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(Original Article)

Sustainable Control of Onion White Rot via Vermicompost-Amended Soil and Microbial Bioagents: Effects on Plant Growth and Rhizosphere Microbiome

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Abstract

White rot disease, caused by *Stromatinia cepivora* (syn. *Sclerotium cepivorum*), presents a major challenge to the production of global onion (*Allium cepa* L.). This study evaluated the effectiveness of various bio-organic treatments, including vermicompost, vermicompost tea, and beneficial microbes (*Pseudomonas fluorescens, Trichoderma harzianum, and Bacillus thuringiensis*), applied individually and in combination under laboratory (*in vitro*), greenhouse, and field (*in vivo*) conditions. The *invitro* assays demonstrated strong antagonistic activity by the microbial agents, with inhibition rates of 85.57%, 84.43%, and 82.57%, respectively. Under greenhouse conditions, the combination of vermicompost and EM1 achieved the highest disease suppression, reducing severity to 15.70% via foliar spraying and 17.40% via soil drenching, with corresponding control efficiencies of 73.98% and 70.87%, respectively.

These treatments also significantly increased bulb diameter (32.44 and 27.33 mm, respectively). Field trials confirmed these findings, with the same combination reducing disease severity to 12.43% and achieving a control efficiency of 78.72%. In addition, the treated plants exhibited improved bulb weight (255.33 g), plant height (25.27 cm), and leaf number (12.44). Soil microbiological analysis revealed enhanced populations of fungi, bacteria, and actinomycetes following bio-organic treatments, while the chemical fungicide Folicure (CC) suppressed soil microbiota. Vermicompost tea-based treatments provided moderate benefits but were less effective than solid microbe-enriched formulations. These results highlight the potential of integrated bio-organic amendments, particularly vermicompost, combined with microbial agents as sustainable alternatives to chemical fungicides for managing WRD, enhancing onion growth, and improving soil health.

Keywords: *Allium cepa, Biocontrol, EM1, Sclerotium cepivorum, Vermicompost.*

Introduction

Onion (*Allium cepa L.*) is a globally significant vegetable crop, particularly important in Egypt due to its high domestic consumption and substantial export value (Abo-Zaid *et al.*, 2020). The country ranks among the leading global producers, benefiting from favorable climatic conditions and extensive agricultural expertise.

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Onion cultivation in Egypt occurs across three main seasons: winter, summer, and Nile, with the winter crop contributing approximately 91% of total production (Ali, 2018).

Despite its economic and nutritional value, onion cultivation faces several challenges, most notably fungal diseases that threaten both yield and quality. White rot (WRD) caused by the soil-borne fungus *Stromatinia cepivora* (*syn. Sclerotium cepivorum* Berk.) is considered one of the most destructive species (Hammad *et al*., 2023). This pathogen infects onion roots and bulbs, leading to wilting, plant death, and severe yield losses (Mardanli and Aliyev, 2025). The long-term survival of the pathogen's sclerotia hardened fungal structures that can remain viable in the soil for over 20 years, even in the absence of a host, is a major concern in managing white rot (Hossain *et al*., 2024; Longjam *et al*., 2024).

Control Strategies for WRD. Various control strategies have been investigated to mitigate the impact of WRD, including chemical treatments (Hossain *et al*., 2024), soil solarization, and cultural practices (Al-Shammary *et al*., 2020). However, growing concerns over environmental sustainability and pathogen resistance development have shifted attention toward biological and organic alternatives.

Biological control methods (Abolmaaty and Fawaz, 2016), soil amendments, and improved agricultural practices (Amin and Fawaz, 2015) have shown promise in the management of WRD. Vermicompost, an organic amendment produced through the activity of earthworms and microorganisms, can suppress various soil-borne pathogens while enhancing soil fertility and structure (Yatoo *et al*., 2021; Gudeta *et al*., 2022). It also improves nutrient availability and promotes overall plant health (Manivannan *et al*., 2009). Additionally, vermicompost tea, a liquid extract obtained by steeping vermicompost in water and allowing microbial fermentation, has been found to increase beneficial microbial populations and enhance plant resistance to diseases (Edwards *et al*., 2006; Arancon *et al*., 2005).

Another eco-friendly strategy involves the use of Effective Microorganisms (EM1) a mixed culture of beneficial microbes that improve soil health, nutrient uptake, and microbial balance in the rhizosphere (Okorski *et al*., 2010). Furthermore, antagonistic biological agents such as *Trichoderma harzianum*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Bacillus thuringiensis*, and *Serratia spp*. have shown promising results in inhibiting phytopathogenic fungi and promoting plant growth through multiple mechanisms (Basco *et al*., 2017).

This study aimed to evaluate the effectiveness of vermicompost, vermicompost tea, effective microorganisms (EM1), and select antagonistic microorganisms (*B. thuringiensis*, *B. subtilis*, *S. marcescens*, *P. fluorescens*, and *T. harzianum*) as resistance inducers against WRD in onions under greenhouse and field conditions. Additionally, the study investigates their impact on growth parameters, yield components of the Giza red onion cultivar, and soil microbial populations (bacteria, fungi, and actinomycetes) compared to untreated controls.

Materials and Methods

1. Isolation, identification, and preparation of *S. cepivorum*

A pathogenic isolate of *S. cepivorum* was obtained from diseased onion plants collected in the Giza Governorate Al-Saf region. The fungus was identified based on its morphological characteristics following the description provided by Mordue (1976). For inoculum preparation, the isolate was cultured on sterilized barley seeds and incubated at 20°C for 3 weeks, as described by Amin *et al*. (2016b); Bipinbhai (2021); Amin and Ahmed (2023).

2. Isolation, identification, and preparation of bioagents isolates:

T. harzianum and four bacterial isolates *B. thuringiensis*, *B. subtilis*, *S. marcescens*, and *P. fluorescens*, from the rhizosphere of healthy onion plants grown in a field naturally infested with *S. cepivorum* in Al-Saf, Giza Governorate, Egypt. Bacterial isolates were identified based on their morphological traits and standard biochemical tests, following the procedures described by Mansour *et al*. (2023). A loopful of each isolate was suspended in 10 mL of sterile distilled water (SDW), and 1 mL of this suspension was inoculated into 300 mL of LB broth medium (1% tryptone, 0.5% yeast extract, and 1% NaCl). The cultures were adjusted to a final concentration of approximately 2.5×10^7 CFU/ml. The fungal isolate (*T. harzianum*) was purified on potato dextrose agar (PDA) according to the method described by Abo-Zaid *et al*. (2025) and prepared as a spore suspension at a concentration of 1×10^7 spores/ml. Before transplanting, onion seedlings were immersed in microbial suspensions for 15 min and then air-dried for 1 h (Ahmed *et al*., 2017). Under greenhouse conditions, two application methods were employed 2 weeks after transplanting: (a) soil drenching with 10 mL of the spore or bacterial suspension, and (b) foliar spraying of the onion plant until complete wet. In contrast, under field conditions, treatments were applied exclusively via foliar spraying.

3. Source and Application of Vermicompost

The vermicompost used in this study was sourced from the Central Laboratory for Agricultural Climate, Agricultural Research Center (ARC), Giza, Egypt, and produced according to the methodology described by Abul-Soud *et al*. (2009). Its chemical composition was as follows: nitrogen (N), 1.71%; phosphorus (P), 0.79%; potassium (K), 1.51%; organic carbon (C), 11.7%; and C/N ratio of 6.84.

Under greenhouse conditions, vermicompost was incorporated into the soil at a rate of 5% (w/w) seven days before transplanting. Under field conditions, it was applied at a rate of 5 tons per feddan during soil preparation, following the recommendations of Abolmaaty and Fawaz (2016).

4. Preparation and application of vermicompost tea (VCT)

Vermicompost tea (VCT) was prepared by mixing high-quality vermicompost with dechlorinated water at a ratio of 1:10 (w/v). The mixture was maintained at 25 °C for seven days, with intermittent aeration provided by an aquarium pump, and was specifically stirred on the fourth day. The suspension was filtered through a fine mesh before application to remove coarse vermicompost particles. During the brewing process, the tea was stored in a dark, cool environment at approximately 4 °C and used within a few days to preserve microbial viability and effectiveness, in accordance with the protocols described by Chaoui *et al*. (2002) and Rajan (2014).

Under greenhouse conditions, VCT was applied either to the soil via irrigation at a rate of 5% (v/w) per pot or as a foliar spray. Applications began post-transplantation and were repeated three times at 2-week intervals, following the recommendations of Helmy and Abu-Hussien (2024).

5. Acquisition and Application of Effective Microorganisms (EM1)

The EM1 solution, which includes lactic acid bacteria, yeasts, and photosynthetic bacteria, was obtained from the Ministry of Agriculture, El-Dokki, Egypt, under license from the Eastern Mediterranean Regional Office, Japan. Before transplanting, onion seedlings were immersed for 15 minutes in an EM1 suspension supplemented with 1% Arabic gum for 15 min and then left to air-dry for 60 min, following the protocol of Abolmaaty and Fawaz (2016). Under greenhouse conditions, two application methods were used beginning two weeks after transplanting: soil drenching and foliar spraying. Conversely, in the field trials, the EM1 treatment was applied solely through foliar spraying.

6. Application of the fungicide folicure

Folicure 25% EC (tebuconazole 25%), a systemic fungicide commonly used for disease management, was selected as the chemical control benchmark in this study to evaluate its efficacy relative to biological treatments. The product was sourced from the Ministry of Agriculture, Egypt. Before transplanting, onion seedlings were treated by immersion in a solution containing 25 mL of Folicure per liter of water for 5 min. Foliar applications were subsequently administered at 6 and 12 weeks post-transplantation, using a concentration of 187.5 mL per 100 L of water.

7. *InVitro* Antagonistic Activity of Biological Isolates

The dual culture technique described by Haveri (2017) was employed to assess the antagonistic potential of *T. harzianum* against *S. cepivorum*. Petri dishes containing PDA medium were inoculated with mycelial discs (5 mm in diameter) from both the antagonist and the pathogen, positioned 7 cm apart and 1 cm from the edge of the plate. Observations were recorded daily until either the two fungal colonies met or one overgrew the other.

The experiments were conducted in triplicate. The percentage of mycelial growth inhibition (I) was calculated using the following formula:

$$\mathbf{I\ (\%) = [(C - T) / C] \times 100}$$

where C represents the pathogen's radial growth in the control plate and T denotes the radial growth in the presence of the antagonist.

A loopful of each bacterial suspension was streaked in a straight line along the margin of PDA plates to evaluate the antagonistic activity of bacterial isolates against *S. cepivorum*. The pathogen's active mycelium was then placed on the opposite side of the plate. Plates were incubated at 27 °C (Hernández, *et al*., 2011) for 7days, after which the percentage inhibition of pathogen growth was determined following the method of El-sharkawy and El-Khateeb (2019).

8. Greenhouse Experiment

- Effect of Different Treatments on Disease Severity and Treatment Efficiency under Greenhouse Conditions

A pot experiment was conducted at the Faculty of Agriculture, Ain Shams University under greenhouse conditions. Plastic pots (30 cm in diameter) were filled with 1:1 mixture of clay and sand (v/v). Soil was artificially infested with *S. cepivorum* inoculum at a concentration of 2% (w/w) seven days before transplantation.

Five pots were allocated to each treatment, and five 60-day-old onion seedlings (cv. Giza red) were transplanted per pot. The experimental design included 23 treatments representing different combinations of vermicompost, vermicompost tea, effective microorganisms (EM1), and antagonistic microbes, including *T. harzianum*, *P. fluorescens*, *S. marcescens*, *B. thuringiensis*, and *B. subtilis* (Table 1).

Table 1. Greenhouse experimental design of combinations treatments

Treatments	Combination application
T1	Vermicompost
T2	Vermicompost + *T. harzianum*
T3	Vermicompost + *P. fluorescens*
T4	Vermicompost + *B. thuringiensis*
T5	Vermicompost + *B. subtilis*
T6	Vermicompost + *S. marcescens*
T7	Vermicompost + EM1
T8	Vermicompost tea
T9	Vermicompost tea + *T. harzianum*
T10	Vermicompost tea + *P. fluorescens*
T11	Vermicompost tea + *B. thuringiensis*
T12	Vermicompost tea + *B. subtilis*
T13	Vermicompost tea + *S. marcescens*
T14	Vermicompost tea + EM1
T15: T20	Individual applications of *T. harzianum*, *P. fluorescens*, *B. thuringiensis*, *B. subtilis*, *S. marcescens*, and EM1
CC	Chemical control with Folicure 25% EC
NC	Negative control (sterilized, non-infested soil)
PC	Positive control (infested soil without treatment)

Two application methods were tested using a handheld sprayer: soil drenching and foliar spraying (Amin, *et al*., 2016a). Water-only spraying served as a control for foliar application. Treatments were administered three times at 2-week intervals starting after transplantation.

Disease severity was assessed at the end of the season, and growth parameters, including plant height, leaf number, bulb diameter, and bulb weight, were recorded. Disease severity was evaluated according to the scale proposed by Cimen *et al. (*2010), with the following ratings:

0 = Healthy plant

1 = Bulb covered with fungal mycelium without decay

2 = 1%–25% of rotted bulb

3 = 26%–50% of rotted bulb

4 = 51%–75% of rotted bulb

5 = 76%–100% of rotted bulb

Disease severity scores were converted to percentage values using the following formula:

$$\textbf{Disease severity (\%)} = \frac{\sum \text{Class rating x class frequency)}}{\text{total number of samples} \times \text{highest rating}} \times 100$$

The percentage of treatment efficiency in reducing disease severity was calculated as follows:

$$\text{Efficiency (\%)} = \frac{A - B}{A} \times 100$$

Where

A = Disease severity (%) in the control group (infested soil without treatment)

B = Disease severity (%) in treated plants

- Effect of Different Treatments on Bulb Diameter and Percentage Increase under Greenhouse Conditions

Bulb diameter (mm) was measured at harvest by a digital caliper. The percentage increase in bulb diameter was calculated relative to the untreated control (infested soil without treatment) using the following equation:

$$\text{Percentage increase (\%)} = \frac{D_c - D_t}{D_c} \times 100$$

Where

$\mathbf{D_t}$= Bulb diameter of the treated plants

$\mathbf{D_c}$= Bulb diameter of control plants (*S. cepivorum*-infested soil)

9. Field Experiment

- Effect of Different Treatments on Disease Severity and Control Efficiency Under Field Conditions

Field experiments were conducted during the 2023/2024 growing season in a naturally *Sclerotium cepivorum*-infested field located at Al-Saf, Giza Governorate, Egypt, to evaluate the efficacy of different treatments in controlling onion WRD. The experiment included seven biological treatments consisting of vermicompost along with various EMs and antagonistic microorganisms. In addition, a chemical fungicide treatment and untreated control were included for comparison (Table 2). The biological treatments integrated vermicompost with *T. harzianum, P. fluorescens, B. thuringiensis, B. subtilis, S. marcescens*, and EM1. Chemical treatment involved the application of Folicur 25% EC. Sixty-day-old onion seedlings (cv. Giza Red) were transplanted into plots arranged in a randomized complete block design with four replicates per treatment. Each plot measured 10.5 m² (3.0 × 3.5 m).

Disease severity (%) was assessed at the end of the season, and treatment efficiency in reducing disease severity was calculated using the previously described formula.

Table 2. Field experimental design of combinations treatments

Treatments	Combination application
T1	Vermicompost
T2	Vermicompost + *T. harzianum*
T3	Vermicompost + *P. fluorescens*
T4	Vermicompost + *B. thuringiensis*
T5	Vermicompost + *B. subtilis*
T6	Vermicompost + *S. marcescens*
T7	Vermicompost + EM1
CC	Chemical control with Folicure 25% EC
NC	Negative control (untreated)

- Effect of Different Treatments on Onion Vegetative Parameters Under Field Conditions

The vegetative performance of onion plants was evaluated at harvest by measuring the bulb weight (g), plant height (cm), and number of leaves per plant. In addition, the percentage increase of each parameter was calculated in comparison to the untreated control.

- Effect of Different Treatments on Total Soil Bacterial, Fungi, and Actinomycetes Counts Under Field Conditions

Microbial populations in the rhizosphere, including total bacteria, fungi, and actinomycetes, were assessed 2 weeks after treatment application using the standard dilution plate technique described by Jangid and Ojha (2025).

Different selective media were used for each microbial group:

- Soil extract agar for the total bacteria
- Potato dextrose agar supplemented with rose bengal for the treatment of fungi
- Oatmeal agar for actinomycetes

Serial dilutions of soil suspensions were plated, and the inoculated Petri dishes were incubated at 27 °C for the following durations:

- Bacteria 2 days
- Actinomycetes 3 days
- Fungi 4 days

After incubation, CFUs were enumerated using a digital colony counter, and results were expressed as CFU per gram of dry soil.

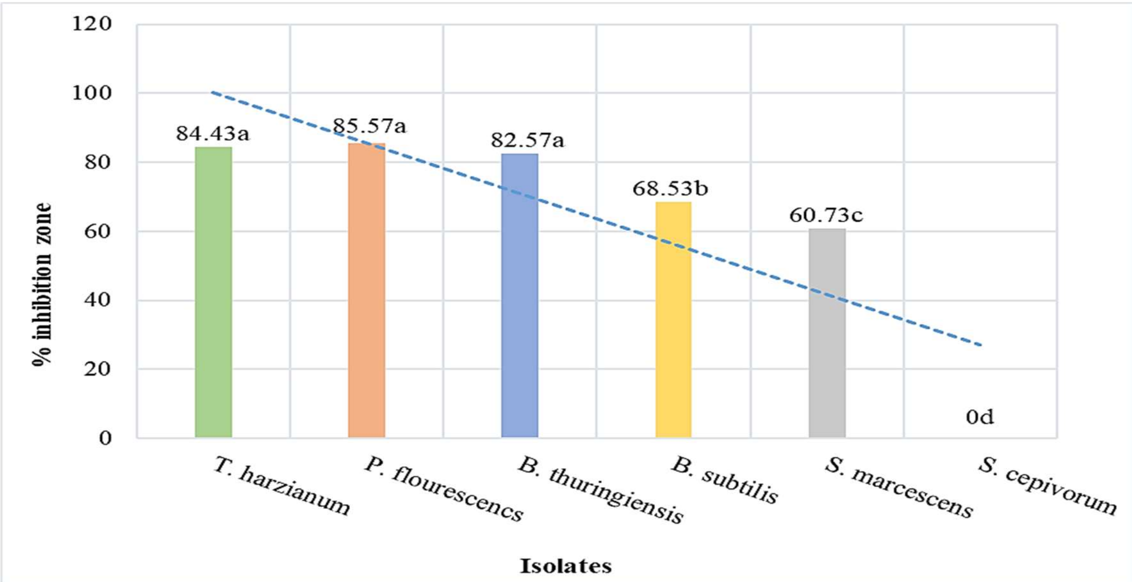
10. Statistical Analysis

All collected data were subjected to statistical analysis through analysis of variance using the general linear model procedure, as outlined in the SAS User's Guide (SAS Institute, 2012). Mean comparisons were performed using Duncan's multiple range test to determine significant differences among treatments at the 5% probability level (Duncan, 1955).

Results

1. *In Vitro* Antagonistic Activity of Biological Isolates:

The in vitro antagonistic activity of various biocontrol agents against *S. cepivorum* was evaluated by measuring the inhibition zone percentage (Figure 1). Among the tested agents, *P. fluorescens* demonstrated the highest antagonistic effect (85.57%), followed closely by *T. harzianum* (84.43%) and *B. thuringiensis* (82.57%). B. subtilis (68.53%) and S. marcescens (60.73%) showed moderate inhibitory effects. These results underscore the significant biocontrol potential of *P. fluorescens*, *T. harzianum*, and *B. thuringiensis* in suppressing white rot under *in vitro* conditions.



Data presented as the means of three replicates ± SD. Different letters refer to significant difference (P ≤ 0.05). L.S.D. at 0.05%

Fig 1. Percentage of mycelial growth inhibition of *S. cepivorum* by different antagonistic microorganisms under *in vitro* conditions.

2. Effect of Different In Vivo Treatments

- Greenhouse Experiment

- Effect of Different Treatments on Disease Severity and Treatment Efficiency under Greenhouse Conditions

The results in Table (3) present notable differences in disease severity and control efficacy among the evaluated treatments (foliar spraying and soil drenching). The combined application of effective microorganisms (EM1) with vermicompost demonstrated the highest efficacy, reducing disease severity to 15.70% and 17.40% under foliar application and soil drenching, respectively. These reductions corresponded to control efficiencies of 73.98% and 70.87%.

Comparable results were observed with *T. harzianum* and *P. fluorescens* when applied in conjunction with VC tea. Specifically, *T. harzianum* achieved control efficiencies of 72.28% (foliar) and 65.80% (soil), whereas *P. fluorescens* achieved control efficiencies of 71.07% and 62.11%, respectively. In contrast, the application of these biological agents without organic amendments resulted in markedly lower efficacy. *T. harzianum* alone yielded control efficiencies of 22.28% (foliar) and 37.77% (soil), whereas *P. fluorescens* alone achieved control efficiencies of 31.90% and 26.17%, respectively.

The positive control treatment (Folicure) also exhibited substantial effectiveness, with disease severities of 21.43% (foliar) and 24.70% (soil), corresponding to control efficiencies of 64.64% and 58.65%, respectively. Conversely, the negative control (infested soil without treatment) exhibited the highest disease severity (60.60% (foliar) and 59.73% (soil) and no disease suppression (0% efficiency), thereby confirming the pathogen's virulence and validating the experimental design.

Overall, treatments administered via foliar spraying consistently resulted in lower disease severity and higher control efficiency than those administered through soil drenching, indicating the superior performance of foliar application in disease management under the conditions tested.

- Effect of Different Treatments on Bulb Diameter and Percentage Increase under Greenhouse Conditions

Table (3) and Figure (2) present the effects of various treatments administered via soil drenching or foliar spraying on onion bulb diameter under pathogen-infested conditions. All treatments significantly improved the bulb diameter relative to the untreated infested control, which recorded the lowest values (8.97 mm for soil drenching and 11.93 mm for foliar spraying).

Soil Drenching Treatments: Among the soil-applied treatments, the combination of *B. thuringiensis* and vermicompost yielded the highest bulb diameter (29.67 mm), followed closely by *P. fluorescens* (29.20 mm) and EM1 (27.33 mm). These treatments corresponded to substantial increases over control (230.77%, 225.53%, and 204.68%), highlighting the synergistic effect of microbial inoculants and organic amendments in enhancing bulb development.

Foliar Spraying Treatments: Foliar spraying demonstrated even greater efficacy. The treatment comprises *T. harzianum* and vermicompost resulted in the highest bulb diameter (35.85 mm), followed by *B. thuringiensis* + vermicompost (35.26 mm) and P. fluorescens + vermicompost (33.70 mm). These treatments achieved increases of 200.50%, 195.56%, and 182.48% over the control, underscoring the potential of foliar bio-stimulants in promoting yield components.

Vermicompost Tea-Based Treatments involving vermicompost tea, either alone or in combination with biocontrol agents, resulted in moderate improvements. Bulb diameters ranged from 14.47 to 22.70 mm under soil drenching and from 26.72 to 31.97 mm under foliar spraying. Notably, *B. subtilis* + vermicompost tea recorded the highest values in both application methods (22.70 and 30.20 mm), representing increases of 153.07% and 153.14% over the control, respectively.

Biocontrol Agents Alone: The results were generally less pronounced when biocontrol agents were applied without organic amendments. However, *S. marcescens* alone still produced notable enhancements in bulb diameter (17.73 mm for soil drenching and 29.20 mm for foliar spraying), corresponding to increases of 97.66% and 144.76%, respectively.

Table 3. Heatmap illustrates significant differences of individual and combined treatments (t1:t2.... etc.as in table1) on white rot disease severity and bulb diameter (mm) & % increase of onion under greenhouse conditions

Treatments	%Efficiency Foliar spraying	%Efficiency Soil drenching	% Disease severity Foliar spraying	% Disease severity Soil drenching	bulb diameter (mm) Foliar spraying	bulb diameter (mm) Soil drenching	% bulb diameter Increase Foliar spraying	% bulb diameter Increase Soil drenching
T1	55.99	42.74	26.60	34.20	30.64	20.27	125.98	156.83
T2	61.27	46.04	23.47	32.23	35.85	23.83	165.66	200.50
T3	70.96	55.75	17.60	26.43	33.70	29.20	225.53	182.48
T4	66.17	47.21	20.50	31.53	35.26	29.67	230.77	195.56
T5	61.68	53.17	23.20	27.97	31.30	21.67	141.58	162.36
T6	66.29	47.72	20.43	31.23	32.47	24.67	175.03	172.17
T7	73.98	70.87	15.70	17.40	32.44	27.33	204.68	171.92
T8	58.37	53.24	25.23	27.93	30.43	14.47	61.32	155.07
T9	72.28	65.80	16.80	20.43	29.93	16.20	80.60	150.88
T10	71.07	62.11	17.53	22.63	29.80	16.47	83.61	149.79
T11	63.42	40.35	22.17	35.63	26.72	16.27	81.38	123.97
T12	65.63	45.87	20.83	32.33	30.20	22.70	153.07	153.14
T13	58.25	39.11	25.30	36.37	31.97	21.83	143.37	167.98
T14	41.70	30.35	35.33	41.60	29.37	22.43	150.06	146.19
T15	22.28	37.77	47.10	37.17	28.80	16.93	88.74	141.41
T16	31.90	26.17	41.27	44.10	25.67	16.83	87.63	115.17
T17	27.18	29.01	44.13	42.40	26.75	12.97	44.59	124.22
T18	37.01	32.14	38.17	40.53	27.47	11.00	22.63	130.26
T19	33.88	29.68	40.07	42.00	29.20	17.73	97.66	144.76
T20	40.99	35.99	36.30	38.23	25.57	12.73	41.92	114.33
CC*	64.64	58.65	21.43	24.70	25.77	10.63	18.51	116.01
NC*	100.00	100.00	0.00	0.00	27.27	17.20	91.75	128.58
PC*	0.00	0.00	60.60	59.73	11.93	8.97	0.00	0.00

* Gradients of frequency key on the right vary from 0% (red) to100% (blue). Different colors mean different significance

*CC: Chemical control with Folicure 25% EC; NC: Negative control (sterilized, non-infested soil); PC: Positive control (infested soil without treatment

Chemical Control and Sterilized Soil: The chemical fungicide Folicure exhibited limited efficacy, with bulb diameters of 10.63 mm (soil drenching) and 25.77 mm (foliar spraying), reflecting modest increases of 18.51% and 116.01% over the control, respectively. The autoclaved soil control yielded intermediate values (17.20 mm and 27.27 mm), partial suppression of pathogen activity due to soil sterilization, although still inferior to several bio-organic treatments.

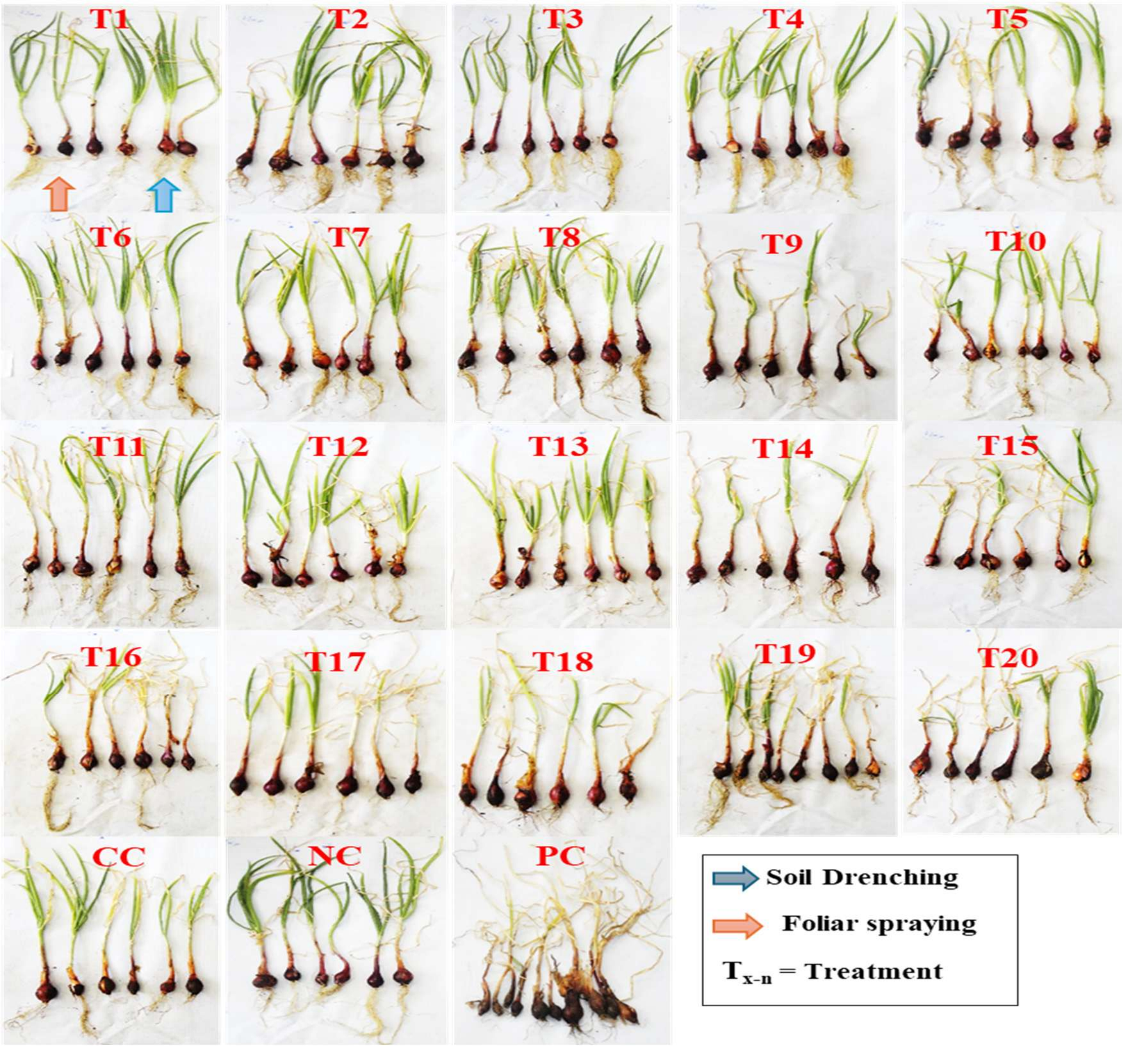


Fig 2. illustrations of Individual and Combined Treatments (T1:T2.... etc.as in Table1) on White Rot of Onion under Greenhouse Conditions.

In conclusion, the integration of microbial biocontrol agents with organic amendments—particularly via foliar application—proved to be the most effective strategy for enhancing onion bulb development under disease pressure. These findings reinforce the importance of adopting integrated, eco-friendly approaches to the management of soil-borne pathogens and the promotion of sustainable crop production (Figure 2).

- Field Experiment

- Effect of Different Treatments on Disease Severity and Control Efficiency Under Field Conditions

As presented in Table (4), all applied treatments under field conditions significantly reduced disease severity compared with the untreated control, which exhibited the highest severity (58.40%) and no control efficiency (0.00%). The most effective treatment was vermicompost combined with EM1, which resulted in the lowest disease severity (12.43%) and the highest control efficiency (78.72%).

Notably, treatments combining vermicompost with *T. harzianum* and *P. fluorescens* also demonstrated substantial efficacy, reducing disease severity to 13.33% and 14.27%, respectively, with corresponding efficiencies of 77.17% and 75.57%, respectively. Additional vermicompost-based treatments incorporating *B. thuringiensis*, *B. subtilis*, and *S. marcescens* achieved disease severity levels ranging from 16.57% to 18.50% and efficiencies ranging from 68.32% to 71.63%. Vermicompost alone reduced disease severity by 20.00%, with a control efficiency of 65.75%.

Table 4. Effect of vermicompost alone and in combination with various treatments on white rot disease severity of onion under field conditions.

Treatments	Disease Severity	%Efficiency
Vermicompost	20.00^{C}±0.28	65.75
+ *T. harzianum*	13.33^{GF}±0.20	77.17
+ *P. fluorescens*	14.27^{F}±0.0.14	75.57
+ *B. thuringiensis*	16.57^{E}±0.29	71.63
+ *B. subtilis*	18.50^{D}±0.28	68.32
+ S. *marcescens*	17.17^{E}±0.17	70.60
+ EM1	12.43^{G}±0.26	78.72
Folicure	27.50^{B}±0.28	52.91
Control(untreated)	58.40^{A}±0.60	0.00

Data presented as the means of three replicates ± SD. Different letters refer to significant difference ($P \leq 0.05$). L.S.D. at 0.05%

In contrast, the chemical fungicide Folicure exhibited moderate effectiveness, lowering disease severity to 27.50% and achieving a control efficiency of 52.91%, which was inferior to most bio-organic treatments evaluated. Figure (3) illustrates the relationship between greenhouse treatments and their impact on disease severity, as well as the alignment of these findings with the treatments implemented under field conditions. The data clearly indicates that the selected treatments were the most effective, demonstrating both statistically significant and positive outcomes compared with alternative options.

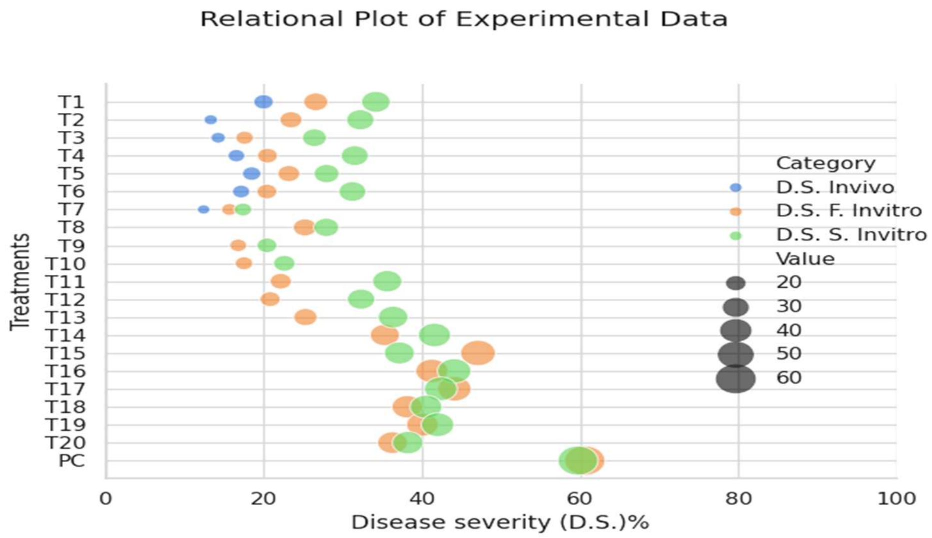


Fig 3. Relation between effect of vermicompost alone and in combination with various treatments on white rot disease severity of onion under field conditions (*in vivo*) and all treatments of greenhouse conditions (*in vitro*).

- Effect of Different Treatments on Onion Vegetative Parameters Under Field Conditions

Bulb Weight: As shown in Table (5) and Figure (4), all treatments significantly increased bulb weight (77.50 g) compared with the untreated control. The most substantial improvement was observed with the combination of vermicompost and EM1, which produced the highest bulb weight (255.33 g), representing a 229.5% increase over the control. Vermicompost + *T. harzianum* (206.00 g; 165.8%) followed by vermicompost + *P. fluorescens* (172.60 g; 122.7%). Moderate enhancements were recorded with combinations involving *S. marcescens* (125.50 g; 61.9%), *B. thuringiensis* (124.20 g; 60.1%), and *B. subtilis* (121.33 g; 56.4%). Vermicompost alone increased the bulb weight to 111.60 g, corresponding to a 44.3% increase. In contrast, the chemical fungicide Folicure resulted in a relatively modest increase (92.36 g; 19.1%), indicating that bio-organic treatments, especially those enriched with beneficial microbes, were more effective in promoting bulb development under field conditions.

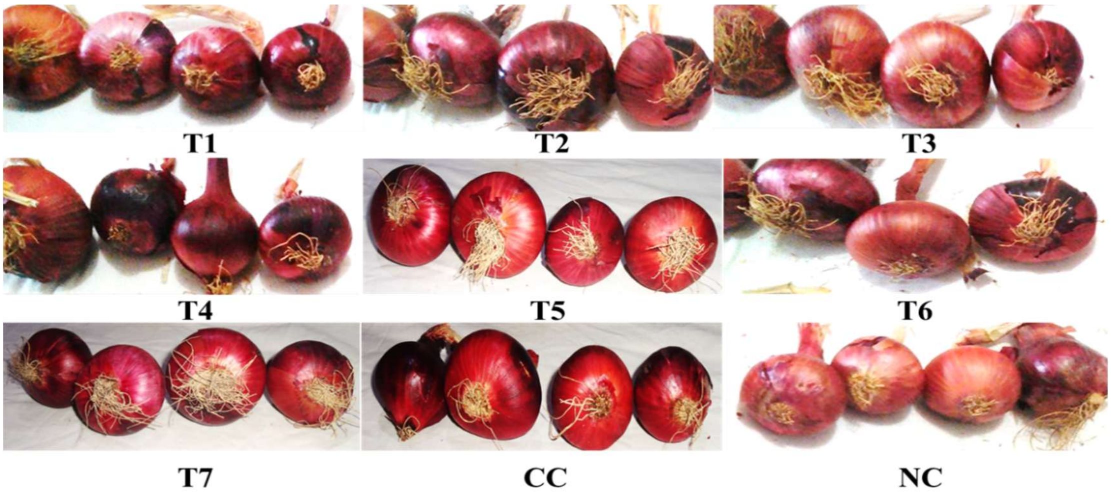


Fig 4. Effect of Individual and Combined Treatments on White Rot of Onion under Field Conditions

Plant Height: Table (5) reveals that all treatments significantly enhanced plant height compared with the untreated control (13.43 cm), which served as the baseline (100%). The greatest increase was again observed with vermicompost + EM1, reaching 25.27 cm (188.72%). Treatments with vermicompost + *T. harzianum* (23.86 cm; 178.20%) and vermicompost + *P. fluorescens* (22.80 cm; 172.18%) also showed strong effects.

Other combinations, such as vermicompost + *B. thuringiensis* (22.76 cm; 171.43%) and vermicompost + *B. subtilis* (21.77 cm; 161.65%), contributed to notable increases in the number of patients. Vermicompost alone resulted in a plant height of 22.13 cm (165.41%). Folicure had the least impact (excluding the control), with a plant height of 21.27 cm (159.40%), further emphasizing the superior performance of bio-organic amendments.

Leaf Number: All treatments significantly increased leaf number compared with the control (6.24 leaves; 100%). The highest leaf count was recorded with vermicompost + *P. fluorescens*, reaching 13.73 leaves (227.57%). This was closely followed by vermicompost + EM1 (12.44 leaves; 204.98%) and vermicompost + *B. subtilis* (12.43 leaves; 204.49%). Vermicompost alone resulted in 11.44 leaves (188.21%), while combinations with *B. thuringiensis* and *S. marcescens* yielded moderate increases (194.35% and 194.85%, respectively). Folicure showed the least improvement among treatments, with 10.73 leaves (177.91%).

The results clearly demonstrate that vermicompost enriched with beneficial microbes, particularly *P. fluorescens*, EM1, and *T. harzianum*, was the most effective in enhancing bulb weight, plant height, and leaf number. These bio-organic treatments outperformed both vermicompost alone and the chemical fungicide Folicure, highlighting their integrated role in promoting plant health and vegetative growth under field conditions (Table 5).

Table 5. Effect of Vermicompost Alone and in Combination with Different Treatments on Bulb Weight, plant high (cm), leaf number and their %Increase in Onion under Field Conditions.

Treatments	Bulb weight (gm)	Increase %	plant high (cm)	Increase %	leaf number	Increase%
Vermicompost	111.60^{G}±0.30	44.3	22.13^{D}±0.18	165.41	11.44^{D}±0.29	188.21
+ T. harzianum	206.00^{B}±0.57	165.8	23.86^{B}±0.08	178.20	12.24^{CB}±0.17	204.98
+ P. fluorescens	172.60^{C}±0.63	122.7	22.80^{C}±0.15	172.18	13.73^{A}±0.29	227.57
+ B. thuringiensis	124.20^{E}±0.43	60.1	22.76^{C}±0.14	171.43	11.73^{CD}±0.14	194.35
+ B. subtilis	121.33^{F}±0.35	56.4	21.77^{DE}±0.14	161.65	12.43^{B}±0.29	204.49
+ S. marcescens	125.50^{D}±0.28	61.9	21.66^{DE}±0.17	160.90	11.71^{CD}±0.17	194.85
+ EM1	255.33^{A}±0.23	229.5	25.27^{A}±0.21	188.72	12.44^{B}±0.29	204.98
Folicure	92.36^{H}±0.23	19.1	21.27^{E}±0.17	159.40	10.73^{E}±0.14	177.91
control (Untreated)	77.50^{I}±0.28	0	13.43^{F}±0.29	100.00	6.24^{F}±0.23	100.00

Data presented as the means of three replicates ± SD. Different letters refer to significant difference (P ≤0.05). L.S.D. at 0.05%

-Effect of Different Treatments on Total Soil Bacterial Fungi, and Actinomycetes Counts Under Field Conditions

Table (6) and Figure (5) indicate significant differences in soil microbial populations across the various treatments.

Fungal Populations (×10^6 CFU/g): The highest fungal colony count was observed in the +EM1 treatment group (26.00), followed closely by +*T. harzianum* (25.33). In contrast, the lowest counts were recorded in the folicure treatment group (7.67) and the untreated control group (9.00).

Bacterial Populations (×10^5 CFU/g): +EM1 also resulted in the highest bacterial count (31.00), significantly exceeding all other treatments. Elevated counts were also noted in +*P. fluorescens* (29.35) and +B. subtilis (29.33) treatments. Folicure treatment showed the lowest bacterial population (13.00).

Actinomycetes Populations (×10^5 CFU/g): The highest actinomycetes count was associated with +EM1 (15.33), followed by +*T. harzianum* (15.00) and +*P. fluorescens* (14.00). The lowest counts were found in the folicure treatment (3.33) and the control (4.67).

Table 6. Effect of Different Treatments on Total Microbial Counts in Soil (Fungi, Bacteria, and Actinomycetes) under naturally infested soil with *S. cepivorum*.

Treatments	Total count (CFU)		
	colony of fungi ($10^6/g^{-1}$)	colony of Bacteria ($10^5/g^{-1}$)	colony of actinomycetes ($10^5/g^{-1}$)
Vermicompost	$16.67^{D}\pm0.88$	$25.33^{B}\pm0.88$	$10.00^{DC}\pm1.15$
+ *T. harzianum*	$25.33^{BA}\pm0.88$	$27.67^{BA}\pm1.45$	$15.00^{BA}\pm0.58$
+ *P. fluorescens*	$22.33^{BC}\pm1.45$	$29.35^{BA}\pm1.45$	$14.00^{BA}\pm1.15$
+*B. thuringiensis*	$21.00^{C}\pm1.15$	$27.33^{BA}\pm1.45$	$12.00^{BC}\pm1.15$
+ *B. subtilis*	$17.33^{D}\pm1.45$	$29.33^{BA}\pm1.76$	$10.67^{DC}\pm0.88$
+ *S. marcescens*	$22.00^{BC}\pm1.53$	$28.67^{BA}\pm1.45$	$10.33^{DC}\pm0.88$
+ EM1	$26.00^{A}\pm1.15$	$31.00^{A}\pm1.53$	$15.33^{A}\pm0.88$
Folicure	$7.67^{G}\pm1.20$	$13.00\ ^{H}\pm1.15$	$3.33^{J}\pm0.88$
Control (untreated)	$9.00^{G}\pm1.15$	$21.00^{G}\pm1.15$	$4.67^{IJ}\pm0.88$

Data presented as the means of three replicates ± SD. Different letters refer to significant difference ($P \leq 0.05$).

L.S.D. at 0.05%

Overall, the application of +EM1 consistently enhanced the populations of all three microbial groups, that exerts a strong stimulatory effect on soil microbial activity. Conversely, Folicure appeared to suppress microbial growth, as further supported by the data presented in Figure 5.

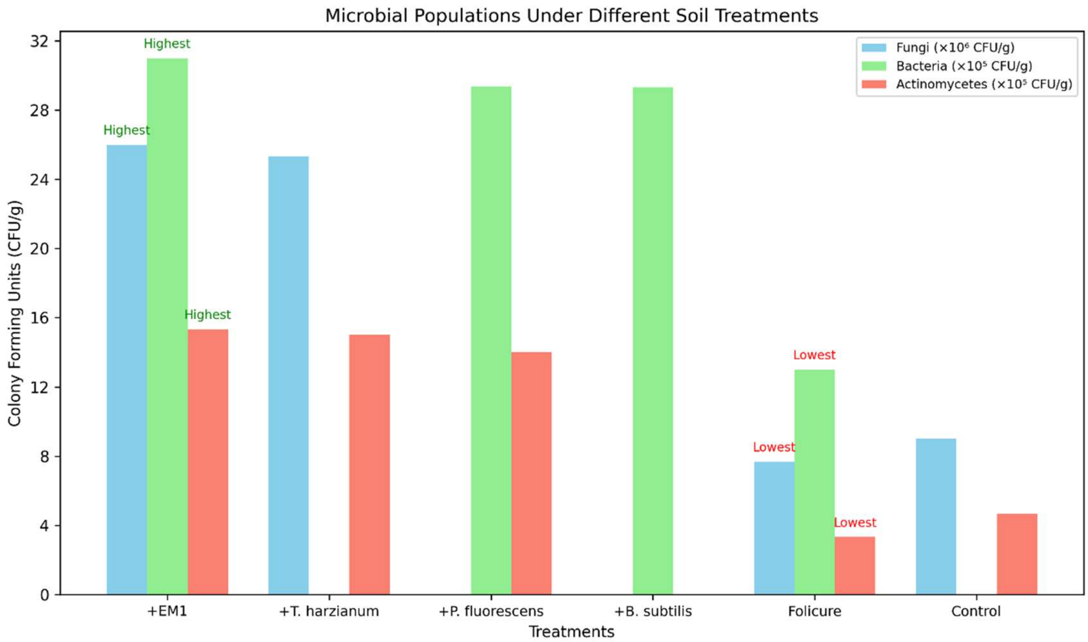


Fig 5. Microbial populations (fungi, bacteria, and actinomycetes) under various soil treatments. The chart highlights the highest and lowest counts for each microbial group.

Discussion

The present study demonstrates that integrated treatments involving biocontrol agents and vermicompost (VC), particularly under foliar application, offer a superior strategy for enhancing bulb development and mitigating the impact of *S. cepivorum* in onion

All tested treatments significantly reduced disease severity compared to the untreated, infested control. This finding aligns with Abolmaaty and Fawaz (2016), who reported that EM1 combined with vermicompost was more effective than individual treatments or chemical fungicides. Combinations such as VC + *T. harzianum* and VC + *P. fluorescens* also exhibited strong biocontrol activity, consistent with integrated disease management studies (Clarkson *et al*., 2006). Additional VC-based treatments involving *Bacillus thuringiensis*, *B. subtilis*, and *S. marcescens* yielded substantial reductions in disease severity, supporting literature that highlights VC's role in enhancing microbial antagonism and soil health (Bisen *et al*., 2023).

Notably, VC alone demonstrated meaningful efficacy, underscoring its intrinsic disease-suppressive properties. This is consistent with field trials showing that onion waste compost significantly reduces sclerotia viability and disease incidence. While the chemical fungicide Folicure showed moderate efficacy, it did not match the suppression achieved by biological and organic combinations. These results support previous findings that integrated organic strategies may outperform chemical controls in disease suppression and crop health (Li *et al*., 2019; Abou El-Yazied *et al*., 2020; Amin and Ahmed, 2023).

Both soil drenching and foliar spraying significantly improved bulb diameter compared to the untreated control. Combined applications, especially those integrating microbial biocontrol agents with organic amendments, consistently outperformed individual treatments, suggesting a synergistic interaction that enhances plant health and soil biological activity.

Under soil drenching, the combination of *B. thuringiensis* and VC achieved the greatest increase in bulb diameter, followed by *P. fluorescens* and EM1. These microbes are known for their plant growth-promoting (PGP) traits, including phytohormone secretion, nutrient solubilization, and pathogen antagonism (El-Nwehy *et al*., 2023). The enhanced bulb development likely results from improved root colonization and increased nutrient availability due to microbial activity and organic matter decomposition.

Foliar applications showed even greater potential in stimulating bulb enlargement. The highest value was recorded with *T. harzianum* + VC, indicating that foliar biostimulants can trigger systemic responses that benefit overall plant development. *T. harzianum* is known to induce systemic resistance and improve plant vigor through enzyme and metabolite production (Joshi *et al*., 2015). VC may also deliver humic substances and micronutrients directly to leaves, enhancing photosynthetic efficiency and metabolic activity (Arancon *et al*., 2004).

Treatments involving VC tea, particularly when combined with *B. subtilis*, showed moderate but meaningful increases in bulb diameter across both application methods. This is consistent with Yatoo *et al*. (2021), who reported that VC extracts suppress pathogens and stimulate growth via bioactive compounds. *B. subtilis* contributes through its biocontrol and PGP traits, including surfactin and antimicrobial peptide production (Bisen *et al*., 2023).

Although single applications of microbial agents such as *S. marcescens* were less effective than combinations, they still resulted in significant improvements over control. This highlights the intrinsic value of beneficial microbes in promoting plant performance, with their impact notably enhanced when combined with organic amendments (Zheng *et al*., 2013).

In contrast, Folicure resulted in the least improvement in bulb diameter, particularly under soil drenching. While chemical fungicides are effective in pathogen suppression, they lack the ability to promote plant growth or enhance soil health (Yatoo *et al*., 2021). Repeated use may also negatively affect beneficial soil microorganisms, limiting long-term sustainability.

The integration of VC with beneficial microbial agents significantly enhanced vegetative growth parameters, including plant height and leaf number, under pathogen stress. These findings align with previous studies that emphasize the synergistic effects of organic amendments and PGPMs in improving plant vigor under biotic stress conditions.

Conclusion

The data suggests that incorporating bio-organic amendments such as EM1 or beneficial microbes not only directly supports plant growth but also enhances the soil's microbiological health. This reinforces the use of microbial based formulations as sustainable alternatives to chemical inputs for long-term soil and crop productivity.

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المكافحة المستدامة لمرض العفن الأبيض في البصل عبر التربة المعدلة بسماد الفيرمي والعوامل الحيوية الميكروبية: تأثيرات على نمو النبات وميكروبيوم منطقة الجذور

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قسم أمراض النبات، كلية الزراعة، جامعة عين شمس، ص.ب 68، حدائق شبرا 11241، القاهرة، مصر.

الملخص

مرض العفن الأبيض، الذي يسببه الفطر *Stromatinia cepivora* (*Sclerotium cepivorum*) ، يمثل تحدياً كبيراً لإنتاج البصل (*Allium cepa* L.) على مستوى العالم. تقيّم هذه الدراسة فعالية مجموعة متنوعة من المعالجات الحيوية العضوية، بما في ذلك سماد الفيرمي (vermicompost) وشاي الفيرمي (vermicompost tea) والميكروبات النافعة (*Pseudomonas fluorescens* و Trichoderma *harzianum* و*Bacillus thuringiensis*) التي تم تطبيقها بشكل فردي ومجتمعة في ظروف المختبر (*in vitro*) والصوبة (greenhouse) والحقل (*in vivo*). أظهرت الفحوصات في المعمل نشاطاً قوياً مضاداً للعوامل الميكروبية، مع معدلات تثبيط بلغت 85.57 و84.43 و82.57% على التوالي. تحت ظروف الصوبة، حقق المزيج المكون من سماد الفيرمي والكائنات الدقيقة الفعالة (EM1) أعلى مستوى من قمع المرض، حيث خفض شدته إلى 15.70% عبر الرش الورقي و17.40% عبر الغمر في التربة، مع كفاءة مكافحة مقابلة بلغت 73.98، و70.87%. كما أدت هذه المعالجات إلى زيادة كبيرة في قطر البصله (32.44 و27.33 مم). أكدت التجارب الحقلية هذه النتائج، حيث أدى المزيج نفسه إلى خفض شدة المرض إلى 12.43% وتحقيق كفاءة مكافحة بلغت 78.72%. بالإضافة إلى ذلك، أظهرت النباتات المعالجة تحسناً في وزن البصله (255.33 جم)، وارتفاع النبات (25.27 سم)، وعدد الأوراق (12.44). كشف التحليل الميكروبيولوجي للتربة عن زيادة في أعداد الفطريات والبكتيريا والأكتينومايسيتات بعد المعالجات الحيوية العضوية، بينما أظهر مبيد الفطريات الكيميائيFolicure تأثيرات مثبطة على الميكروبات في التربة. قدمت المعالجات المعتمدة على شاي الفيرمي فوائد معتدلة، ولكنها كانت أقل فعالية من التركيبات الصلبة المعززة بالميكروبات. تسلط هذه النتائج الضوء على إمكانات التعديلات الحيوية العضوية المتكاملة، لاسيما سماد الفيرمي، بالاشتراك مع العوامل الميكروبية كبدائل مستدامة لمبيدات الفطريات الكيميائية لمكافحة مرض العفن الأبيض، وتعزيز نمو البصل، وتحسين صحة التربة.

الكلمات المفتاحية: *Allium cepa*، *المكافحة الحيوية*، *Sclerotium cepivorum*، *EM1* ، *سماد الفيرمي*.