Research Article

Preparation of Chitosan Microgels Reinforced with Chemical and Biological Inhibitors for Controlling *Rhizoctonia solani* the Causal Agent of Pepper Root Rot

Sara H. Aftean and Awf A. Ahmed Al-Jbory*

Department of Plant Protection, College of Agriculture, University of Tikrit, Iraq.

Abstract | Given the worsening problem of soilborne pathogens and the difficulty of combating them through the rapid disappearance of environmentally friendly pesticides,The experiments were conducted in the laboratories of the Plant Protection Department, College of Agriculture, University of Tikrit, during the academic year 2024-2025. The initial laboratory work involved isolating some pathogenic agents from the roots of pepper plants, represented by the following isolates*: Rhizoctonia solani, Alternaria sp., Fusarium solani, Macrophomina phaseolina*, and *Fusarium oxysporum*, which were identified morphologically. The pathogenicity of these isolates was then tested on pepper seeds in Petri dishes. The isolate *R. solani* showed the lowest germination rate of 6.66% compared to 76.66%, 63.33%, 10%, and 50% for the other isolates, respectively. The fungicide Moncut significantly outperformed the fungicides Topsin and Beltanol, as the radial growth of the most pathogenic isolate *R. solani* reached 1.83 and 0 cm^2 at concentrations of 0.025% and 0.50%, respectively. The element boron also significantly outperformed organic copper and zinc, as the radial growth of *R. solani* reached 3.83 and 2 cm^2 at concentrations of 0.025% and 0.50%, respectively. Chitosan microgels were prepared with particle sizes ranging from 10 to 50 microns. Different concentrations of Chitosan and Chitosan microgels showed effects on the radial growth of *R. solani*, where Chitosan and Chitosan microgels achieved the lowest radial growth of the isolate, reaching 4.16 cm^2 and 2.83 cm^2, respectively, at 100%, and 0.08 $cm^2$for both at the same concentration, compared to the control treatment which recorded an inhibition percentage of 9 cm^2.When Chitosan microgels were reinforced with boron, bacterial filtrate (*P. fluorescens*), and the fungicide Moncut, the treatment Moncut + Chitosan microgels significantly outperformed the other treatments, achieving a radial growth of *R. solani* of 0 cm^2 compared to 1.83 cm^2 and 2.41 cm^2 for boron and bacterial filtrate reinforced with Chitosan microgels, respectively.

Received | June 22, 2025; **Accepted** | August 08, 2025; **Published** | November 10, 2025
***Correspondence** | Awf A. Ahmed Al-Jbory, Department of Plant Protection, College of Agriculture, University of Tikrit, Iraq; **Email:** Awfabd91@tu.edu.iq
Citation | Aftean, S.H. and A.A.A. Al-Jbory. 2025. Preparation of chitosan microgels reinforced with chemical and biological inhibitors for controlling *Rhizoctonia solani* the causal agent of pepper root rot. *Pakistan Journal of Agricultural Research,* 38(4): 24-32.
DOI | https://dx.doi.org/10.17582/journal.pjar/2025/38.4.24.32
Keywords | Chitosan microgel, Hydrogel, *Rhizoctonia solani*, Pepper root rot, Biological control, Sustainable agriculture

Introduction

Chitosan is one of the components of chitin found in the exoskeletons of crustaceans, fungi, and insects. It strengthens cell walls after being extracted from chitin, appearing as a white powder with a slight reddish tint. It is insoluble in water but dissolves in organic acids (Chakraborty *et al*., 2019). Chitosan is a derivative of chitin, which ranks second after cellulose as the most abundant natural biopolymer (Moran *et al*., 2018). Chitosan is distinguished from other polysaccharides by possessing amino groups in its structural backbone, granting it unique properties when used in biological applications. Chitosan is composed of two compounds: D-glucosamine and N-acetyl-D-glucosamine (Kheiri et al., 2017). In agricultural applications, chitosan has been studied and used to promote plant growth and stimulate plant defenses under biotic stress conditions. Studies have shown that the use of chitosan enhances plant resistance to pests and pathogenic infections (Fan *et al*., 2022). Excessive use of insecticides and fungicides can be harmful to humans, animals, and plants (Tsalidis, 2022). To reduce or avoid the use of chemical substances, natural elicitors such as chitosan can serve as *alternatives* for pest and disease management. Chitosan is used in many agricultural applications as well as an antifungal agent, a biopesticide, and in seed treatment. It is also used in the food industry and in medicine as a drug delivery agent, antibacterial agent, and in pharmaceuticals as a packaging material (Liburdi *et al*., 2016). Microgel represents one of the most recent applications in converting polymers into gel-like structures capable of absorbing water in three-dimensional networks. Microgel can absorb water up to 400 times its dry weight and has the ability to gradually release water and the compounds loaded onto it, while chitosan's antifungal effects are well documented, limited studies have addressed its synergistic microgel form combined with bio-chemical inhibitors against *R. solani* in peppers (Michael *et al*., 2022). This study aimed to prepare chitosan microgel and use it as an inhibitor, carrier, and aid for the controlled release of loaded inhibitors, thereby reducing dependence on chemical fungicides in suppressing plant pathogens. Moreover, it is an environmentally friendly and biodegradable material.

Materials and Methods

Medium preparation

Preparation of potato dextrose agar (PDA): Potato Dextrose Agar medium was prepared by adding 19.5 g of the nutrient medium to 500 ml of distilled water in a sterilized 1000 ml flask. The mixture was stirred well using a hotplate. The flask opening was sealed with sterile cotton and aluminum foil and then placed in an autoclave at 121˚C and 1 bar/cm^2 pressure for 15 minutes. After sterilization, the medium was cooled, and 500 mg of the antibiotic Amiflux was added and mixed thoroughly. The medium was then poured into 9 cm diameter Petri dishes inside the inoculation room after sterilizing the dishes with alcohol (Al-Jbory *et al*., 2023).

Preparation of nutrient broth: Nutrient broth medium was prepared by adding 13 g of the nutrient medium to 1000 ml of distilled water in a sterilized 2000 ml flask. The mixture was stirred well using a hotplate. The flask opening was sealed with sterile cotton and aluminum foil and then placed in an autoclave at 121˚C and 1 bar/cm^2 pressure for 15 minutes (Al-Jbory *et al*., 2025).

Isolation and identification of the causal agents of pepper root rot from different areas of Salah Al-Din Governorate

Fungi associated with the roots of pepper plants (*C. annum*) showing symptoms of infection, such as yellowing, wilting, plant weakness, and root rot, were isolated. Infected samples were collected from various areas in Salah Al-Din (Al-Alam, Tikrit, Samarra, Al-Duluiya, and Al-Dujail) and transported to the postgraduate laboratory at the College of Agriculture, University of Tikrit for isolation procedures. The plant roots were thoroughly washed with water to remove soil and debris. Then, they were cut into small pieces (1 cm diameter) using scissors sterilized with alcohol. The pieces were surface sterilized with 3% sodium hypochlorite solution (NaClO) for one minute, washed with distilled water, and transferred to the inoculation room. Each piece was plated onto a Petri dish containing the nutrient medium PDA and incubated at 25 ± 2˚C for five days. The isolates were then purified by transferring a portion from the colony margin onto new Petri dishes containing PDA medium.

Pathogenicity test of the isolated pathogens on pepper seeds

The pathogenicity of the fungi isolated from the roots of infected pepper plants (*Rhizoctonia solani*, *Alternaria* sp., *Fusarium solani*, *Macrophomina phaseolina*, *Fusarium* oxysporum) was tested on pepper seeds. The seeds were surface sterilized with 3% sodium

hypochlorite solution (NaOCl) for one minute, as described in section (1.3), and then transferred to the inoculation room. The seeds were evenly sown near the edge of Petri dishes containing the nutrient medium BDA, with 10 seeds per dish. A 1.5 cm diameter mycelial disc was placed in the center of the dish, cut from the margin of a 7-days old pure fungal colony grown on BDA medium. Three replicates were used for each fungal isolate, in addition to a control treatment where sterilized pepper seeds were planted in dishes without fungal inoculation, using the same number of replicates. The plates were incubated at 25 ± 2°C for five days. The germination percentage was calculated after the seeds in the control treatment had fully germinated, as follows:

$$100 \times \frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ seeds} = Seed\ germination\ percentage$$

Preparation of chitosan microgel

The hydrogel compound was prepared by utilizing various polymeric materials capable of forming a three-dimensional structure, including chitosan. This was achieved by adding 2 ml of 6% acetic acid to 120 mg of chitosan, then mixing the solution using a connecting tube between two syringes, as illustrated below, for 30 seconds. After mixing, 100 microliters of genipin solution were added to the prepared mixture, followed by a second round of mixing using the dual-syringe system for another 30 seconds. The mixture was then poured into a Petri dish, covered with its lid, and incubated at 30°C for 24 hours. After incubation, the hydrogel was cut into the desired size or converted into microgel particles by passing it through a syringe equipped with a filter of known pore size, creating the required particle sizes by pushing the mixture through the filter. The resulting chitosan microgel particles were collected in a 50 ml Erlenmeyer flask, to which 20 ml of distilled water were added, and the mixture was stirred to obtain a chitosan microgel suspension. The chitosan microgel was resuspended in 10 ml of 70% ethanol and stirred for 5 minutes. Centrifugation was performed at 1000 rpm for 5 minutes, the supernatant was discarded, and the pellet was retained. The washing process was repeated three times with distilled water to remove residual ethanol. Finally, the compound was sterilized by exposure to ultraviolet light for one hour to make it ready for biological evaluation and the addition of other inhibitors (Michael *et al.*, 2022).

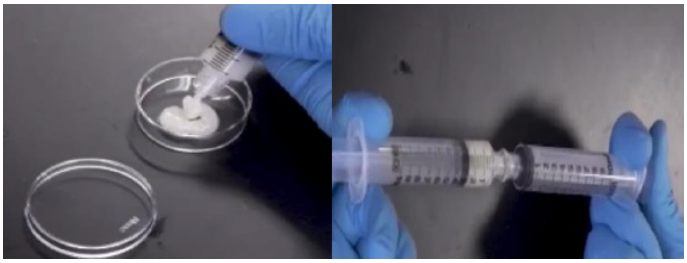

Figure 1: *Preparation of chitosan microgel.*

Testing the efficacy of biological agents in inhibiting the most pathogenic causal agent

This test was conducted by culturing Bacillus subtilis and Pseudomonas fluorescens in the nutrient broth (N.B) medium, prepared as described in section (1.1.2). The cultures were incubated in a shaking incubator at 37°C for 24 hours. The bacterial filtrate was prepared using 9 cm diameter filter paper. Ten milliliters of the liquid N.B medium containing the bacteria were placed onto the filter paper fixed on the opening of a sterile 100 ml glass flask. The filtrate was then collected using a syringe equipped with a Mollipore filter to obtain a sterile filtrate. Petri dishes containing BDA medium were inoculated with 1 ml of the bacterial filtrate, and the plates were swirled gently to evenly distribute the bacterial filtrate. A 0.5 cm diameter disc was then taken from the margin of a 7-days old pure fungal colony grown on BDA medium and placed at the center of each plate. Three replicates were used for each bacterial type, in addition to three replicates for the control treatment. The plates were incubated at 25 ± 2°C for five days until the fungal growth in the control treatment reached the edge of the plate. The colony diameter was measured by recording the dimensions of the fungal colony.

Testing the efficacy of chemical agents in inhibiting the most pathogenic causal agent

Three types of chemical elements (organic copper, boron, and zinc) were tested. For each element, two concentrations were used: (0.025) and (0.05). Each concentration was dissolved in 100 ml of pre-prepared PDA medium in a 250 ml flask, with a total of 7 flasks (100 ml for each concentration of each element). The media containing the above concentrations were poured into Petri dishes inside a sterile laminar flow hood. A 0.5 cm diameter disc was taken from the margin of a pure *R. solani* colony grown on PDA medium and placed in the center of each plate. Three replicates were prepared for each concentration. The seventh flask was left without any additive to serve as a control treatment. The plates were labeled with

codes and numbers to identify the element and concentration, sealed with parafilm, and incubated at 25 ± 2°C for 5 days until the fungal colony in the control treatment reached the edge of the plate. The colony diameter was measured by recording the dimensions of the growing colony.

Pesticide screening experiment

Three types of reputable and widely available fungicides (Beltanol, Moncut, and Topsin) were tested against the most pathogenic causal agent. For each fungicide, two concentrations were used: (0.025) and (0.05). Each concentration was dissolved in 100 ml of pre-prepared PDA medium in a 250 ml flask, with a total of 7 flasks (100 ml for each concentration of each fungicide). The media containing the above concentrations were poured into Petri dishes inside a sterile laminar flow hood. A 0.5 cm diameter disc was taken from the margin of a pure *R. solani* colony grown on PDA medium and placed in the center of each plate. Three replicates were prepared for each concentration. The seventh flask was left without any additive to serve as a control treatment.

The plates were labeled with codes and numbers to identify the type of fungicide and concentration, sealed with parafilm, and incubated at 25 ± 2°C for 5 days until the fungal colony in the control treatment reached the edge of the plate. The colony diameter was measured by recording the dimensions of the growing colony.

Enhancement of chitosan microgel with biological and chemical inhibitors and its use as a controlled-release agent for inhibitors, and evaluation of its single and combined inhibitory efficiency

The PDA medium was prepared as described in section (1.1) in 5 flasks of 250 ml capacity, with 100 ml per flask. The Chitosan Microgel compound was fortified with three treatments as follows, leaving the fourth treatment with Chitosan Microgel alone, while the fifth treatment was left without any addition:

1. (0.025) ml Chitosan Microgel + (0.025) ml of Pseudomonas fluorescens bacterial filtrate
2. (0.025) ml Chitosan Microgel + (0.025) ml Moncut fungicide
3. (0.025) ml Chitosan Microgel + (0.025) ml Boron (B)
4. (0.05) ml Chitosan Microgel
5. Control treatment

The above treatments were added separately to each flask. Then, the culture media containing the inhibitors were poured into Petri dishes. After the medium solidified, a 0.5 cm disc was taken from the margin of a pure *Rhizoctonia solani* colony grown on PDA medium and placed at the center of the plate. Three replicates were made for each treatment, while ensuring a control plate without any additives was included. The plates were labeled with codes and numbers to identify the type of compound and concentration, sealed with parafilm, and incubated at 25 ± 2°C for 5 days until the colony in the control treatment reached the edge of the plate. The colony diameter was measured by recording the dimensions of the growing colony.

Statistical analysis

Experimental data resulting from the *complete random design* (C.R.D) were analysed using the SAS programme and The least significant difference test was used to compare the means at a probability level of (0.05%).

Results and Discussion

Effect of different fungi on pepper seed germination percentage in petri dishes

The results of testing the effect of fungi on pepper seed germination percentage in Petri dishes Figure 2 showed that most fungal isolates caused a significant reduction in the germination percentage of pepper seeds, which ranged between 6.66 % and 96.6% compared to the control treatment, which reached 96.6 %. The *Rhizoctonia solani* isolate was superior, preventing pepper seed germination with a rate of 6.66 %, followed by *Macrophomina phaseolina*, *Fusarium solani* and *Fusarium oxysporum*, with germination rates of 63.33%, 50 %, and 10 %, respectively. The highest germination rate (76.66%) was recorded with the *Alternaria alternata* isolate.

The variation in the effect of fungal isolates on seed germination is attributed to differences in their pathogenicity, toxin production, the quantity and type of these toxins, their genetic makeup, and their ability to secrete enzymes such as cellulase, pectinase, and protease, which contribute to seed decay and prevent germination. In contrast, non-pathogenic fungal isolates lack these enzymes (Hashi, 2020; Avan *et al.*, 2021). El-Kazzaz *et al.* (2022) reported that *R. solani* secretes metabolites and cellulase and pectinase

enzymes during the initial infection stage, playing a vital role in degrading the cell wall to facilitate host penetration. The more enzymes and toxins an isolate produce, the more pathogenic it is to the plant host.

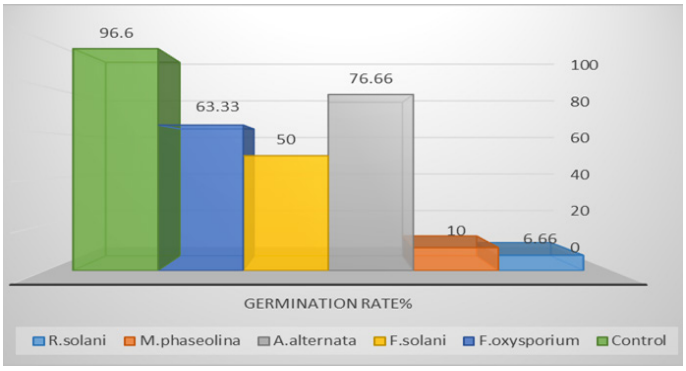

Figure 2: *The effect of different fungi on the germination percentage of pepper seeds in Petri dishes, noting that the value of (L.S.D = 2.05).*

Testing the effect of different fungicides on the radial growth of R. solani (cm²)

The results of testing the fungicides on the fungal growth of *Rhizoctonia solani* Figure 3 showed inhibition of fungal growth, with varying effects among the fungicides Beltanol, Moncut, and Topsin. The chemical fungicide Moncut was the most effective, achieving complete inhibition of the pathogenic fungal colony, with a radial growth of 0 cm^2 at a concentration of 0.05, which was significantly different from the radial growth of 1.83 cm^2 at a concentration of 0.025, compared to the control treatment where the inhibition percentage was 0.0%. Beltanol followed, with a radial growth of 0.33 cm^2 at 0.05 concentration compared to 0.66 cm^2 at 0.025 concentration. The fungicide Topsin recorded the highest radial growth among the fungicides, with values of 3 cm^2 and 1.5 cm^2 at 0.025 and 0.05 concentrations, respectively. The superiority of the fungicide Moncut in inhibiting the pathogenic fungus is attributed to its ability to inhibit respiration by affecting the fungal mitochondria, leading to the inhibition of NADH oxidase activity and causing the fungal cell death. It is considered a protective and curative fungicide effective against basidiomycete fungi (Yang and Li, 2012). This study agrees with Al-Jbory *et al.* (2019) and Abedalred *et al.* (2019) regarding the inhibitory capacity of fungicides against various fungal pathogens, including *R. solani*.

Testing the effect of different chemical elements on the fungal growth of R. solani (cm²)

The results in Figure 4 showed the effect of different chemical elements on the fungal growth of *R. solani*. All treatments caused a significant reduction in the colony diameter. The boron treatment at a concentration of 0.05 resulted in the lowest radial growth, reaching 2 cm^2, which was significantly different compared to the 0.025 concentration that resulted in a radial growth of 3.83 cm^2, compared to the control treatment which recorded a radial growth of 9 cm^2.

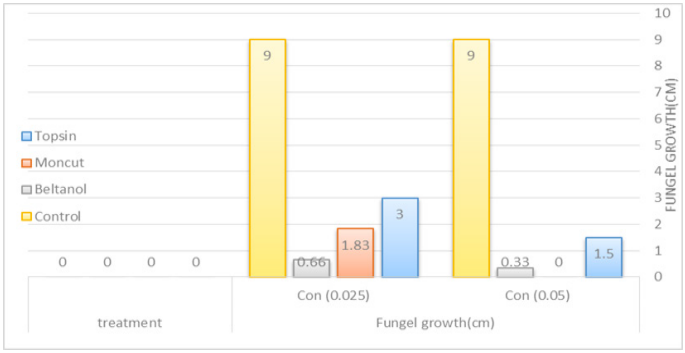

Figure 3: *The effect of different fungicides on the radial growth of R. solani (cm²), noting that the value of (L.S.D = 0.21).*

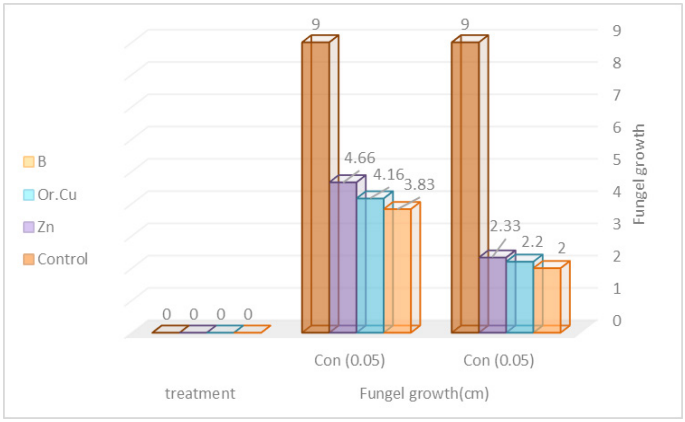

Figure 4: *The effect of different chemical elements on the radial growth of R. solani (cm²), noting that the value of (L.S.D = 0.19).*

Next was the copper treatment, which achieved a radial growth of 2.2 cm^2 at 0.05 concentration compared to 4.16 cm^2 at 0.025 concentration. The zinc treatment recorded the highest radial growth among the treatments, with 2.33 cm^2 at 0.05 concentration compared to 4.66 cm^2 at 0.025 concentration. These results are in agreement with the study by Erper *et al.* (2019), who used a 1% concentration of boron against *R. solani* isolates, leading to a reduction in fungal growth by (95.07%–97.61%). The results indicate that boron may serve as an alternative to synthetic fungicides against root rot. Madusanka *et al.* (2024) also reported, in their study on several elements against *R. solani*, the ability of copper to inhibit the

fungus, achieving the highest inhibition rate among the tested elements at 73.43% at a concentration of 1000 mg/L. Mahmoud (2024) similarly noted, in his study on root and stem rot caused by *R. solani* in faba bean, the inhibitory effect of zinc on the fungus, showing a significant reduction in fungal growth.

Testing the effect of P. fluorescens and B. subtilis bacterial isolates on the radial growth of R. solani (cm²)

The experiment results in Figure 5 illustrate the effect of the two bacterial isolates on the radial growth of *R. solani*. The bacterium *P. fluorescens* gave the highest inhibition of the fungal radial growth, reaching 3.1 cm² and 6.7 cm² at concentrations of 0.05 and 0.025 respectively, compared to the control treatment, which recorded an inhibition rate of 0.0%. This was followed by *B. subtilis*, which gave a lower inhibition rate of fungal colony growth, reaching 3.7 cm² and 7.1 cm² at concentrations of 0.05 and 0.025, respectively.

P. fluorescens produces several secondary metabolites such as antibiotics like pyrrolnitrin, phenazine, and DAPG (Raaijmakers and Mazzola, 2012). Antagonistic bacteria act by producing antifungal substances that inhibit the hyphal growth of pathogenic fungi; *P. fluorescens* produces many antifungal compounds such as pyrrolnitrin, pyoluteorin, phenazines, siderophores, cyanide, and 2,4-diacetylphloroglucinol (Al-Jbory *et al.*, 2025).

This study is consistent with Mohamed *et al.* (2021) regarding the effect of some bacterial isolates in inhibiting fungal growth in vitro and studying their antagonistic activity, where *B. subtilis* gave the highest reduction in pathogen radial growth, with an inhibition rate of 74.76%.

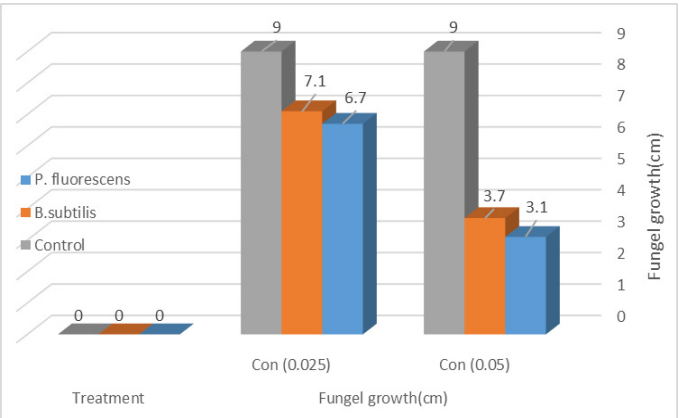

Figure 5: *The effect of the bacterial isolates P. fluorescens and B. subtilis on the radial growth of R. solani (cm²), noting that the value of (L.S.D = 0.39).*

Testing the effect of different concentrations of chitosan and chitosan microgel on the radial growth (cm²) of R. solani

The results in Figure 6 showed the effect of different concentrations of Chitosan and Chitosan Microgels on the radial growth of *R. solani*. Both Chitosan and Chitosan Microgels resulted in the lowest radial growth of the fungal isolate, reaching 4.16 cm² and 2.83 cm², respectively, at concentrations of 100% and 0.08 each, compared to the control treatment, which showed a growth of 9 cm². This was followed by Microgel concentrations of 75% and 50%, with inhibition values of 5.33 cm² and 4 cm², and Chitosan at concentrations of 0.04 and 0.06, with inhibition values of 7 cm² and 5.33 cm², respectively. The lowest reduction in fungal growth was recorded at a concentration of 25% for Microgels (6.33 cm²) and 0.02 for Chitosan (7.83 cm²).

This study agrees with Freddo *et al.* (2014), who evaluated the effectiveness of Chitosan in reducing the growth of *R. solani*, using four different concentrations of Chitosan (0, 0.25, 0.5, 2%), showing a significant effect on fungal growth *in vitro*, with inhibition increasing as the concentration increased. Fungi tend to produce acids at varying levels, making the growth medium acidic, which stimulates protonation of the amino group in Chitosan, leading to the destruction of biomolecules. Chitosan activity against fungi is higher at low pH values due to its increased solubility compared to other media (Sathiyabama and Muthukumar, 2020; Ahmed *et al.*, 2025).

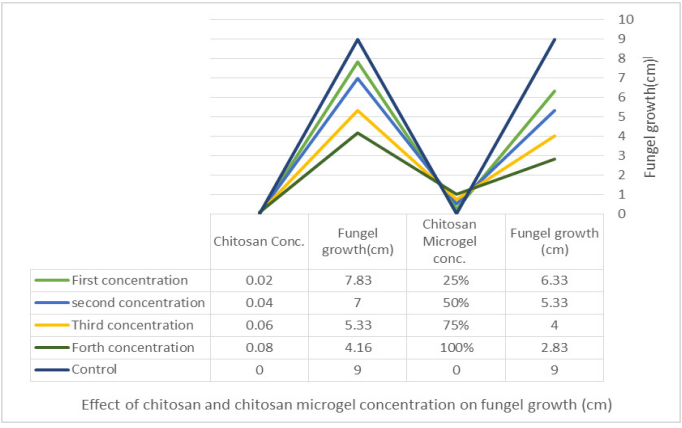

Figure 6: *The effect of different concentrations of Chitosan and Chitosan Microgel on the radial growth of R. solani (cm²), noting that the value of (L.S.D=1.01).*

Results of the effect of several treatments with chitosan microgel on the radial growth of R. solani (cm²)

The results in Figure 7 showed a significant reduction

in the radial growth of the fungal colony in all treatments, with values ranging from 0 to 2.66 cm^2, compared to the control treatment, which showed a radial growth of 9 cm^2.The treatment Chitosan Microgel + pesticide gave the lowest radial growth of the pathogenic fungus, reaching 0 cm^2, followed by Chitosan Microgel + Boron and Chitosan Microgel + Bacteria with radial growths of 1.83 cm^2 and 2.41 cm^2, respectively.

The Microgel pure treatment recorded the highest radial growth compared to the other treatments, reaching 2.66 cm^2. This is consistent with Mahdi *et al.* (2024), who indicated that the use of hydrogels reduces the impact of plant pathogens. These results also align with several studies that concluded that using multiple control agents has a greater effect in inhibiting the pathogen through a synergistic interaction of these agents. Additionally, they contribute to making the environment unsuitable for the pathogen's growth by limiting its access to nutritional requirements, as well as inhibiting and restricting its growth (Al-Mashhadani, 2022).

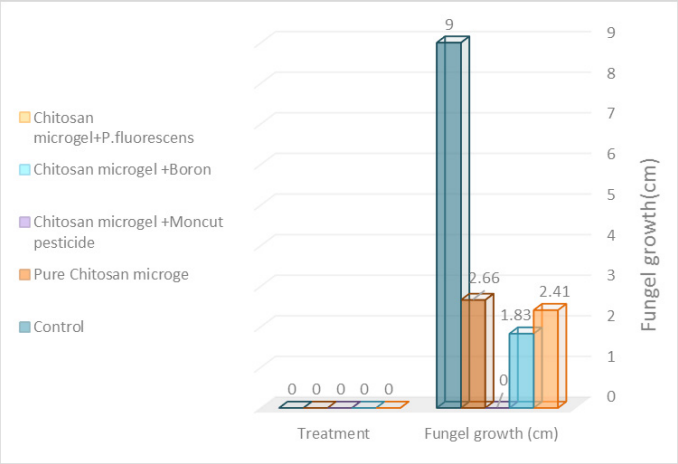

Figure 7: *The effect of various treatments with Chitosan Microgel on the radial growth of R. solani (cm^2), noting that the value of (L.S.D = 0.73).*

Conclusions

From the above, it can be concluded that the Chitosan Microgel compound has a high inhibitory efficacy against the pathogen *R. solani*, and that its inhibitory efficiency can be enhanced by loading it with chemical inhibitors and biological filtrates. This makes it an inhibitory agent and a carrier material that aids in the controlled release of inhibitors, in addition to being environmentally friendly and biodegradable and This approach may reduce synthetic fungicide dependency in greenhouse and field conditions.

Acknowledgment

We are greatly thankful to our staff of Plant Protection Department, College of Agriculture, Tikrit University for their help and support.

Novelty Statement

This manuscript represents a new aspect in the development of natural compounds and their conversion into gel form for use in combating plant diseases.

Author's Contribution

Sara H. Aftean: The first author did the work Conceived the idea, Wrote abstract, Methodology.
Awf A. Ahmed Al-Jbory: The second author did the work SPSS analysis, Conclusion, Technical Input at every step, Overall Management of the article, Data collection, Result and discussion, introduction, References.

Funding
This study is funded by the researchers' own account.

Generative AI and AI-assisted technology statement
The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest
The authors have declared no conflict of interest.

References

Abd El-Hack, M.E., M.T. El-Saadony, M.E. Shafi, N.M. Zabermawi, M. Arif, G.E. Batiha, A.F. Khafaga, Y.M. Abd El-Hakim and A.A. Al-Sagheer. 2020. Antimicrobial and antioxidant properties of chitosan and its derivatives and their applications: A review. Int. J. Biol. Macromol., 164: 2726-2744. https://doi.org/10.1016/j.ijbiomac.2020.08.153

Abedalred, E.M., W.M.H. Ismail, R.G. Abdulmoohsin and M.A.J. Al-Karhi. 2019. First molecular identification of *Fusarium fujikuroi* causing pollen rot of palm trees (*Phoenix dactylifera* L.) in Iraq and evaluation efficacy of some nanoparticles against it. IOP Conf. Ser.

Earth Environ. Sci., 388: 012007. https://doi.org/10.1088/1755-1315/388/1/012007

Ahmed, W.K., A.T. Mohammed and A.A.A. Al-Jbory. 2025. *In vitro* antifungal activity of capparis spinosa extract against soil-borne plant pathogens. Plant Prot., 9(2): 273-280. https://doi.org/10.33804/pp.009.02.5581

Ali, N.A.A., 2023. The integrated efficacy of the biological control agents *Trichoderma harzianum*, *Pseudomonas fluorescens*, and chelated iron in controlling wilt disease in pepper. Department of Plant Protection, Plant Pathology, College of Agriculture, University of Baghdad.

Al-Jbory, A.A.A., A.K. Mohammed, R.S. Mahmood and E.E. Wagih. 2025. Effect of using *Bacillus subtilis*, *Ganoderma lucidum*, and *Spirulina platansis* Alga on bean yellow mosaic virus (BYMV) infection in two varieties of broad bean. Tikrit J. Agric. Sci., 25(1): 57-68. https://doi.org/10.25130/tjas.25.1.5

Al-Jbory, A.A.A., K.A. Mohamad and Z.S. Ahmed. 2019. Effect of some biological control agents and their integration with Tachigaren fungicide for the control of Rhizoctonia rot on cucumber plant. Arab J. Plant Prot., 37(3): 259-265. https://doi.org/10.22268/AJPP-037.3.259265

Al-Jbory, A.A.A., K.A. Mohammed and S.M. Ismaeel. 2023. Isolation of the pectinase enzyme produced by the fungus Penicillium spp. that causes citrus fruit rot disease and evaluation of the inhibitors efficacy for its control. Arab J. Plant Prot., 41(2): 190-196. https://doi.org/10.22268/AJPP-41.2.190196

Al-Mashhadani, S.J., 2022. The effectiveness of integrated control in managing some root rot pathogens on Celosia argentea. Master's thesis, Department of Plant Protection, College of Agriculture, University of Karbala.

Avan, M., G. Palacıoğlu, C. Aksoy, R. Kaya, H. Bayraktar, Y. Katırcıoğlu and S. Maden. 2021. Characterization and pathogenicity of Rhizoctonia species causing root rot and damping-off on sugar beet in Turkey. Curr. Microbiol., 78(1): 39-48. https://doi.org/10.1007/s00284-021-02470-4

Baharin, K.W., S. Zakaria, A.V. Ellis, N. Talip, H. Kaco, S. Gan, F.D. Zailan and S.N.A.S. Hashim. 2018. Factors affecting cellulose dissolution of oil palm empty fruit bunch and kenaf pulp in NaOH/urea solvent. Sains Malays., 47: 377–386.

Chakrabarti, A., B.L. Campbell and V. Shonkwiler. 2019. Eliciting consumer preference and willingness to pay for mushrooms: A latent class approach. J. Food Distribut. Res., 50(1): 46-62. Chandra, P., 2021. Aflatoxins: Food Safety, Human Health Hazard

Chen, H., W. Ouyang, B. Lawuyi, C. Martoni and S. Prakash. 2020. Reaction of chitosan with genipin and its fluorogenic Materials Advances chitosan hydrogels: Structure, pH responsiveness, gelation kinetics, and rheology. J. Appl. Polym. Sci., 137(41).

Dakhil, F.A., 2021. Integration between biological agents and chemical fungicides in the control of root diseases of ornamental plants in the nurseries of Karbala and Babylon provinces. Master's thesis, College of Agriculture, University of Karbala, Iraq.

Darpkova, D., 2022. Chitosan: A review of its pharmacological and cosmetic applications. J. Cosmet. Dermatol., 21(2): 148-155.

El-Kazzaz, M., K. Ghoneim, A. Helmy, S. Behiry, A. Abdelkhalek, S. Hamzah, A. Al-Askar and A. Arishi. 2022. Suppression of pepper root rot and wilt diseases caused by *Rhizoctonia solani and Fusarium oxysporum*. Curr. Res. Microb. Sci.,12(4): 587-589. https://doi.org/10.3390/life12040587

Erper, I., E. Yildirim and M. Türkkan. 2019. Antifungal effect of boron compounds against three *Rhizoctonia solani* AG-4Subgroups causing root and crown rot. Gesunde Pflanzen, 71(1): 61-71. https://doi.org/10.1007/s10343-019-00442-0

Fan, J., Johannes, A.J., Ann, R. and Anne, L.N., 2022. First evidence of CPGV resistance of coding moth in the USA. Insects, 13(10): 1-12. https://doi.org/10.3390/insects14010015

Faqir, Y., J. Ma and Y. Chai. 2021. Chitosan in modern agriculture production. Plant Soil Environ., 67: 679–699. https://doi.org/10.17221/332/2021-PSE

Freddo, Á.R., S.M. Mazaro, E.J. Brun and A.W. Júnior. 2014. A quitosana comofungistático no crescimento micelial de Rhizoctonia solani Kuhn. Ciencia Rural, Gesunde Pflanzen, 71(1): 61-71. https://doi.org/10.1590/S0103-84782014000100001

Hashi, M.A.K., 2020. Study of pathogenic *Rhizoctonia* spp. isolates on potato and their

control by soil biofumigation. Master's thesis, College of Agricultural Engineering Sciences, University of Baghdad, pp. 21.

Hassnain, A. Basit, M. Alam, I. Ahmad, I. Ullah, N. Alam and M. Shair. 2020. Efficacy of chitosan on performance of tomato (*Lycopersicon esculentum* L.) plant under water stress condition. Pak. J. Agric. Res., 33(1): 27-41. https://doi.org/10.17582/journal.pjar/2020/33.1.27.41

Kheiri A. Moosawi Jorf SA Malihipour A. Saremi H and Nikkhah M. 2017. Synthesis and characterization of chitosan nanoparticles and their ef- fect on Fusarium head blight and oxidative activity in wheat. Int J Biol Macromol., 102: 526–538. doi: 10.1016/j.ijbiomac.2017.04.034

Liburdi, K., I. Benucci, F. Palumbo and M. Esti. 2016. Lysozyme immobilized on chitosan beads: Kinetic characterization and antimicrobial activity in white wines. Food Contr., 63: 46-52. https://doi.org/10.1016/j.foodcont.2015.11.015

Madusanka, H.K.S., A.G.B. Aruggoda, J.A.S. Chathurika and S.R. Weerakoon. 2024. Evaluation of the antifungal effect of green synthesized metal oxide nanoparticles against plant pathogenic *Rhizoctonia* species. Am. J. Life Sci. Innov., 3(2): 63–72. https://doi.org/10.54536/ajlsi.v3i2.4281

Mahdi, M.M., A.A.R.A. Al-Jbory and S.M. Asmaeel. 2024. The effect of using hydrogel and some biological and chemical treatments on controlling charcoal rot disease in soybeans under field experiment conditions. In: IOP conference series: Earth and environmental science, 1371(3): 032039. https://doi.org/10.1088/1755-1315/1371/3/032039

Mahmoud, R., 2024. Effect of zno-nps on rhizoctonia solani causing root and stem rot on broad bean (*Vicia faba* I.). Sabrao J. Breed. Genet., 56(6): 2461-2460. https://doi.org/10.54910/sabrao2024.56.6.27

Michael, A.S., Christopher.B.E., Karin A.P., and Melissa D.K. 2022. Preparation and characterization of chitosan hydrogels for biomedical applications. J. Biomed. Mater. Res. A, 110(5): 1241-1253.

Mohamed, A.B., M.M. El-Sheikh Aly and R.M.I. El-Sharkawy. 2021. Antifungal activity of bioagents and plant extracts against certain fungal diseases of potatoes. J. Phytopathol. Dis. Manage., 8(1): 29-45.

Moran, H.B., J.L. Turley, M. Andersson and E.C. Lavelle. 2018. Immunomodulatory properties of chitosan polymers. Biomaterials, 184: 1-9. https://doi.org/10.1016/j.biomaterials.2018.08.054

Neethu, T.M., P.K. Dubey and A.R. Kaswala. 2018. Prospects and applications of hydrogel technology in agriculture. Int. J. Curr. Microbiol. Appl. Sci., 7: 3155–3162. https://doi.org/10.20546/ijcmas.2018.705.369

Novakovic, K., S. Matcham and A. Scott. 2018. Hydrogels, gels horizons: From science to smart materials, pp. 1-28. https://doi.org/10.1007/978-981-10-6077-9_1

Raaijmakers, J.M. and M. Mazzola. 2012. Diversity and natural functions of antibiotics produced by beneficial and plant pathogenic bacteria. Annu. Rev. Phytopathol., 50: 403-424. https://doi.org/10.1146/annurev-phyto-081211-172908

Sathiyabama, M. and S. Muthukumar. 2020. Chitosan guar nanoparticle preparation and its in vitro antimicrobial activity towards phytopathogens of rice. Int. J. Biol., 53: 297-304. https://doi.org/10.1016/j.ijbiomac.2020.03.001

Sun, X., Q. Wu, D.H. Picha, M.H. Ferguson, I.E. Ndukwe and P. Azadi. 2021. Comparative performance of bio-based coatings formulated with cellulose, chitin, and chitosan nanomaterials suitable for fruit preservation. Carbohyd. Polymers, 259: 117764. https://doi.org/10.1016/j.carbpol.2021.117764

Tsalidis, G.A., 2022. Human health and ecosystem quality benefts with life cycle assessment due to fungicides elimination in agriculture. Sustainability, 14(2): 846. https://doi.org/10.3390/su14020846

van der Burgh, A.M. and M.H.A.J. Joosten. 2019. Plant immunity: Thinking outside and inside the box. Trends Plant Sci., 24(7): 587–601. https://doi.org/10.1016/j.tplants.2019.04.009

Yang, G. and C. Li. 2012. General description of *Rhizoctonia* species complex. In: Plant pathology. Intech Open. https://doi.org/10.5772/39026