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Enhancing Pepper Plant Resistance to Root Rot Disease by Encapsulating Roots with Biological and Chemical Inducers

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Abstract

Pepper (*Capsicum annuum*) is an important global crop but is highly vulnerable to root rot diseases caused by *Rhizoctonia solani*, *Fusarium solani*, and *Pythium debaryanum*, resulting in substantial yield losses. This study investigated the effectiveness of root encapsulation with biological and chemical inducers in controlling root rot and enhancing plant resistance. Conducted under glasshouse conditions, the experiment compared encapsulated and non-encapsulated root treatments. Encapsulated roots were coated with an alginate-based biopolymer containing *Trichoderma asperellum*, *Bacillus subtilis*, clove oil, ascorbic acid, or potassium sorbate, while non-encapsulated roots received direct treatments. Disease incidence, defense enzyme activities (peroxidase, polyphenol oxidase, and chitinase), and biochemical constituents (total phenolics) were evaluated. The findings revealed that root encapsulation significantly enhanced disease suppression, boosted defense enzyme activity, and increased key biochemical compounds. Among treatments, encapsulated clove oil application provided the highest disease resistance. These results highlight root encapsulation as a promising strategy for sustainable disease management and improved pepper cultivation.

Keywords: Pepper (*Capsicum annuum*) - Root rot disease - *Rhizoctonia solani* - Root encapsulation - Biological control - Chemical inducers - *Trichoderma asperellum* - *Bacillus subtilis* - Clove oil - Ascorbic acid - Potassium sorbate - Alginate-based biopolymer.

Introduction

Pepper (*Capsicum annuum*) is one of the most economically important vegetable crops worldwide, valued for its high nutritional content, culinary versatility, and commercial significance. However, pepper production faces serious challenges due to soil-borne fungal pathogens that cause root rot disease, particularly *Rhizoctonia solani*, *Fusarium solani*, and *Pythium debaryanum*. These pathogens invade the root system, leading to wilting, chlorosis, root necrosis, and ultimately plant death, resulting in substantial yield losses and economic damage to pepper growers **[1, 2].**

Conventional disease management strategies primarily rely on synthetic fungicides, which, despite their effectiveness, pose several environmental and agronomic concerns, including the development of fungicide-resistant pathogen strains, accumulation of toxic residues in soil and water, and disruption of beneficial soil microbiota **[3].** As a result, there is an increasing demand for sustainable and eco-friendly alternatives for controlling root rot diseases in pepper cultivation. One promising approach involves the use of biological control agents (BCAs) such as *Trichoderma asperellum* and *Bacillus subtilis*, which are known for their antagonistic activity against soil-borne pathogens through mechanisms such as competition, mycoparasitism, antibiosis, and induction of plant systemic resistance **[4, 5].** Additionally, biochemical inducers like clove oil, ascorbic acid, and potassium sorbate have shown potential in enhancing plant defense responses and reducing disease incidence through their antimicrobial and antioxidant properties **[6, 7].**

A major limitation in applying of BCAs and biochemical inducers is their reduced stability and persistence in the rhizosphere, which can limit their long-term effectiveness **[8].** Encapsulation technology has emerged as an innovative solution to enhance the efficacy, stability, and controlled release of these agents. Alginate-based encapsulation, in particular, provides a protective matrix that extends the viability of BCAs, shields them from environmental stressors, and ensures their gradual release in the root zone **[9].** Recent studies have demonstrated that encapsulated microbial and chemical treatments significantly improve disease suppression, plant growth, and crop yield compared to non-encapsulated applications **[10, 11].**

This study aims to evaluate the impact of root encapsulation with biological and chemical inducers on disease suppression, enzyme activity, biochemical responses, and overall yield in pepper plants infected with Rhizoctonia root rot under glasshouse conditions. Unlike previous studies that focused solely on the encapsulation of BCAs or chemical agents, this research investigates the effects of directly encapsulating the roots of pepper seedlings in an alginate-based matrix enriched with

biocontrol and biochemical inducers. By comparing encapsulated root treatments with non-encapsulated applications, the study provides new insights into the potential benefits of root coating technology as a sustainable and effective disease management strategy in pepper cultivation. Understanding the interaction between root encapsulation, plant defense responses, and pathogen suppression is crucial for optimizing the application of biocontrol-based formulations and promoting environmentally friendly agricultural practices.

MATERIALS AND METHODS

Survey and Collection of Infected Pepper Plants

A field survey was conducted during the 2021 and 2022 growing seasons to assess the prevalence of root rot disease in pepper (Capsicum annuum) plants. Diseased plants exhibiting typical symptoms of root rot, including wilting, yellowing, and root discoloration, were collected from five agricultural regions: Qalyubia (Moshtohor), Cairo (Alf-Maskan), Giza (Mansoria), Gharbiya (Mit-Ghazal), and Sharkiya (Kafr-Sakr). The collected samples were placed in sterile plastic bags, labeled with location details, and transported to the laboratory for further analysis.

Isolation, Purification, and Identification of Pepper Root Rot Pathogen and Antagonistic Agents

Root samples exhibiting symptoms of root rot disease were collected from five different governorates in Egypt were thoroughly washed, surface sterilized with 0.5% sodium hypochlorite for three minutes, rinsed with sterile distilled water, and dried on sterilized filter papers. Small sections of the roots were aseptically transferred onto potato dextrose agar (PDA) plates and incubated at 25±2°C for 3-5 days. Emerging fungal colonies were sub-cultured for purification following **[12, 13].** The Rhizoctonia solani isolates were identified morphologically and microscopically based on key characteristics, including right-angled branching hyphae and septation patterns, following **[14, 15, 16].** For the isolation of antagonistic microorganisms, Trichoderma asperellum and Bacillus subtilis were obtained from the rhizosphere of healthy pepper plants using the serial dilution method **[17].** Soil suspensions were plated on PDA (for fungi) and nutrient agar (NA) (for bacteria) and incubated at 25±2°C (fungi) and 30°C (bacteria) for 7 days and 24 hours, respectively. Colonies with characteristic Trichoderma and Bacillus morphologies were repeatedly sub-cultured to ensure purity. Identification was based on cultural and microscopic characteristics for *T. asperellum* **[18]** and biochemical tests such for *B. subtilis* **[19].**

Pathogenicity Tests and Disease Evaluation

To confirm the pathogenicity of the isolated fungi, a glasshouse experiment was conducted at Benha University. The fungal isolates were cultured on sterilized barley grains for 14 days at 25°C. The inoculum was mixed with sterilized soil at a concentration of 5% (w/w) to ensure adequate infection levels. Three-weeks-old healthy pepper seedlings (cv. Baladi) were transplanted into the infested soil. A positive control group was inoculated with pathogens but left untreated. Disease severity was assessed 45 days post-inoculation using a disease rating scale from 0 (healthy) to 4 (severely diseased) following **[20].**

Encapsulation Process

The encapsulation process of pepper seedling roots was performed using an alginate-based root coating technique to enhance disease resistance through the gradual release of tested biocontrol agents and chemical inducers. A 2.5% sodium alginate solution was prepared and mixed with one of the tested biological or chemical agents, including *T. asperellum* (1×10^7 spores/mL), B. subtilis (1×10^8 CFU/mL), clove oil (0.5%), ascorbic acid (1 g/L), potassium sorbate (10 g/L), and the fungicide Rizolex-T (50% tolclofos-methyl) at a concentration of 3 g/L. Pepper seedlings were immersed in the alginate solution containing the selected treatment and then transferred into a 50 mM calcium chloride solution for 30 minutes to form a stable gel matrix around the roots. After encapsulation, the coated seedlings were carefully removed, rinsed with sterile water to eliminate excess calcium chloride, and air-dried at room temperature before transplantation. For non-encapsulated treatments, freshly prepared microbial suspensions, natural and chemical inducers solutions of tested treatments were directly applied to the roots by immersion before planting. This method allowed for immediate interaction between the applied agents and plant roots but lacked the controlled release effect provided by encapsulation. The inclusion of Rizolex-T fungicide in both encapsulated and non-encapsulated treatments facilitated a direct comparison between conventional chemical control and bio-based treatments in managing Rhizoctonia root rot disease in pepper plants.

Experimental Design and Treatment Groups

A pot glasshouse experiment was conducted using a completely randomized block design (CRBD) to evaluate the impact of root encapsulation on Rhizoctonia root rot disease suppression, defense enzyme activities (peroxidase, polyphenol oxidase, and chitinase), and biochemical constituents (total phenolics) under artificial infection conditions. Treatments were divided into two main groups:

encapsulated root treatments and non-encapsulated root treatments. Encapsulation was performed using an alginate-based root coating technique to improve the persistence and bioavailability of the applied treatments. In contrast, non-encapsulated applications involved immersing plant roots in suspensions or solutions of the tested treatments, providing an immediate but potentially less sustained effect. Within each treatment group, applications were conducted either individually or in combination to assess different disease control strategies. *T. asperellum* was tested alone and in combination with B. subtilis, clove oil, ascorbic acid, or potassium sorbate to evaluate its biocontrol efficacy and potential synergistic interactions. Additionally, Rizolex-T fungicide was tested independently to compare its effectiveness with the tested treatments. Each treatment was replicated three times, with three plants per replicate, ensuring statistical reliability. To assess the impact of the treatments, infected control treatments were included for both encapsulated and non-encapsulated conditions. The encapsulated infected control consisted of plants whose roots were encapsulated in alginate without any biocontrol agents, natural compounds, or chemical inducers. The non-encapsulated infected control consisted of infected plants that received no treatment or encapsulation. These controls were essential for evaluating whether the encapsulation process itself had any direct effect on disease progression. The experimental design allowed for a comprehensive evaluation of Trichoderma treatments, both individually and in combination with B. subtilis, clove oil, ascorbic acid, or potassium sorbate. This provided insights into the benefits of root encapsulation, interactions between biocontrol agents (*T. asperellum* and *B. subtillis*), the tested natural compound (clove oil), and chemical inducers (ascorbic acid and potassium sorbate), as well as their overall effects on disease incidence, defense enzyme activities (peroxidase, polyphenol oxidase, and chitinase), and biochemical constituents (total phenolics). The inclusion of biological agents, a natural compound, and chemical inducers allowed for direct comparisons between conventional fungicide applications and novel disease control approaches, contributing to the development of sustainable disease management strategies in pepper cultivation.

Data Collection and Measurements

Disease Assessment

To evaluate the effectiveness of different treatments, disease severity (DS%), representing the intensity of infection within affected plants, was assessed using a modified scale described by **[21].** Disease severity was calculated for each treatment using the following formula **[22].**

Disease severity (DS%) = (∑ (P x Q)" ")/(M x N) ×" 100"; where: P = severity score assigned to each plant based on the modified scale, Q = number of infected plants corresponding to each severity score, M = total number of assessed plants in each treatment, N = maximum disease severity score (4 in this study). On the other hand, disease incidence (DI%), which quantifies the proportion of infected plants within the total population, was determined following the method **[23].** The disease severity percentage (DI%) was calculated using the formula:

Disease incidence (DI%) = "Number of infected plants " /"Total number of plants" ×" 100" ; where: Number of infected plants = plants exhibiting visible root rot symptoms based on the severity scale, Total number of assessed plants = all plants included in the experiment.

Biochemical Analysis

To assess plant defense responses, key enzymatic activities were investigated. Peroxidase (PO), polyphenol oxidase (PPO), and chitinase activities were measured using spectrophotometric **[24, 25].** Total phenolic content was determined using the Folin-Ciocalteu reagent **method [26].** To quantify the stimulatory effect of tested treatments, the efficacy percentage (Efficacy%) in tested treatments was calculated using the provided formula:

Efficiency percentage (Efficiency%) = (Treatment-Infected Control" ")/"Treatment" ×" 100"

Statistical Analysis

All collected data were subjected to one-way analysis of variance (ANOVA), followed by the Least Significant Difference (LSD) test at a 5% significance level to determine statistically significant differences between treatments following **[27].**

Results and discussion

Pathogenicity and Virulence Assessment of Fungal Isolates

The pathogenicity assessment of fungal isolates responsible for root rot in pepper (*Capsicum annuum*) plants (cv. Baladi) demonstrated significant variations in virulence levels among the tested isolates. This variability in disease incidence (DI%) and disease severity (DS%) reflects the differential ability of these fungal pathogens to establish infection and induce damage in pepper plants. The results in **Table 1** indicated that *Rhizoctonia solani* isolate RH3, obtained from Alf-Maskan (Cairo), was the most virulent, achieving a 100% disease incidence and an 80.3% disease severity score. This aligns with previous studies where *R. solani* has been

identified as a highly aggressive pathogen causing root and crown rot in various solanaceous crops **[28].** The second most virulent isolate, RH2 from Moshtohor (Qalyubia), also exhibited high pathogenicity with DI% and DS% values of 88.8% and 66.6%, respectively. These findings emphasize the capacity of *R. solani* to cause severe infections under conducive environmental conditions, particularly in moist, warm soils where it thrives **[29].** Among the *Fusarium solani* isolates, Fu4 from Mit-Ghazal (Gharbiya) was the most pathogenic, with DI% and DS% values of 88.8% and 69.4%, respectively. This isolate exhibited significantly higher disease severity than Fu1 (DI% 77.7%, DS% 33.2%) and Fu3 (DI% 77.7%, DS% 58.3%), suggesting that symptom intensity varies within the species. Such variability in *F. solani* virulence has been reported in prior studies, where genetic diversity among isolates has been linked to differences in pathogenic potential and toxin production **[30].** The ability of *F. solani* to produce cell-wall-degrading enzymes and mycotoxins contributes to its pathogenicity, which explains the high DS% observed in Fu4 compared to the other isolates **[31].** The lowest pathogenicity was observed in the *Pythium debaryanum* isolate (Py) from Kafr-Sakr (Sharkiya), which recorded DI% and DS% values of 22.2% and 11.1%, respectively. This significantly lower impact on disease progression suggests that *P. debaryanum* plays a secondary role in root rot complex diseases, often taking advantage of pre-existing plant stress or wounds rather than initiating severe infections on its own **[32].** The ability of Pythium species to infect plants under excessive soil moisture conditions is well documented, but their pathogenicity can be overshadowed by more aggressive pathogens such as Rhizoctonia and Fusarium **[33].** The control treatment, which was not inoculated with any fungal pathogen, remained free from disease symptoms (0.0% DI% and 0.0% DS%), reinforcing the validity of the pathogenicity assessment. This confirms that the disease symptoms observed in infected plants were directly attributable to fungal infection rather than environmental factors or pre-existing plant stress.

Control studies of *Rhizoctonia solani in vivo*

Impact of Encapsulated Root Treatments on Disease Suppression

The results in **Table 2** highlight the impact of encapsulated and non-encapsulated root treatments on Rhizoctonia root rot disease incidence in pepper plants under glasshouse conditions, showing significant variation among treatments. These findings emphasize the effectiveness of biological and chemical control measures, particularly when combined with encapsulation. Encapsulation appears to stabilize bioactive compounds, prolong microbial viability, and ensure sustained release, reinforcing its role in improving plant disease management strategies. *T. asperellum* alone reduced disease incidence from 22.22% in non-encapsulated roots to 11.11% upon encapsulation, supporting studies demonstrating its ability to suppress soil-borne pathogens through antagonism, enzyme production, and systemic resistance induction **[34, 4].** Encapsulation likely enhanced Trichoderma colonization in the rhizosphere, allowing for prolonged disease suppression. However, when *T. asperellum* was combined with B. subtilis or clove oil, disease incidence remained at 11.11% regardless of encapsulation, indicating that these combinations naturally offer strong disease suppression without requiring encapsulation for stability. This aligns with research suggesting that Trichoderma and *B. subtilis* act synergistically in enhancing plant defenses and competing against pathogens **[20, 5].** Similarly, antifungal properties of clove oil, attributed to eugenol, disrupt fungal cell membranes and inhibit pathogen growth **[6, 7]**, reinforcing its effectiveness regardless of formulation. The *T. asperellum* + ascorbic acid combination showed improved disease suppression upon encapsulation, reducing disease incidence from 22.22% to 11.11%, suggesting that ascorbic acid enhances Trichoderma activity under controlled-release conditions. This could be due to antioxidant properties ascorbic acid, which may protect microbial viability and boost plant resistance **[35].** In contrast, the *T. asperellum* + potassium sorbate combination showed no improvement upon encapsulation, with disease incidence remaining at 22.22%, suggesting that potassium sorbate does not complement Trichoderma-based biocontrol mechanisms **[11].** The fungicide Rhizolex-T significantly reduced disease incidence from 22.22% to 11.11% upon encapsulation, suggesting that encapsulation enhances its efficacy by prolonging availability in the root zone. This aligns with studies reporting systemic and contact fungicidal activity of Rhizolex-T against soil-borne pathogens **[36, 37].** The improved performance of encapsulated Rhizolex-T fungicide supports the potential of controlled-release formulations in optimizing fungicide efficiency while minimizing environmental risks associated with excessive application. The infected control recorded the highest disease incidence (100% in non-encapsulated and 88.88% in encapsulated plants), confirming the high susceptibility of untreated plants to Rhizoctonia infection.

Table (1) Pathogenicity assessment of *Rhizoctonia solani*, *Fusarium solani*, and *Pythium debaryanum* isolates in pepper plants:

Governorates	Locality	Isolated fungus	Code	DI %	DS %
Qalyubia	Moshtohor	*Fusarium solani*	Fu2	66.60	24.90
		Rhizoctonia solani	RH2	88.80	66.60
Cairo	Alf-Maskan	*Fusarium solani*	Fu3	77.70	58.30
		Rhizoctonia solani	RH3	100.00	80.30
Giza	Mansoria	*Fusarium solani*	Fu1	77.70	33.20
Gharbiya	Mit-Ghazal	*Fusarium solani*	Fu4	88.80	69.40
Sharkiya	Kafr-Sakr	*Pythium debaryanum*	Py	22.20	11.10
Control				**0.00**	**0.00**
LSD at 5%				**34.006**	**23.208**

DI%= Disease incidence percentage, **DS%**=%Disease severity percentage.

Table (2) Effect of encapsulated root treatments on Rhizoctonia root rot disease incidence percentage in pepper plants:

Treatments	Non-encapsulated Root	Encapsulated Root	Mean
Trichoderma asperellum	22.22	11.11	**16.67**
Trichoderma asperellum+B. subtilis	11.11	11.11	**11.11**
Trichoderma asperellum+Clove Oil	11.11	11.11	**11.11**
Trichoderma asperellum+Ascorbic Acid	22.22	11.11	**16.67**
Trichoderma asperellum+Potassium sorbate	22.22	22.22	**22.22**
Rhizolex-T	22.22	11.11	**16.67**
Control	**100.00**	**88.88**	**94.44**
L.S.D at 5% for: Treatments= 31.633, Root Encapsulation= 31.633, Interaction = 25.123			

The slight reduction in the encapsulated infected control suggests some passive suppression, possibly due to minor environmental factors or slow-release effects of encapsulation. The study highlights that encapsulation significantly enhances disease suppression in *T. asperellum*, Trichoderma + ascorbic acid, and Rhizolex-T fungicide treatments. However, the stability and effectiveness of *B. subtilis* and clove oil in both encapsulated and non-encapsulated forms indicate that these treatments do not require additional formulation adjustments. These findings align with research suggesting that biocontrol agents and plant-derived antifungal compounds exhibit sustained efficacy when applied in optimized combinations **[8].** Overall, the study reinforces the potential of encapsulated biological and chemical inducer treatments in sustainable disease management strategies. The superior performance of Trichoderma-based treatments, particularly in encapsulated formulations, highlights the benefits of controlled-release strategies in optimizing biocontrol efficiency. The encapsulation of Rhizolex-T fungicide demonstrates the potential for chemical fungicide optimization, suggesting that controlled-release formulations can reduce environmental impact while maintaining strong disease suppression **[9, 10].** These findings contribute to the ongoing development of integrated disease management strategies that balance biological control with chemical interventions, offering sustainable solutions for Rhizoctonia root rot management in pepper cultivation.

Impact of Encapsulated Root Treatments on Defense-related Enzymes

The findings in **Table 3** reveal significant variations in the activity of key defense-related enzymes; peroxidase (PO), polyphenol oxidase (PPO), and chitinase among different treatments, emphasizing the role of biocontrol agents and encapsulation in boosting plant defense mechanisms. Encapsulation generally led to higher enzyme activity compared to non-encapsulated treatments, which is likely due to the prolonged bioavailability of active compounds and beneficial microbes, ensuring sustained activation of plant immune responses. Previous studies have confirmed that sodium alginate encapsulation enhances microbial viability and allows for the controlled release of bioactive agents, thereby improving plant resistance to soil-borne pathogens [8]. The application of *T. asperellum* alone led to a moderate increase in PO (50% to 72.51%), PPO (14.86% to 17.88%), and chitinase (56.92% to 70.64%) activity upon encapsulation. This aligns with previous reports that Trichoderma spp. induce systemic resistance by upregulating oxidative stress-related enzymes and reinforcing plant cell walls

through chitinase activity **[4, 34].** However, the relatively modest changes in PPO and chitinase activity suggest that Trichoderma alone may not provide the strongest defense response, requiring additional biocontrol agents or chemical inducers for greater effectiveness. A significant enhancement in enzymatic activity was observed when *T. asperellum* was combined with B. subtilis, with PO increasing from 250% to 275.49%, PPO from 98.64% to 118.54%, and chitinase maintaining high activity (84.46% to 79.13%) upon encapsulation. These findings suggest a synergistic effect between *T. asperellum* and *B. subtilis*, leading to enhanced oxidative stress regulation and pathogen inhibition [20, 5]. The greater peroxidase activity in encapsulated treatments reinforces the hypothesis that controlled release formulations prolong the stimulation of plant defense enzymes, ensuring a more sustained resistance against *R. solani* infection. The highest levels of enzymatic activity were recorded in the treatment combining *T. asperellum* with clove oil, particularly in PO (257.69% to 342.38%) and PPO (100.67% to 188.74%) upon encapsulation, while chitinase levels remained stable (85.23% to 81.29%). The strong role of clove oil in boosting oxidative defense mechanisms is likely due to its antifungal properties and ability to trigger plant immune responses through bioactive compounds such as eugenol **[6, 7].** The substantial increase in peroxidase and PPO activity upon encapsulation suggests that the controlled release formulation maintains the bioavailability of clove oil, leading to a prolonged activation of plant defense pathways. When *T. asperellum* was combined with ascorbic acid, enzyme activity followed a distinct pattern, with PPO activity increasing significantly from 55.40% to 90.72%, while PO slightly declined from 208.46% to 199.33% upon encapsulation, and chitinase remained stable (74.15% to 73.38%). These findings suggest that ascorbic acid primarily enhances PPO-related pathways, likely by modulating redox balance and scavenging reactive oxygen species (ROS), which play a role in plant defense signaling **[35].** However, the absence of a strong increase in chitinase activity implies that ascorbic acid does not directly enhance fungal cell wall degradation, unlike clove oil or *B. subtilis* treatments. A moderate enhancement in enzyme activity was observed in the *T. asperellum* + potassium sorbate treatment, with PO increasing from 114.61% to 188.74% and PPO from 39.86% to 66.22%, while chitinase activity remained nearly unchanged (63.07% to 63.45%) upon encapsulation.

These results indicate that potassium sorbate plays a role in oxidative stress regulation, but its effect on chitinase activity is limited. The moderate increase in PO and PPO suggests that encapsulation improves potassium sorbate availability, allowing for a prolonged induction of plant defense enzymes. However, enzyme levels in this treatment remained lower than those in clove oil or *B. subtilis* combinations, suggesting a less pronounced role in stimulating plant immunity **[11].** The fungicide Rizolex-T resulted in notable increases in enzymatic activity upon encapsulation, with PO rising from 50% to 105.29%, PPO from 10.81% to 13.90%, and chitinase from 52.15 to 74.10. Although these values were lower than those recorded for biological treatments, they indicate that the fungicide contributes to pathogen suppression by enhancing plant defense responses. These findings align with previous studies demonstrating that fungicides can indirectly induce plant resistance by mitigating pathogen stress and reducing disease severity **[38].** The results demonstrate that encapsulation significantly enhances the induction of defense-related enzymes in most treatments, with the strongest effects observed in *T. asperellum* + clove oil, *T. asperellum* + *B. subtilis*, and *T. asperellum* + ascorbic acid treatments. The substantial increases in peroxidase and PPO activity in encapsulated treatments suggest that controlled release formulations lead to a more sustained activation of plant defense responses, supporting previous studies that highlight the importance of encapsulation in optimizing biocontrol efficiency **[8].**

Table (3) Effect of encapsulated root treatments on efficacy percentage of enzyme activities in pepper plants infected with Rhizoctonia root rot disease:

Treatments	**Non-encapsulated Root**			**Encapsulated Root**		
	PO	**PPO**	**Chitinase**	**PO**	**PPO**	**Chitinase**
Trichoderma asperellum	50.00	14.86	56.92	72.51	17.88	70.64
Trichoderma asperellum+B. subtilis	250.00	98.64	84.46	275.49	118.54	79.13
Trichoderma asperellum+Clove Oil	257.69	100.67	85.23	342.38	188.74	81.29
Trichoderma asperellum+Ascorbic Acid	208.46	55.40	74.15	199.33	90.72	73.38
Trichoderma asperellum+Potassium sorbate	114.61	39.86	63.07	188.74	66.22	63.45
Rizolex- T	50.00	10.81	52.15	105.29	13.90	74.10
Control	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

Impact of Encapsulated Root Treatments on Total Phenolics

The data in **Table 4** highlight the effects of encapsulated and non-encapsulated root treatments on total phenolics accumulation in pepper plants infected with Rhizoctonia root rot disease under glasshouse conditions. These biochemical compounds play essential roles in plant defense, with phenolics contributing to structural reinforcement and pathogen resistance. The results reveal a general trend where total phenolics are more strongly induced by specific treatments upon encapsulation. The application of *T. asperellum* alone led to moderate induction of total phenolics (39.61%) in non-encapsulated roots, whereas encapsulation slightly reduced total phenolics (37.56%). These findings align with previous studies reporting that *Trichoderma* spp. enhances antioxidant capacity, mineral uptake, and resistance to biotic stress **[39].** A notable increase in total phenolics was observed when *T. asperellum* was combined with B. subtilis, reaching 264.08% in non-encapsulated roots, though decreasing to 206.78% in encapsulated roots. This suggests that *B. subtilis* enhances phenolic biosynthesis and antioxidant activity, consistent with findings by **[40]**, who reported that *B. subtilis* significantly improved phenolics accumulation and disease resistance in pepper plants. The decline in total phenolics upon encapsulation suggests that slow-release mechanisms result in prolonged, rather than immediate, antioxidant responses. The highest total phenolics accumulation was recorded in *T. asperellum* + clove oil treatment, with values reaching 303.39% in non-encapsulated roots, though decreasing to 212.92% in encapsulated roots. This suggests that clove oil strongly stimulates phenolic biosynthesis, likely due to its antimicrobial properties and ability to trigger oxidative stress responses **[6, 7].** The decline in total phenolics upon encapsulation suggests that clove oil acts more effectively in free form, where its immediate release induces a stronger defense response. The combination of *T. asperellum* with ascorbic acid resulted in 200.98% total phenolics in non-encapsulated roots, whereas encapsulation significantly reduced these values to 124.20% and 31.15%, respectively. These results suggest that free ascorbic acid application provides an immediate antioxidant effect, whereas encapsulation alters its bioavailability, leading to a less pronounced increase in plant defense responses **[41].** The combination of *T. asperellum* with potassium sorbate exhibited moderate total phenolic accumulation (113.59%) in non-encapsulated roots, increasing slightly to 119.36% in encapsulated roots. These findings indicate that potassium sorbate plays a role in oxidative stress regulation but does not significantly enhance phenolic biosynthesis. A clear trend emerges, where total phenolic accumulation is strongly induced by Trichoderma-based treatments, particularly when combined with *B. subtilis* or clove oil upon encapsulation. These findings support the potential of Trichoderma-based biocontrol strategies in enhancing plant biochemical defenses, with encapsulation playing a key role in modulating response timing and intensity. While clove oil and *B. subtilis* combinations show strong potential in promoting phenolic accumulation. Future studies should investigate long-term effects on fruit quality, stress resilience, and disease suppression under field conditions, ensuring that biocontrol formulations maximize both plant health and crop nutritional value.

Table (4) Effect of encapsulated root treatments on efficacy percentage of total phenolic contents in pepper plants infected with Rhizoctonia root rot disease:

Treatments	Non-encapsulated Root	Encapsulated Root
Trichoderma asperellum	39.61	37.56
Trichoderma asperellum+B. subtilis	264.08	206.78
Trichoderma asperellum+Clove Oil	303.39	212.92
Trichoderma asperellum+Ascorbic Acid	200.98	124.20
Trichoderma asperellum+Potassium sorbate	113.59	119.36
Rizolex- T	28.22	30.53
Control	**0.00**	**0.00**

CONCLUSION

This study highlights the significant impact of root rot disease on pepper production in Egypt, identifying *Fusarium solani*, *Rhizoctonia solani*, *Fusarium oxysporum*, and *Pythium deparyanum* as the primary pathogens responsible for the disease. Among these, *R. solani* demonstrated the highest virulence, causing substantial damage to the crop. The results also confirm the potential of biological control agents, particularly *Trichoderma asperellum* as effective strategies for managing root rot. Additionally, essential oils, such as clove oil, proved to be highly effective in reducing disease severity when used in combination with *Trichoderma asperellum*. The encapsulation of these treatments further enhanced their efficacy, suggesting that this approach could provide a more sustainable and efficient solution for disease control. Biochemical analyses indicated that the treatments not only reduced disease symptoms but also stimulated the defense mechanisms of plants, as evidenced by the increased activity of enzymes such as peroxidase, polyphenol oxidase, and chitinase. Overall, the integration of *T. asperellum* and clove oil, especially in encapsulated form, offers a promising environmentally friendly alternative to conventional chemical fungicides, contributing to the development of more sustainable pest management practices for pepper cultivation.

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