Shahid Bahonar University of Kerman
Agricultural Biotechnology Journal
Iranian Biotechnology Society
Print ISSN: 2228-6705 Online ISSN: 2228-6500

Evaluation of the effectiveness of the *Trichoderma harzianum* and extract of clove (*Syzygium aromaticum*) against *Rhizoctonia solani* the causal agent of tomato root rot disease

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Abstract

Objective

Root rot is a serious disease of tomato (*Solanum lycopersicum* L.). It caused by the soil-borne fungus *Rhizoctonia solani*. This disease leads to major yield losses in tomato production worldwide. The aim of the present study was to isolate and identify the most virulent isolate of *R. solani*. Moreover, we aimed to evaluate the biocontrol ability of *Trichoderma harzianum* and aqueous clove (*Syzygium aromaticum*) extract. The aim was to evaluate both individually and in combination against tomato root rot under laboratory and greenhouse conditions.

Materials and methods

Several isolates of *R. solani* were obtained and tested for pathogenicity. Among them, isolate R4 showed the highest virulence. It has 100% disease incidence and 98.67% disease severity. So, it was selected for further experiments. Aqueous clove extract was prepared at concentrations of 5% and 10%. *Trichoderma harzianum* was cultured and applied as a spore suspension. Dual culture assays in vitro were used to evaluate the antifungal effects of these agents firstly. After that, greenhouse experiments were conducted to assess their effects on disease incidence, disease severity, and plant growth parameters. LSD test at a 5% significance level was statistically used to analyze data.

Results

In vitro results showed that both *T. harzianum* and clove extract significantly inhibited the growth of *R. solani*. Complete inhibition of fungal growth was observed when both agents were applied together. In greenhouse experiments, the combined treatment of *T. harzianum* and clove extract significantly reduced disease incidence (13.33%) and disease severity (9.33%), in comparison to the infected control. In addition, this treatment improved tomato plant growth. Because it resulted in higher fresh weight (43.33 g), dry weight (9.33 g), shoot length (46.33 cm), and root length (10.33 cm).

Conclusion

The findings of the current study shows that *Trichoderma harzianum* and clove extract work synergistically to control *Rhizoctonia solani*. This natural and eco-friendly method can effectively reduce tomato root rot. It also can increase plant growth. This combination shows strong potential as a safe alternative to chemical fungicides. Therefore, it is better to further evaluate it under field and laboratory conditions.

Keywords: clove extract, root rot, *Rhizoctonia solani*, tomato, *Trichoderma harzianum*

Paper Type: Research Paper.

Citation: Shamran, Z. T., Alwan, R. K., & Rdhaiw, G. A. (2026). Evaluation of the effectiveness of the *Trichoderma harzianum* and extract of clove (*Syzygium aromaticum*) against *Rhizoctonia solani* the causal agent of tomato root rot disease. *Agricultural Biotechnology Journal,* 18(2), 255-266.

Agricultural Biotechnology Journal, 18(2), 255-266. DOI: 10.22103/jab.2026.26628.1833

Received: Decembre 04, 2025. Received in revised form: January 23, 2026.

Accepted: January 24, 2026. Published online: February 28, 2026.

Publisher: Shahid Bahonar University of Kerman & Iranian Biotechnology Society.

Introduction

Tomatoes (*Solanum lycopersicum* L.) are significant nutritional and economic importance in addition of using it in the food industry. They contain important compounds such as lycopene, beta-carotene, vitamins (A, C, and E), flavonoids, and phenolic acids, making them vital for promoting human health and supporting global food security (Collins et al., 2022). Many tomato-producing countries suffer economic losses due to diseases, particularly fungal infections endemic

to their soils. They cause various diseases most notably root rot (Ptaszek et al., 2023). Undoubtedly, *Rhizoctonia solani* is one of the most soil-borne fungi. It affects tomato crops. It infects its root and stopped water and nutrient absorption. This result symptoms such as stunted growth, yellowing leaves and surly causing death for the plant (Arasu & Al-Dhabi, 2023; Meshram & Adhikari, 2024). Traditional control methods which always used chemical fungicides have been used. Although effective, these methods are harmful to the environment and, in addition to posing potential risks to human health, contribute to the production of pesticide-resistant pathogens (Bakkali et al., 2008; Liu et al., 2026). To avoid these problems, we must develop environmentally friendly alternative methods, such as biological control agents and plant extracts (Ongena and Jacques, 2008). *Trichoderma harzianum* is a well-established biocontrol fungus known for its multiple antagonistic mechanisms, including mycoparasitism, antibiosis, enzyme secretion (e.g., chitinases, glucanases), and competition for nutrients and space (Harman et al., 2004; Guzmán-Guzmán et al., 2019). On the other hand, clove (*Syzygium aromaticum*) extract, particularly rich in the bioactive compound eugenol, has shown strong antifungal properties against a broad spectrum of phytopathogens (Pitiwittayakul et al., 2025; Giménez-Santamarina et al., 2025). This study was designed to isolate and identify the most virulent *R. solani* isolate and evaluate the individual and combined effectiveness of *T. harzianum* and aqueous clove extract under in vitro and greenhouse (wooden shade) conditions. The study implemented two extract concentrations (5% and 10%) and applied statistical analysis to assess treatment efficacy.

Materials and methods

Isolation, identification and pathogenicity tests of Rhizoctonia solani: Tomato Root samples showing symptoms of root rot were collected from 5 fields in Babylon gouvernante**, Iraq**. Infected root tissues were cut into small segments (0.5-1 cm), surface-sterilized with 70% ethanol for 30 seconds, followed by immersion in 1% sodium hypochlorite for 2 minutes, and then rinsed three times with sterile distilled water. Sterilized segments were cultured on Potato Dextrose Agar (PDA) medium and incubated at $25 \pm 2°C$ for 5 days. The fungal colony's properties, the mycelium's nature and the structures it creates, and the accepted taxonomic keys were used to diagnose the fungus down to the genus and species level (Domsch & Gams, 1980)**.** Emerging fungal colonies were purified and sub-cultured. Pathogenicity tests were performed on healthy tomato seedlings to determine the most virulent isolate. The percentage of germination then computed using the following equation:

$$\text{Germination (\%)} = \frac{\text{No. germinated seeds}}{\text{Total number of seeds}} \times 100$$

Preparation of Aqueous Clove Extract (*Syzygium aromaticum*): The clove aqueous extract was prepared using the soaking method. Dried flower buds of *S. aromaticum* (100 g) were ground and soaked in 1 L of hot sterile distilled water (80°C) for 24 hours with intermittent stirring. The extract was filtered through sterile muslin cloth followed by Whatman No. 1 filter paper. The filtrate was stored in sterilized glass bottles at 4°C until use. Two concentrations (5% and 10%) were prepared and applied in all bioassays.

In vitro evaluation of the antifungal activity of *T. harzianum* and clove extract: The antagonistic effect of *Trichoderma harzianum* and clove extract against *R. solani* (isolate R4) was assessed using the dual culture technique on PDA medium. A 5 mm mycelial disc of *R. solani* was placed in the center of a Petri dish, while either *T. harzianum* or clove extract was applied 2.5 cm away from the pathogen. Plates were incubated at 25 ± 2°C for 5 days. Fungal colony diameters were measured and the inhibition percentage was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Colony diameter in control treatment - Colony diameter in treatment}}{\text{Colony diameter in control treatment}} \times 100$$

Evaluation under wooden shade (greenhouse conditions): Greenhouse trials were conducted using 25 cm plastic pots filled with soil artificially infested with *R. solani* (R4). Tomato seedlings aged 21 days were transplanted into the pots. Treatments were arranged in a completely randomized design (CRD) with three replicates per treatment. The treatments included: R4 = (R. *solani),* T = (*Trichoderma harzianum*), C = (Clove extract), Bel = (fungicide beltanol), and Con.= (control) and considered as following:

R4, T+R4, C+R4, T+C+R4, R4+Bel, T, C, T+C*,* Con.

After 45 days of treatment, plants were evaluated based on disease incidence (%), disease severity (%), fresh and dry plant weight (g), Shoot and root length (cm).

Statistical Analysis: All data were subjected to one-way analysis of variance (ANOVA) using a completely randomized design (CRD). Means were compared using the Least Significant Difference (LSD) test at a 5% level of significance ($P \leq 0.05$). Statistical analyses were performed using SPSS software version 25 or its equivalent.

Results and discussion

The results of the laboratory assay revealed clear antifungal activity of both the aqueous extract of clove (*Syzygium aromaticum*) and the biocontrol fungus *Trichoderma harzianum* in inhibiting the growth of *Rhizoctonia solani*, the primary causal agent of root rot disease in tomato plants. The most virulent isolate was selected from among 5 isolates of the pathogenic fungus by

testing the effect of the fungus on the germination rate of tomato seeds, where isolate R4 gave the lowest germination rate and complete death of the seeds (Table 1).

Table 1. comparison between isolates of *Rhizoctonia solani to* choose the most aggressive one

Isolations	Seeds Germination
R1	33.3
R2	16.3
R3	25.3
R4	0.00
R5	18.6
Control	96.6
L.S.D. (0.05)	8.674 *

*: P≤0.05

Efficacy of clove extract in inhibiting the pathogen: The results (Table 2) showed that the clove extract at 5% concentration reduced the colony diameter of the pathogen to 1 cm, with an inhibition rate of 88.8%, while the higher concentration (10%) completely inhibited fungal growth (100%), with statistically significant differences confirmed by LSD test at the 0.05 probability level ($P \leq 0.05$). This confirms the presence of eugenol compound and other active compounds in clove extract which have an inhibitory effect to R.solani (Giménez-Santamarina et al., 2025; Pitiwittayakul et al., 2025).

Table 2. Effect of the Clove Extract and Biocontrol Fungus on R. *solani* in Laboratory

Treatment	Concentration (%)	Fungal Colony Diameter (cm)	Pathogenic Fungus Inhibition (%)
Clove Extract (*Syzygium aromaticum)* + R4	5	1	88.8
	10	0	100
Trichoderma +R4		0	100
Control		9	0
L.S.D. (0.05)		2.074 *	9.437 *

*: P≤0.05

These findings suggest that clove extract can serve as a safe and effective botanical alternative to chemical fungicides in managing *R. solani*. This aligns with Bakkali et al. (2008), who emphasized the antifungal potential of plant extracts rich in phenolic compounds.

Efficacy of *Trichoderma harzianum* Against *R. solani:* The dual culture assay between *T. harzianum* and *R. solani* (*Trichoderma* + R4) resulted in complete inhibition of pathogen growth (100%), with no fungal colonies observed in the culture medium. This confirms the high antagonistic activity of T. *harzianum*, which operates through several mechanisms, including: secretion of hydrolytic enzymes such as chitinases and β-1,3-glucanases, competition for nutrients and space, and production of antifungal metabolites (Guzmán-Guzmán et al., 2019; Shabani et

al., 2024). These results are consistent with previous studies, including Yao et al. (2023), which demonstrated the ability of T. *harzianum* to suppress *Rhizoctonia* growth through direct mycoparasitism and antagonism. While in the control treatment the fungal colony diameter reached 9 cm with 0% inhibition, indicating the pathogen's normal growth in the absence of any suppressive agents. This serves as the baseline confirming the effectiveness of the tested treatments.

Results of wooden shade experiment: The results of the shade house experiment demonstrated a significant efficacy of both biological agent and clove extract treatments in mitigating the harmful effects of *Rhizoctonia solani* on tomato plants, compared to the infected control treatment (R4), as shown in Table 3. It recorded the highest disease incidence (100%) and severity (98.67%). This confirm that R. *solani* is the main causative fungi of root rot in tomato plants. It reflects the high pathogenic potential of R. *solani*, as previously reported by Nassary (2025) and Hussein et al. (2025). They reported 85% and 56% as infection and infection Severity, respectively.

Table 3. the effectiveness of treatments on rate and severity of infections under wooden shade conditions

Treatments	Infection (%)	Infection Severity (%)
R4	100	98.67
T+R4	40.00	28.00
C+R4	46.67	32.00
T+C+R4	13.33	9.33
R4+Bel	6.67	4.00
T	6.67	5.33
C	6.67	5.33
T+C	0.00	0.00
Con.	0.00	0.00
L.S.D. (0.05)	9.514 *	8.730 *

*: P≤0.05

Effectiveness of treatments in reducing disease incidence and severity: The combined treatment (T+C+R4) significantly reduced both disease incidence and severity, reaching 13.33% and 9.33%, respectively. These results are shown in Table 3 with statistically significant differences based on LSD test (P≤0.05). This indicates a synergistic interaction between the biological agent and the plant extract in suppressing disease development. The individual treatments (T+R4 and C+R4) also showed notable reductions and were 40% ,46% for infection and 28%, 32% for severity respectively. But the greatest effect was achieved when both were applied together. These findings are consistent with Christova et al. (2024), who reported that combining biocontrol agents with plant-derived compounds can enhance antifungal efficacy.

Shade house experiment: The combined treatment (T+C) recorded the highest values for root length (27.67 cm) and shoot length (45.83 cm), followed by R4+Bel and T+C+R4. In contrast, the infected control (R4) exhibited the shortest root (0.667 cm) and shoot (9.17 cm) lengths (Table 4). It indicates the damaging effect of the pathogen on plant development.

Table 4. The effect of treatments on shoot and root length of tomato plants under wooden shade conditions

Treatments	Shoot Length (cm)	Root Length (cm)
R4	9.17	0.667
T+R4	35.67	7.80
C+R4	39.67	7.20
T+C+R4	46.33	10.33
R4+Bel	46.33	11.33
T	41.67	18.00
C	39.50	19.00
T+C	45.83	27.67
Con.	30.67	5.17
L.S.D. (0.05)	8.263 *	6.449 *

*: P≤0.05

These results support the findings of Shabani et al. (2024), who explained that *T. harzianum* promotes plant growth by producing natural growth regulators and improving nutrient uptake, while the role of clove extract as Thabet and Khalifa (2018) findings which is prophylactic, which reflects positively with plant health and improved growth indicators. The (T+C) treatment also resulted in the highest fresh weight (71.00 g) and dry weight (14.13 g), followed by R4+Bel and T+C+R4. Conversely, the infected control (R4) showed the lowest values for fresh (1.23 g) and dry weight (0.233 g), as shown in Table 4. It highlights the severe root damage caused by the pathogen and its role in stunting plant growth. These findings underline the crucial role of biological and botanical treatments in alleviating physiological stress induced by fungal infection.

It is noteworthy that treatments such as T, C, and T+C resulted in plant growth and development metrics that surpassed even those of the healthy control, indicating not only a protective role against disease but also a stimulatory effect on plant vigor. This observation is in line with what recent reviews have reported, like that by Guzmán-Guzmán et al. (2019) and Arif et al. (2024). They noted the plant growth-promoting properties of *Trichoderma* spp. They briefly discussed the role of *Trichoderma* spp. as major growth promoters around hormonal regulation, particularly the auxin signaling pathway in plants as well as improving the physiological response of plants in reaction to harsh environmental conditions.

Table 4. The effect of treatments on fresh weight and dry weight in tomato plants under wooden shade conditions

Treatments	Fresh Weight (g)	Dry Weight(g)
R4	1.23	0.233
T+R4	27.67	6.27
C+R4	22.00	4.43
T+C+R4	43.33	9.33
R4+Bel	67.67	11.67
T	45.67	8.43
C	53.33	9.30
T+C	71.00	14.13
Con.	10.67	5.76
L.S.D. (0.05)	12.079 *	5.122 *

*: P≤0.05

Conclusion: This study shows that *Trichoderma harzianum* fungus and clove extract (*Syzygium aromaticum*) have a good inhibition effect against to fungus *Rhizoctonia solani*. It proves their ability to be as a part of integrated disease management with sustainability since they are considered safe alternatives to chemical pesticides. While the effectiveness of the plant extract alone was dependent on the concentrations used, the fungus used as bio-agent completely inhibited the pathogen. We therefore, infer that when used together these agents will cause an even higher effect on controlling root rot in tomatoes for further researches. Our findings provide that biocontrol agent either individually or together can control this pathogen infection on tomato plants. There was also observed high efficacy when used in combination with significant improvement all parameters measured. We therefore suggest further field researches towards improving the efficacy of this agent and develop it as biopesticide for field use.

Authors' contributions

Z.T.S. contributed to the study methodology and experimental design. G.A.R. performed data collection and statistical analysis. R.K.A. and Z.T.S. drafted the original manuscript. All authors reviewed, discussed, and approved the final version of the manuscript.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgment

The authors would like to thank the Department of Plant Production / Al-Mussaib Technical Institute, Al-Furat Al-Awsat Technical University

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethical Approval

Not applicable.

Conflict of Interest

All authors declare that they have no competing interests or financial conflicts of interest related to this study.

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مجله بیوتکنولوژی کشاورزی
شاپا چاپی: ۶۷۰۵-۲۲۲۸ شاپا الکترونیکی: ۶۵۰۰-۲۲۲۸

ارزیابی اثربخشی *Trichoderma harzianum* و عصاره میخک (*Syzygium aromaticum*) علیه *Rhizoctonia solani* عامل بیماری پوسیدگی ریشه گوجه‌فرنگی

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چکیده

هدف: پوسیدگی ریشه یکی از بیماری‌های مهم و خسارت‌زای گوجه‌فرنگی (*Solanum lycopersicum* L.) است که توسط قارچ خاکزاد *Rhizoctonia solani* ایجاد می‌شود و منجر به کاهش شدید عملکرد گوجه‌فرنگی در سراسر جهان می‌گردد. هدف از این مطالعه، جداسازی و شناسایی مهاجم‌ترین ایزوله *R. solani* و همچنین ارزیابی توان کنترل زیستی *Trichoderma harzianum* و عصاره آبی میخک(*Syzygium aromaticum*) بود. این عوامل به‌صورت جداگانه و تلفیقی، در شرایط آزمایشگاهی و گلخانه‌ای علیه پوسیدگی ریشه گوجه‌فرنگی بررسی شدند.

مواد و روش‌ها: چندین ایزوله از *R. solani* جداسازی و از نظر بیماری‌زایی مورد ارزیابی قرار گرفتند. در میان آن‌ها، ایزوله R4 بیشترین حدت بیماری‌زایی را نشان داد، به‌طوری که میزان بروز بیماری ۱۰۰٪ و شدت بیماری ۹۸/۶۷٪ بود. ازاین‌رو، برای آزمایش‌های بعدی انتخاب شد. عصاره آبی میخک در غلظت‌های ۵٪ و ۱۰٪ تهیه گردید. قارچ *Trichoderma harzianum* کشت داده شده و به‌صورت سوسپانسیون اسپور مورد استفاده قرار گرفت. ابتدا اثرات ضدقارچی این عوامل با استفاده از آزمون کشت دوگانه در شرایط درون‌شیشه‌ای (in vitro) بررسی شد. سپس آزمایش‌های گلخانه‌ای به‌منظور ارزیابی تأثیر آن‌ها بر میزان بروز

بیماری، شدت بیماری و شاخص‌های رشد گیاه انجام گرفت. داده‌ها با استفاده از آزمون LSD در سطح معنی‌داری ۵٪ تجزیه و تحلیل آماری شدند.

نتایج: نتایج آزمایشگاهی نشان داد که هر دو عامل *T. harzianum* و عصاره میخک به‌طور معنی‌داری رشد *R. solani* را مهار کردند. در حالتی که هر دو عامل به‌صورت هم‌زمان به‌کار رفتند، مهار کامل رشد قارچ مشاهده شد. در آزمایش‌های گلخانه‌ای، تیمار تلفیقی *T. harzianum* و عصاره میخک به‌طور معنی‌داری میزان بروز بیماری (۱۳/۳۳٪) و شدت بیماری (۹/۳۳٪) را در مقایسه با شاهد آلوده کاهش داد. علاوه بر این، این تیمار موجب بهبود رشد گیاه گوجه‌فرنگی شد، به‌طوری که بیشترین وزن تر (۴۳/۳۳ گرم)، وزن خشک (۹/۳۳ گرم)، طول ساقه (۴۶/۳۳ سانتی‌متر) و طول ریشه (۱۰/۳۳ سانتی‌متر) به‌دست آمد.

نتیجه‌گیری: یافته‌های این مطالعه نشان می‌دهد که *Trichoderma harzianum* و عصاره میخک به‌صورت هم‌افزا در کنترل *Rhizoctonia solani* عمل می‌کنند. این روش طبیعی و سازگار با محیط زیست می‌تواند به‌طور مؤثری پوسیدگی ریشه گوجه‌فرنگی را کاهش داده و رشد گیاه را افزایش دهد. این ترکیب پتانسیل بالایی به‌عنوان جایگزینی ایمن برای قارچ‌کش‌های شیمیایی دارد. بنابراین، انجام مطالعات تکمیلی در شرایط مزرعه‌ای و آزمایشگاهی توصیه می‌شود.

کلمات کلیدی: پوسیدگی ریشه، گوجه‌فرنگی، عصاره میخک، *Rhizoctonia solani*، *Trichoderma harzianum*

نوع مقاله: پژوهشی

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Publisher: Shahid Bahonar University of Kerman & Iranian Biotechnology Society.