum, Sclerotinia sclerotiorum* on bentgrass plants in greenhouse.

Table (8): Evaluation of the most active tested treatments in reducing percentages of pre and post emergence damping–off disease of bentgrass under greenhouse conditions.

Treatments	Effect of treatments on:								
	Fusarium thapsinum			*Rhizoctonia solani*			*Sclerotinia sclerotiorum*		
	Pre-emergence (%)	Post-emergence (%)	Plant survival (%)	Pre-emergence (%)	Post-emergence (%)	Plant survival (%)	Pre-emergence (%)	Post-emergence (%)	Plant survival (%)
Potassium silicate	44	21	35	48	23	29	40	13	47
VMC	40	13	47	44	21	35	36	18	46
VMC + *Azospirillum* sp.	36	12	52	40	20	40	32	11	57
VMC + *A. vinelauvi*	32	11	57	36	18	46	28	16	56
VMC + *B. subtilis*	28	16	56	32	17	51	24	15	61
VMC + *T. harzianum*	8	8	84	12	9	79	8	8	84
VMC + *T. viride*	12	9	79	16	14	70	12	9	79
Vitavax 200	4	4	92	8	4	88	8	8	84
Control	48	33	19	52	41	7	44	35	21
L.S.D.at 5%									
Treatments (T)	0.40	0.66	1.2	0.50	0.76	1.4	0.60	0.88	1.6
Fungi (F)	0.62	0.77	0.97	0.12	0.43	1.2	0.54	0.32	1.2
T × F	0.98	0.54	0.1.0	0.39	0.78	0.12	0.65	0.43	1.7

Table (9): Bentgrass growth parameters 60 days after sowing in infested soil under greenhouse conditions.

Treatments	*Fusarium thapsinum*				*Rhizoctonia solani*				*Sclerotinia sclerotiorum*			
	Plant height / Plant (cm)	Fresh weight / Plant(g)	Dry weight / Plant (g)	Total Chlorophyll (mg/g)	Plant height / Plant (cm)	Fresh weight / Plant(g)	Dry weight / Plant (g)	Total Chlorophyll (mg/g)	Plant height / Plant (cm)	Fresh weight / Plant(g)	Dry weight / Plant (g)	Total Chlorophyll (mg/g)
Potassium silicate	10.0	15.6	3.5	6.844	11.0	16.5	4.2	7.744	9.3	14.5	5.3	5.824
VMC	15.0	18.2	6.0	10.655	16.2	19.1	7.3	10.665	14.2	17.6	5.4	9.605
VMC + *Azospirillum* sp.	16.0	19.2	6.5	11.200	17.6	19.7	7.6	11.250	15.6	18.3	6.4	10.250
VMC + *A. vinelauvi*	17.0	19.8	6.6	12.432	18.5	20.7	6.8	13.472	16.0	20.6	7.4	11.402
VMC + *B. subtilis*	16.0	20.9	7.5	13.546	16.6	19.9	7.6	14.544	17.4	19.8	6.6	12.506
VMC + *T. harzianum*	25.5	25.2	8.0	22.321	26.4	26.3	9.4	23.361	24.4	27.3	9.5	20.301
VMC + *T. viride*	18.0	20.6	6.9	16.258	19.2	20.5	7.7	17.278	17.5	18.9	6.5	15.248
Vitavax 200	30.0	30.4	10.5	20.693	31.3	31.5	11.6	21.673	29.3	30.0	10.2	21.653
Control	6.0	7.6	2.5	4.564	7.5	8.5	3.2	5.504	5.3	6.5	2.2	4.504
L.S.D.at 5%												
Treatments (T)	3.32	2.54	0.23	1.33	2.80	1.44	0.22	1.00	1.43	1.23	0.45	0.98
Fungi (F)	2.98	3.65	0.46	1.45	2.54	1.87	0.43	1.65	1.24	1.54	0.86	0.32
T × F	3.00	2.98	0.87	1.32	2.77	1.21	0.65	1.43	1.85	1.33	0.76	0.44

4.3. Field experiment:

4.3.1. Influence of control treatments of bentgrass at area infected and turf quality, grown under naturally infested field conditions after 4,6,8 and 10 weeks (w), after planting in Beni-Suef governorate, 2021 season:

Data (Table, 10 and Fig., 6) show that all treatments significantly decreased the percentages of diseased areas. The lowest percentages of infected area (4.6%) and (5%) were recorded as a result of treatment with each of Vitavax 200 and VMC plus *Azospirillum* sp. Accordingly, Vitavax 200 (4.6%) was the best treatment followed by VMC plus *Azospirillum* sp. (5.0%) and VMC plus *T. harzianum* (5.9%), respectively. On the other hand, VMC (15.2) and potassium silicate (16.3) were the least effective treatments.

The highest turf quality in this experiment was significantly associated with VMC plus *T. harzianum*. VMC plus *T. harzianum* treatments, the obtained measurements of turf quality were significantly higher than those of the other treatments.

Table (10): Area infected and turf quality, grown under naturally infested field conditions after 4,6,8 and 10 weeks (w) after planting, in Beni-Suef governorate, 2021 season.

Treatments	Area infected (%)					Turf quality*				
	4w	6w	8w	10w	M	4w	6w	8w	10w	M
Potassium silicate	16.8	16.6	16.0	15.9	16.3	1.0	2.0	2.0	1.0	1.5
VMC	15.3	15.2	15.0	15.5	15.2	2.0	2.0	2.0	3.0	2.2
VMC + *Azospirillum* sp.	8.5	5.0	4.3	2.4	5.0	2.0	3.0	3.0	4.0	3.0
VMC + *A. vinelauvi*	9.0	6.4	5.2	3.5	6.0	2.0	2.0	3.0	3.0	2.5
VMC + *B. subtilis*	9.4	7.5	6.3	4.3	6.9	2.0	2.0	3.0	4.0	2.7
VMC + *T. harzianum*	6.0	6.3	6.4	5.0	5.9	5.0	5.0	6.0	5.0	5.2
VMC + *T. viride*	10.0	9.3	8.0	6.0	8.3	5.0	4.0	5.0	5.0	4.7
Vitavax 200	5.0	4.5	4.7	4.4	4.6	5.0	6.0	6.0	6.0	5.7
Control	20.9	25.8	23.7	22.0	23.1	1.0	1.0	1.0	1.0	1.0
L.S.D.at 5%	0.10	0.55	0.66	0.98	0.88	0.44	0.76	0.87	0.87	0.11

***** On a scale of 1 to 6, with 6 indicating no disease and 1 denoting the worst overall appearance

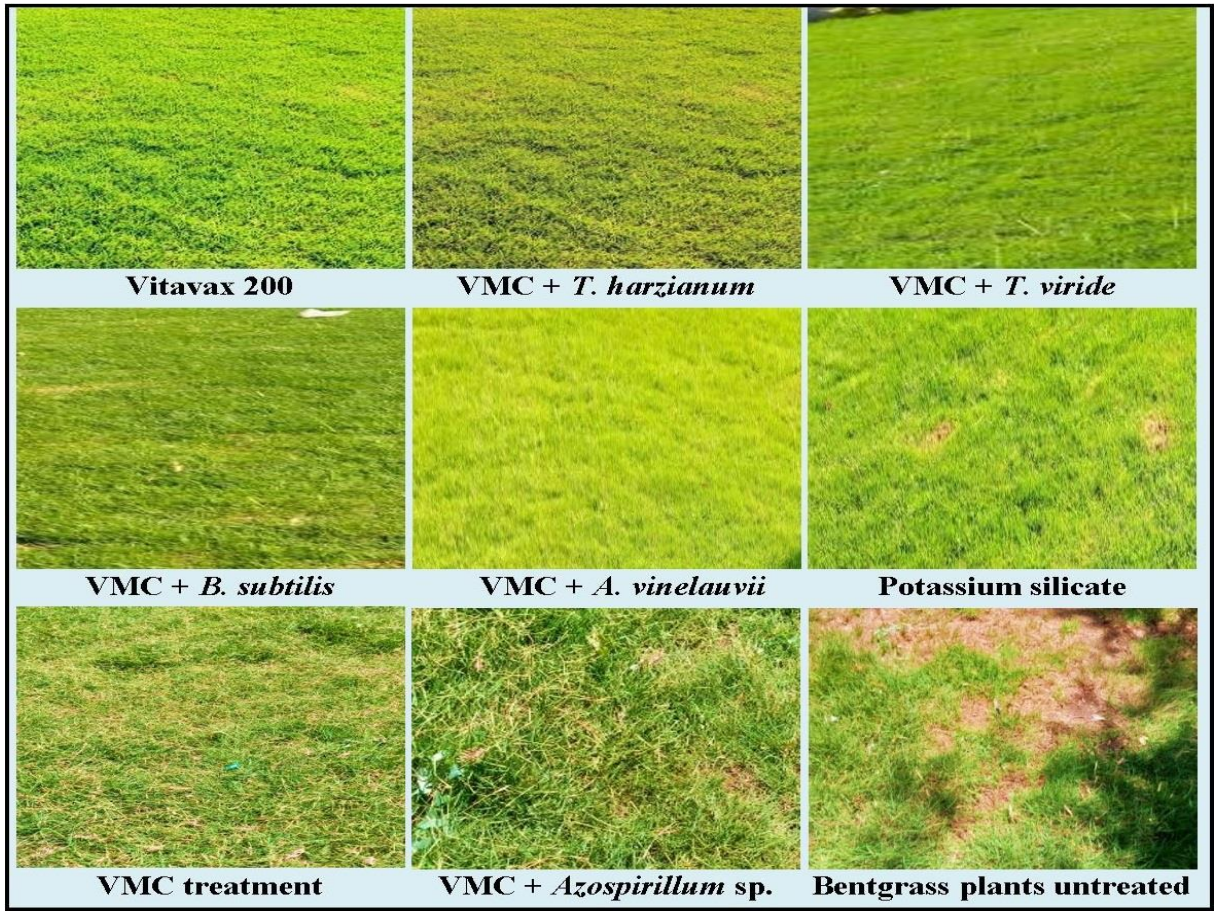

Fig. (6): Effect of various treatments on bentgrass quality.

4.3.2. Influence of control treatments at area infected (%) and turf quality, grown under naturally infested field conditions during 2022 season in Beni-Suef governorate:

Data presented in Table (11) show that treatments significantly decreased the percentages of diseased area. Vitavax 200 showed the lowest percentages of diseased area, being (5.4%) on the average in Beni-Suef governorate at season 2022. Thus, Vitavax 200 proved to be the best treatment, followed by VMC plus *T. harzianum* (6.2%) and VMC plus *A. vinelauvi* (6.4%) in that season, respectively. On the other hand, plants treated with VMC (8.2), and potassium silicate (8.3) were the least effective treatment.

However, turf quality was significantly increased by all treatments, potassium silicate, Vitavax 200, VMC and its combinations with *T. harzianum*, *T. viride*, *B. subtilis*, *A. vinelauvii*, and *T. harzianum*. Vitavax 200 caused the highest turf quality in this study, significantly outperforming the other treatments. Vitavax 200 application, however, showed measurements of turf quality higher than those of the other treatments.

4.3.3. Influence of control treatments on the parameters of bentgrass plants grown at Beni-Suef governorate under natural field conditions, 2021 and 2022 seasons:

Data (Table, 12) illustrates that all treatments significantly increased the estimated plant parameters. Moreover, Vitavax 200, VMC plus *T. harzianum*, VMC plus *T. viride* and VMC plus *B. subtilis* were the most effective treatments in Beni-Suef governorate during 2021 and 2022 seasons, Potassium silicate treatment was the least efficient on plant parameters and other concerned criteria as well.

Table (11): Influence of control treatments on area infected (%) and turf quality, grown under naturally infested field soil conditions after 4,6,8,10 weeks after planting during 2022 season in Beni-Suef governorate.

Treatments	Area infected (%)					Turf quality				
	4w	6w	8w	10w	M	4w	6w	8w	10w	M
Potassium silicate	8.3	8.2	8.4	8.3	8.3	2.0	2.0	3.0	2.0	2.2
VMC	8.0	8.2	8.9	8.0	8.2	2.0	2.0	3.0	3.0	2.5
VMC + *Azospirillum* sp.	9.3	5.5	5.0	3.5	6.9	2.0	2.4	3.0	3.6	2.6
VMC + *A. vinelauvi*	9.5	6.6	5.5	3.9	6.4	2.0	2.0	3.2	4.0	2.5
VMC + *B. subtilis*	10.0	8.0	6.6	4.8	7.3	2.0	2.0	3.0	3.0	2.5
VMC + *T. harzianum*	6.3	6.5	5.5	6.6	6.2	6.0	6.0	5.0	5.0	5.5
VMC + *T. viride*	7.5	7.5	7.6	7.8	7.6	5.0	5.0	5.0	4.0	4.7
Vitavax 200	5.0	5.5	5.9	5.4	5.4	6.0	6.0	6.0	5.0	5.7
Control	13.0	14.0	14.5	13.5	13.7	1.0	1.0	1.0	1.0	1.0
L.S.D.at 5%	0.44	0.64	0.55	0.39	0.28	0.76	0.83	0.11	0.63	0.21

Table (12): Influence of control treatments on the parameters of bentgrass plants grown at Beni-Suef governorate under natural field conditions, 2021 and 2022 seasons.

Treatments	2021			2022		
	Plant height / plant (cm)	Fresh weight / plant (g)	Dry weight / plant (g)	Plant height / plant (cm)	Fresh weight / plant (g)	Dry weight / plant (g)
Potassium silicate	25.0	6.0	2.3	25.0	5.0	1.3
VMC	30.0	7.0	2.0	29.5	6.5	1.5
VMC + *Azospirillum* sp	35.0	7.8	2.5	34.5	7.5	2.2
VMC + *A. vinelauvi*	39.0	8.0	2.6	37.0	8.0	2.5
VMC + *B. subtilis*	42.0	8.5	1.5	39.0	9.5	2.9
VMC + *T. harzianum*	45.0	10.5	5.7	40.0	11.0	3.5
VMC + *T. viride*	36.0	8.9	1.5	35.0	8.5	2.7
Vitavax 200	49.0	12.5	5.2	45.0	13.0	4.3
Control	22.0	4.5	1.9	23.0	4.0	1.0
L.S.D.at 5%	4.43	0.65	0.33	5.44	1.20	0.44

4.3.4. Effect of control treatments on total phenols and defense-related enzymes of bentgrass grown in Beni-Suef governorate under natural field conditions, 2021 and 2022 seasons:

The tested treatments, (VMC) and the combinations, VMC plus *T. harzianum,* VMC plus *T. viride*, VMC plus *B. subtilis*, VMC plus A. *vinelauvii*, VMC plus *Azospirillum* sp. also, potassium silicate and Vitavax 200 as single treatments were found to have greater phenols compounds, peroxidase, and polyphenol oxidase activity than those in control (Table 13). In this regard, VMC plus *T. harzianum* recorded 6.30 mg/g fresh weight total phenol, 2.70 enzyme unite/mg Peroxidase, 0.90 enzyme unite/mg Polyphenol oxidase, and 1.90 mg/g fresh weight Conjugated phenols, respectively, while Vitavax 200 displayed, total phenols 2.03, Peroxidase 0.90, Polyphenol oxidase 0.24, and 0.94 Conjugated phenols.

Table (13): Influence of different control measures on total phenols and defense-related enzymes of bentgrass grown in Beni-Suef governorate under natural infection during 2021 and 2022 seasons.

Treatments	Phenols		Enzymatic activities	
	Total phenol (mg/g fresh weight)	Conjugate phenol (mg/g fresh weight)	Peroxidase (Enzyme unite/mg)	Polyphenol oxidase (Enzyme unite/mg)
Potassium silicate	2.60	0.90	1.20	0.30
VMC	3.50	0.95	1.30	0.55
VMC + *Azospirillum* sp	5.40	1.30	1.80	0.80
VMC + *A. vinelauvi*	5.20	1.60	1.30	0.70
VMC + *B. subtilis*	4.15	1.40	1.10	0.65
VMC + *T. harzianum*	6.30	1.90	2.70	0.90
VMC + *T. viride*	5.90	1.20	1.60	0.44
Vitavax 200	2.03	0.94	0.90	0.24
Control	1.04	0.80	0.60	0.10
LSD at 5%	0.10	0.15	0.20	0.06

DISCUSSION

Bentgrass is an important and essential element from the environmental point of view as it works to reduce pollution and recreation. During the growing season, bentgrass plants are liable to infection by root-rots. The recovered *F. thapsinum* strain, however, had 100 identities and 100 coverage for *F. thapsinum* obtained from the GenBank, according to molecular analysis. These results are consistent with those of Zakaria (2023) and Abd-Elsalam (2009). Chemical control of persistent soil-borne diseases is not advisable since the chemicals are expensive and dangerous to the environment. According to previous studies, several bio control agents were utilized in a variety of crops as part of an environmental strategy (Mishra *et al*., 2015 and Sarma *et al*., 2015,). Organisms' potential may be due to their ability to release enzymes, different antibiotics, or antifungal. Trichoderma produces a variety of antimicrobial metabolites (Keswani *et al*. 2015).

High-quality compost is made by a variety of different microorganisms (Attia *et al*., 2022). The vermicomposting populations of microorganisms were evaluated before being strengthened with more microorganisms. The most common microbial species found in vermicomposting often match those listed by Navagallemma *et al*. (2004). In vermicomposting, *Bacillus* spp. predominate, according to (Navagallemma *et al*., 2004). According to Dhir (2017) the infection with *R. solani* was extremely well treated by *Azotobacter brasilensis*. This result was attributed to a reduction in the population density of the Rhizosphere. Moreover, Brown (2012) reported that Azotobacter might create fungal antibiotics.

The lower malonicaldehyde concentration in silicon-supplied plants significantly reduced lipid peroxidation for the banana, *F. oxysporum* f. sp. *cubense*, cotton, *Ramularia areola*, and rice, *P. oryzae* (Debona *et al*., 2014 and Domiciano *et al*., 2015).

Vitavax 200 fungicide treatment was significantly the best among all the tested treatments. According to Chaurasia *et al*. (2017) antimicrobial chemicals function as inhibitors of essential processes inside the cell's membrane, which may be the cause of the inhibition in mycelial growth. The ability of *T. harzianum* to prevent pathogenic fungi may be a result of the substance's production (Ismail, 2017). Azotobacter, however, was found to produce ether-soluble fungi static substances (Youssef and Asmahan, 2015).

Soaking bentgrass seeds in the solutions of the tested treatments significantly decreased pre and post emergence damping-off when the seeds were experimentally infested with *F. thapsinum*, *R. solani*, and *S. sclerotiorum*. The greatest percentage values of survival plants were obtained by VMC plus *T. harzianum* and Vitavax 200, followed by VMC plus *T. viride*. Fresh weight, plant height, dry weight, and total chlorophyll values were increased by Vermicompost (VMC) and its combinations, VMC plus *T. harzianum*, VMC plus *T. viride*, VMC plus *B. subtilis*, $_{VMC}$ plus *A. vinelauvii*, VMC plus *Azospirillum* sp. also, potassium silicate and Vitavax 200 as single treatments. *F. thapsinum*, *R. solani*, and *S. sclerotiorum* linear growth was decreased by all the antagonists *in vitro*. The greatest reduction was brought on by *T. harzianum*, followed by *T. viride*. These results concur with those of Ragab *et al*. (2015) and Abd-El-Khair *et al*. (2011) who showed that *T. hamatum* and *T. harzianum* effectively inhibited the mycelial growth of *R. solani*. *T. harzianum* drastically reduced the growth areas of *F. solani* and *R. solani*, by more than 90.6 and 87.4% (El-Mougy *et al*., 2013). Sarhan *et al*. (2001) reported that *B. subtilis* cell free culture filtrate decreased spore germination of *F. oxysporum*.

Area infected was significantly decreased by any of the tested treatments, the findings agree with those reported by El-Mougy *et al*., (2013), who indicated that root rot incidence of beans and cowpeas caused by *F. solani* and *R. solani* was decreased by using *T. harzianum*, similar findings were reported by Abd-El-Khair *et al*. (2011) and Ragab *et al*. (2015), who found that, *T. koningii* and *T. harzianum* protected bean seedlings from the harmful effect of *R. solani* and *Fusarium* sp. Data collected during field studies indicated positive outcomes for managing soil-borne pathogenic fungi. VMC plus *T. harzianum* was the most successful treatment. Bentgrass plants were significantly improved under field conditions, fresh weight, plant height, turf quality, and dry weight, moreover, also reduced the area infected (%). These findings agree with those reported by Ragab *et al*. (2015) and support the data of the present investigation. The findings of our study showed that essential elements of plants' defensive mechanisms against pathogen invasion are greatly boosted using bio agents. Results showed that treated bentgrass plants had higher levels of enzymatic activity than untreated ones. These findings are in agreement with those reported by Abd-El-Khair *et al*. (2011) who stated that treatment with *Trichoderma* spp. as bioagents caused the increase of several enzymes in treated snap bean plants, including chitinase, peroxidase and polyphenol oxidase. Enzymes like chitinase and -1, 3 glucanase are crucial for plants' defense against fungi because they hydrolyze their cell walls (Barilli *et al*., 2010). Polyphenol oxidase was found after treatments with different antioxidants (Abdel-Monaim, 2008) and (Anonymous, 2017).

CONCLUSION

According to the results of this study, controlling plant diseases with vermicomposting and bio-agents with beneficial microbes would be environmentally friendly practices that might have a big impact on sustainable agriculture. Vermicomposting treatment follows bio-agents using *B. subtilis* and *T. harzianum* which improve growth and plant quality. These findings suggest that bio-agents and vermicomposting could be used for larger-scale open field diseases control and ultimately, sustainable disease management.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

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