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Research Article

In Vitro Efficacy of Chitosan Gel Combined with Organic and Inorganic Inhibitors against *Rhizoctonia solani*

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ABSTRACT

Rhizoctonia solani is an important soil-borne pathogen causing severe root and stem diseases in numerous crops, necessitating the evaluation of effective antifungal treatments. The present study evaluated the *in vitro* efficacy of selected fungicides, chemical elements, organic compounds, chitosan, and chitosan microgel-based combinations against *R. solani*. Among the fungicides, Moncut showed the highest inhibition, with 1.41% and 2.86% inhibition at 0.05% and 0.10%, respectively, followed by Beltanol (0 and 0.73%) and Topsin (3.58 and 4.75%). Among the chemical elements, boron was the most effective, producing radial colony diameters of 1.30, 2.46, and 3.66 cm at 0.05%, 0.10%, and 0.15%, respectively, whereas zinc was least effective, with inhibition of 2.80%, 4.13%, and 5.70% at 0.05%, 0.10%, and 1.0%, respectively. Organic copper completely inhibited fungal growth at all tested concentrations, while biochar and humic acid produced maximum inhibitions of 6.20% and 4.13%, respectively. Chitosan inhibited fungal growth in a concentration-dependent manner, reducing radial growth to 5.50, 4.36, and 1.96 cm at 0.05%, 0.10%, and 0.15%, respectively, compared with 9.0 cm in the untreated control. The chitosan microgel combinations exhibited markedly greater inhibition than the individual treatments. Chitosan microgel combined with Beltanol and copper completely inhibited fungal growth, while the chitosan microgel + boron treatment restricted radial growth to 0.66 cm. Overall, chitosan microgel, particularly in combination with selected chemicals or organic treatments, demonstrated promising *in vitro* antifungal activity against *R. solani*. Further greenhouse and field studies are recommended to validate their efficacy and determine appropriate application rates and treatment combinations.

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Introduction

Watermelon belongs to the Cucurbitaceae family (Jeffrey, 2001) and has global economic importance. It is widely cultivated to meet domestic demand and for export, due to its high popularity among consumers because of its high water and vitamin content, especially in the summer (FAO, 2023). Iraq's watermelon production reached approximately 420,000 tons in 2023, compared to 440,000 tons in 2022. Watermelon plants are susceptible to several diseases, including root rot, a fungal disease that directly impacts plant growth and yield. There are several soil-borne fungi that have been shown to cause this disorder, such as *Fusarium* and *Pythium* species, as well as *Macrophomina phaseolina* and *Rhizoctonia solani*. These organisms reside in the soil and infect the roots of plants; however, disease

onset is usually encouraged by too much moisture and poor soil aeration (Agrios, 2005).

Many methods have been used to combat the pathogens of this disease, including chemical pesticides. However, the drawbacks of pesticides and their environmental pollution have prompted plant protection specialists to search for alternative or complementary methods to synthetic chemical pesticides, such as biological control methods. These include the use of certain organic compounds in the control process, such as organocopper, chitosan, humic acid, and biochar, as well as the use of various chemical compounds like copper, zinc, and boron. A recent trend in research has focused on the use of hydrogels, which have proven effective in carrying inhibitors and facilitating their controlled release. Furthermore, they are biodegradable and do not pollute the environment (Mahdi et al., 2024). Among these hydrogels, chitosan hydrogels stand out as a key component in this research. This importance stems from their inhibitory efficacy, biodegradability, and lack of side effects on humans, animals, or plants. Chitosan is a natural chemical compound derived from chitin after the removal of the acetyl group, leaving the amine group free. Chitin is converted into a substance soluble in dilute weak acids, such as acetic acid. This substance is called chitosan (Wick and Bade, 1988). Chitosan is a naturally occurring polysaccharide made up primarily of glucosamine monomers along with some N-acetyl D-glucosamine residues. The desirable properties of chitosan, including nontoxicity and biodegradability, among others, have played a role in making it a suitable candidate as an antimicrobial compound. It is one of the most abundant natural polymers with a dual action. It controls pathogenic microorganisms by inhibiting fungal spore growth and germination, disrupting cell function, and inducing various defensive responses in the host plant (Kheiri et al., 2017).

In agriculture, chitosan-based gels have received much interest due to their antifungal and biocidal activities and application in seed/grain dressing and other food industry applications. Moreover, the chitosan gel is one of the promising polymers which can create a three-dimensional network that can hold large amounts of water (Liburdi et al., 2016). It can be used as a carrier for the selected bio/chemical agents. Therefore, the current research was performed to develop a chitosan gel and investigate the possibilities of its usage both as an inhibitor and as a carrier for organic and chemical compounds to combat the fungus causing root rot in watermelon plants under *in vitro* conditions.

Materials and Methods

Isolation of fungal cultures

An already identified isolate of *Rhizoctonia solani* maintained in the Plant Pathology Laboratory, College of Agriculture, Tikrit University, was used in the study (Mohammed et al., 2025). The fungal culture was grown on Potato Dextrose Agar (PDA) and maintained at 4°C until further use.

Chitosan microgel fabrication

Chitosan as a three-dimensional polymeric material was used to prepare chitosan microgel according to the preparation method described by Aftean and Al-Jbory (2025). In brief, 120 mg of chitosan was dissolved in 2 mL of 6% acetic acid to get the homogeneous chitosan solution. The preparation obtained was added to a double syringe system and homogenized for 30 sec. Genipin solution (100 µL) was then added and the formulation was re-homogenized for an additional 30 sec using the dual syringe system.

The mixture was poured into a Petri dish and after covering with a lid placed in an incubator for 24 h at 30°C for the formation of gel. The formed gel was then placed in a syringe with a 0.22-µm filter, and the gel material was extruded through the filter to form microgel particles. The prepared microgel particles were moved to a 50 mL conical flask and 20 mL of distilled water was added to it. Consequently, 10 mL of 70% ethanol was added and stirred for 5 min.

The mixture was centrifuged for 5 min at 1000 rpm. The supernatant was discarded and the microgel was retained after centrifugation. To wash off the traces of ethanol, the microgel was rinsed with distilled water three times. Lastly, the microgel was disinfected by exposing it to ultraviolet (UV) light for 1 h prior to using it in bioassays and adding the inhibitory agents (Aftean and Al-Jbory, 2025).

Fungicide screening experiment

Three commercial fungicides viz., Beltanol, Moncut and Topsin were chosen for testing their inhibitory effects against *R. solani* using two concentrations i.e., 0.10% and 0.05%. Each concentration of each fungicide was added individually to 100 mL of autoclaved PDA medium. The amended PDA media of each concentration of each fungicide were then dispensed into 250 mL flasks.

Fungicide amended PDA media were aseptically poured

into sterile Petri dishes. Mycelial plugs (0.5-cm diameter) cut from the actively growing edge of a pure culture of *R. solani* grown on PDA were inserted in the middle of the Petri dishes. For each fungicide concentration, three replications were made. PDA without fungicide amendment served as the untreated control.

The Petri dishes were labeled properly, sealed with Parafilm and kept at 25 ± 2°C for five days or until the growth of the fungus reached the edge of the Petri dish in the control plates. The diameter of the fungal colony was used to evaluate the antifungal activity of each fungicide at the recommended and half-recommended concentrations of 0.10% and 0.05%, respectively.

Evaluation of different chitosan concentrations

Four 250-mL flasks were filled with 100 mL of autoclaved PDA medium as described above. Three concentrations of chitosan (0.15%, 0.10% and 0.05%) were prepared and added individually to the respective PDA preparations. The modified media was well agitated and then aseptically poured into sterile Petri plates.

Mycelial plugs (0.5 cm diameter) were cut from the actively growing edge of a purified culture of *R. solani* growing on PDA and transferred into the center of each Petri dish. For each concentration of chitosan, three replicate plates were prepared. PDA plates without chitosan served as the untreated control. The inoculated Petri dishes were kept in an incubator for 5 days at 25 ± 2°C. The diameter of each of the fungal colonies was measured, when the growth of the fungus reached the edge of the Petri dishes in the control plates.

Evaluation of chitosan microgel as a carrier for inhibitory agents

The chitosan microgel was tested as carrier system for delivery against *R. solani*. The activity of the inhibitors of the test pathogen was investigated for both individual and combined treatments. Five 250-mL flasks containing 100 mL of PDA medium were prepared following the procedure outlined above. Similarly, the chitosan microgel prepared as mentioned above was supplemented with the following treatments:

1. 0.025 mL chitosan microgel + 0.025 mL Beltanol
2. 0.025 mL chitosan microgel + 0.025 mL boron (B)
3. 0.025 mL chitosan microgel + 0.025 mL copper (Cu)
4. 0.025 mL chitosan microgel alone
5. Untreated control

Each treatment was added individually to the respective flask containing PDA medium. These modified culture media were then poured into Petri dishes aseptically and left for solidification. Mycelial plugs, measuring 0.5 cm diameter, were cut from the actively growing edge of a purified culture of *R. solani* maintained on PDA and transferred into the center of each Petri dish. For each treatment, three replicate plates were used while the fifth flask without any treatment was kept as the control. The Petri dishes were labeled properly according to treatments, sealed with Parafilm and kept in an incubator at 25 ± 2°C for five days or until the growth of the fungus reached the edge of the Petri dish in the control plates. The growth of fungus was determined by measuring the diameter of the fungal colony.

Experimental design and statistical analysis

The treatments were analyzed under Completely Randomized Design (CRD) in which three independent replicates per treatment were included. The analysis of variance (ANOVA) was done using SAS software, version 9.4. Further, the differences among treatment means were analyzed using Fisher's Least Significant Difference (LSD). The statistical significance was defined at ≤ 0.05. probability level.

Results and Discussion

Effect of different fungicides on the radial growth of *R. solani*

The results in Figure 1 showed that the effect of the fungicides tested on the radial growth of *R. solani* varied. The fungicide Moncut was the most inhibitory of the three evaluated fungicides viz., Beltanol, Moncut and Topsin. Moncut at 0.05% and 0.10% concentrations gave 1.41% and 2.86% inhibition respectively when compared with untreated control which showed no inhibition. Beltanol exhibited the second highest inhibitory activity with inhibition values of 0% and 0.73% at 0.05% and 0.10%, respectively. Topsin had the lowest inhibitory activity with inhibition values of 3.58% and 4.75% respectively at 0.05% and 0.10% concentrations.

The comparatively higher percentage efficacy of Beltanol against *R. solani* is probably related to its antifungal activity and inhibitory effect on fungal growth and development. *R. solani* typically causes damping-off, girdling, stem lesions, and root rot in the crown and basal stem areas. Pathogen suppression through the use of fungicide can then limit mycelial growth and disease development. The observed inhibitory effect of Beltanol is in agreement with previous studies which showed its effectiveness against various phytopathogenic fungi such

as *R. solani* (Abedalred et al., 2019). The mechanisms suggested for systemic movement, disruption of vascular tissues, mineral deficiency and recovery by infected roots, however, need direct experimental proof and should not be deduced from this *in vitro* growth assay alone.

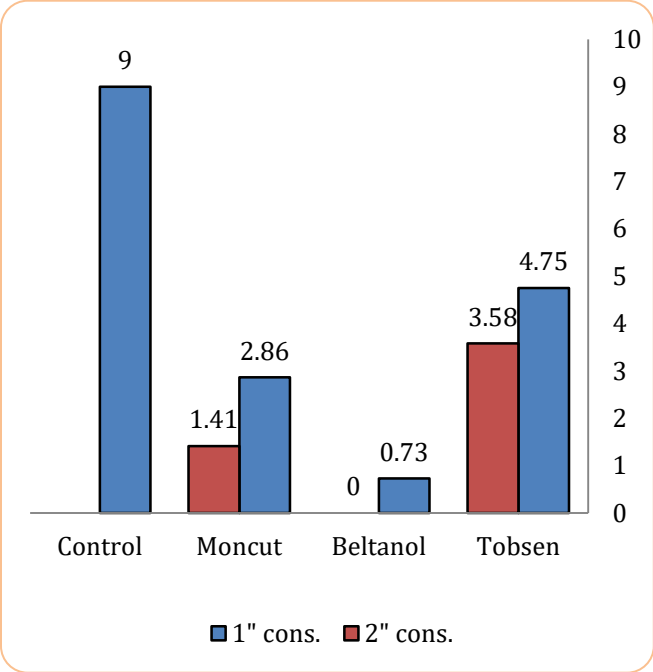


Figure 1. Influence of various fungicides on the radial growth (cm) of R. solani (LSD = 0.39).

Effects of different chemical elements on the growth of *R. solani*

The data shown in Figure 2 showed that the tested chemical elements significantly influenced the growth of mycelia of *R. solani*. The fungus growth was inhibited by all treatments when compared to the untreated control, although the inhibitory effect was quite different for each treatment. Boron was the most inhibitory among the test elements. For the concentrations of 0.05%, 0.10% and 0.15%, the colony diameters recorded were 1.30 cm, 2.46 cm and 3.66 cm respectively, with the untreated control showing no inhibition.

The second best treatment against *R. solani* was copper. On the other hand, among the tested elements, zinc was found to be the least inhibitory with reported inhibitions of 2.8%, 4.13% and 5.7% at 0.05%, 0.10% and 1% concentrations respectively. The present results are similar to those reported by Erper et al., (2019), who tested 1% boron against an isolates of *R. solani* and found high inhibition of the fungal growth from 95.07% to 97.61%. These results indicate that boron could be used as a component of integrated management strategies for root rot caused by *R. solani*, however more experiments are needed under greenhouse and field conditions to validate its practical effectiveness and its concentration.

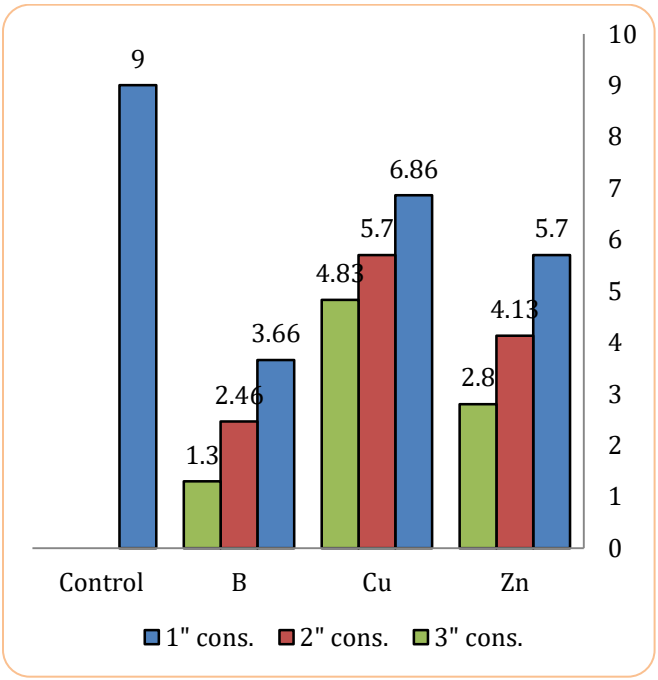


Figure 2. Radial growth (cm) of *R. solani* in the presence of various inorganic elements (LSD = 0.29).

These current results on copper are also corroborated by Madusanka et al. (2024) who studied the antifungal activity of various agents against *R. solani* and found that copper showed strong inhibitory effect. Copper showed the maximum inhibition of 73.43% at 1000 mg L^{-1} among the tested agents. Likewise, Mahmoud (2024) reported significant growth inhibition of *R. solani* with zinc treatment, confirming the probable antifungal activity of zinc-based treatments against the diseases caused by this pathogen.

Effect of different organic compounds on the growth of *R. solani*

The effects of the tested organic compounds on the growth of *R. solani* are shown in Figure 3. It is evident from the figure that all the organic compounds tested had a significant effect on the growth of the fungus. All of the treatments caused a decrease in the growth of the fungal colonies relative to the untreated control, but by varying levels. Organic copper was found to be the most

effective treatment, with 100% control of fungal growth at all the applied rates. At the concentrations of 0.05%, 0.10%, and 0.15%, the fungal growth was inhibited with a radial inhibition of 0 cm, while the non-treated sample did not show any inhibition.

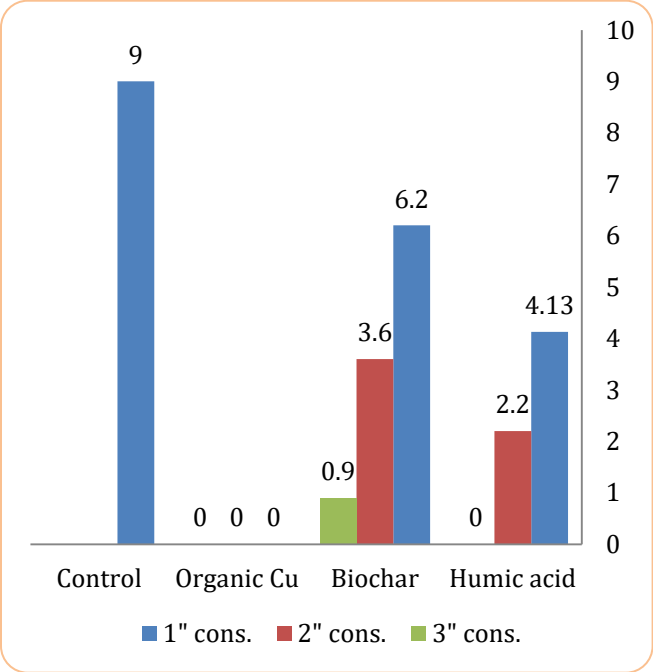


Figure 3, Impact of various organic treatments on the radial growth (cm) of *R. solani* in culture media (LSD = 0.20).

The second most effective treatment was biochar which exhibited inhibition values of 0.9%, 3.6% and 6.2% at concentrations of 0.05%, 0.10% and 0.15%, respectively. The lowest inhibitory activity was seen with humic acid with the corresponding inhibition values of 0%, 2.2% and 4.13% at the three concentrations respectively.

The antifungal activity of copper based compounds against *R. solani* has been revealed previously. It has been reported by Al-Jbory et al. (2023) and Ahmed et al. (2025) that organocopper can have inhibitory effects on fungal structures such as mycelial development and spore morphologies leading to reduction in fungal growth. The same trend was observed by Abdelkhalek (2022) who found significant inhibition of *R. solani* by organocopper to the tune of 73.43% at 1000 mg L^{-1}. In the same way, Jaiswal et al. (2017) showed that biochar could be used as a suppressive agent against the development of root and stem rot in broad bean caused by *R. solani*. Together these results suggest a possible role of organic and copper based products in the disease management program of *R. solani*.

Effect of different chitosan concentrations on the radial growth of *R. solani*

The impact of various concentrations of chitosan on radial growth of *R. solani* is shown in Figure 4. The growth of fungus was inhibited in a concentration-dependent manner by chitosan. The highest concentration of 0.15% yielded the maximum inhibition of the radial growth to 1.96 cm. On the other hand, the fungal growth was 4.36 cm and 5.50 cm at 0.10% and 0.05% concentrations respectively with 9.0 cm growth in the untreated control.

The higher chitosan concentrations resulted in greater inhibitory effect, which could be ascribed to higher interaction between chitosan and fungal cell surfaces. Chitosan has a negative effect on cell-membrane integrity, changes cell permeability and disrupts critical cell functions thus impeding mycelial growth. The present findings support Sathiyabama and Muthukumar (2020) who showed antifungal activity of chitosan against different phytopathogenic fungi and found that the efficacy of chitosan is improved with the increase in concentration.

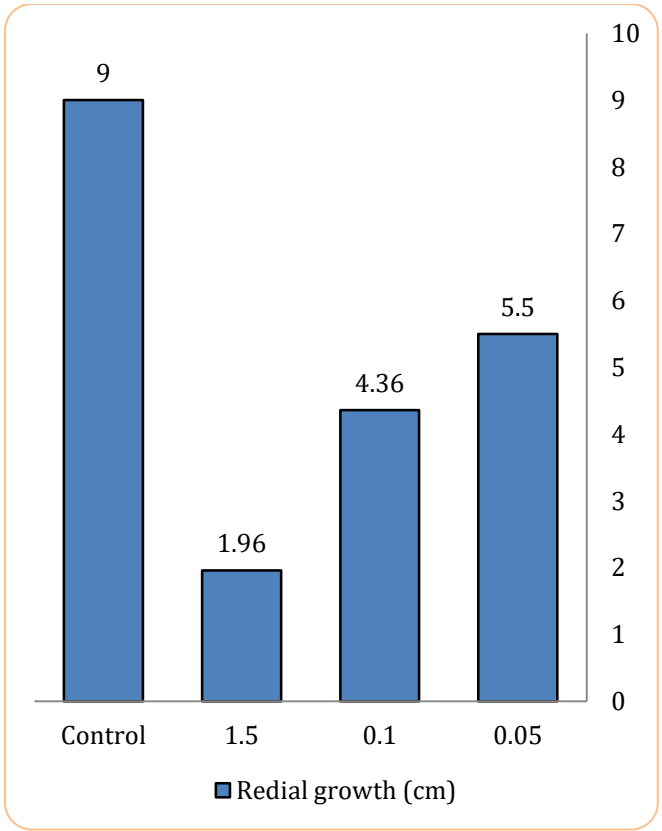


Figure 4. Influence of various concentrations of chitosan on radial mycelial growth (cm) of *R. solani* in culture media (LSD = 0.52).

Effect of combined organic and chemical treatments with chitosan microgel on the radial growth of *R. solani*

Inhibitory activity of the treatments composed of chitosan microgel against *R. solani* is presented in the results shown in Figure 5. Fungal growth was significantly inhibited by all the combinations of treatments with radial growths of 0 to 1.30 cm as compared with 9.0 cm in the untreated control. The 'chitosan microgel + Beltanol' treatment completely inhibited fungal growth with a radial growth of 0 cm. 'Chitosan microgel + Boron' showed a radial growth of 0.66 cm, while the 'chitosan microgel + copper' exhibited a radial growth of 1.30 cm. The combined treatment also completely checked the growth of fungus (0 cm).

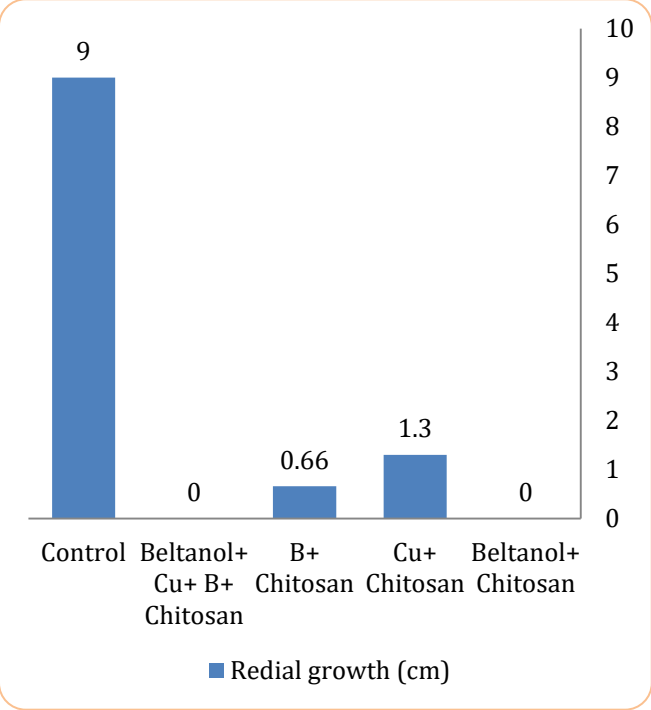


Figure 5. Radial growth (cm) of *R. solani in* culture medium under the influence of chitosan-based treatments (LSD = 0.20).

The improved inhibitory effect of the combined treatments could be due to synergistic or complementary effects between the different antifungal agents. The chemical and organic components might have supplementary antifungal effects via different mechanisms whereas chitosan might be responsible for the inhibition via disrupting cell membranes and other cellular processes. These combinations could thus be more effective in controlling *R. solani* than any of the individual components. Combining biological, chemical and organic agents has also been reported to improve the suppression of pathogens, and possibly activate plant defense mechanisms such as the induction of pathogenesis-related proteins and other defense-associated responses (Al-Mashhadani, 2022).

The results show that chitosan microgel, especially when used in conjunction with the selected chemical or organic treatments, has significant potential to inhibit the radial growth of *R. solani*. However, the proposed synergistic effects need to be substantiated by proper factorial experiments and interactions analysis using suitable statistics, and not only be assumed from the observed decrease in radial growth.

Conclusion

The tested treatments varied in their ability to suppress the radial growth of *R. solani in vitro*. Moncut, boron, organic copper, and chitosan showed notable inhibitory activities, while chitosan microgel combined with Beltanol completely inhibited fungal growth. These findings indicate the potential of chitosan-based combinations for managing *R. solani*.

Recommendations

The most promising treatments, particularly chitosan microgel + Beltanol, should be further evaluated under greenhouse and field conditions. Dose optimization, phytotoxicity, and interaction studies are also recommended to confirm their efficacy and practical suitability for integrated disease management.

Authors' Contributions

KSB and AAAA conceptualized and designed the experiment. WKA conducted the field investigations and carried out the experimental work. AAAA provided overall supervision and ensured methodological rigor and analytical consistency throughout the study. WKA also contributed as a co-supervisor, providing critical guidance on experimental design, data interpretation, and research refinement at various stages of the project. All authors critically reviewed the manuscript, contributed to its intellectual refinement, verified the data and analyses, and approved the final version for submission.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger
SDG 12: Responsible Consumption and Production
SDG 15: Life on Land

Policy Addressed

The findings support integrated pest management and sustainable pesticide-use policies by promoting chitosan-based formulations as potential biodegradable alternatives or complements to conventional fungicides. Field validation, dose optimization, and environmental safety assessment are recommended before practical adoption.

References

Abedalred, A., Al-Abedy, A.N., Al-Fadhal, F.A., 2019. Inhibitory capacity of Beltanol against various types of phytopathogenic fungi. International Journal of Agricultural Science 4, 151–157.

Aftean, S.H., Al-Jbory, A.A.A., 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.

Agrios, G.N., 2005. Plant Pathology, 5th ed. Elsevier Academic Press.

Ahmed, W.K., Mohammed, A.T., Al-Jbory, A.A.A., 2025. *In vitro* antifungal activity of *Capparis spinosa* extract against soil-borne plant pathogens. Plant Protection 9(02), 273–280.

Al-Jbory, A.A.A., Mohammed, K.A., Ismaeel, S.M., 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 Journal of Plant Protection 41(2), 190–196.

Al-Mashhadani, S.J., 2022. The effectiveness of integrated control in controlling some root rot pathogens on *Celosia argentea* (Doctoral dissertation, University of Karbala).

Dong, X., Zhao, B., 2006. Nested multiplex PCR-a feasible technique to study partial community of arbuscular mycorrhizal fungi in field-growing plant root. Science in China Series C: Life Sciences 49, 354-361.

Erper, I., Yıldırım, E., Türkkan, M., 2019. Antifungal effect of boron compounds against three *Rhizoctonia solani* AG-4 subgroups causing root and crown rot. Gesunde Pflanzen 71(1), 61–71.

FAO, 2023. World watermelon production and economic importance. Food and Agriculture Organization of the United Nations, Statistics Division.

Jaiswal, A.K., Elad, Y., Graber, E.R., Cytryn, E., 2017. *Rhizoctonia solani* suppression and plant growth promotion in biochar-amended soils. Plant and Soil 418(1), 313–325.

Jeffrey, C., 2001. Cucurbitaceae. In: Hanelt, P., et al. (Eds.), Mansfeld's Encyclopedia of Agricultural and Horticultural Crops, pp. 1510–1557.

Kheiri, A., Jorf, S.A.M., Malihipour, A., Saremi, H., Nikkhah, M., 2017. Synthesis and characterization of chitosan nanoparticles and their effect on *Fusarium* head blight and oxidative activity in wheat. International Journal of Biological Macromolecules 102, 526–538.

Liburdi, K., Benucci, I., Esti, M., 2016. Industrial, medical, and agricultural applications of chitosan: A review. Journal of Food Science and Technology 53(7), 2880–2892.

Madusanka, H.K.S., Aruggoda, A.G.B., Chathurika, J.A.S., Weerakoon, S.R., 2024. Evaluation of the antifungal effect of green synthesized metal oxide nanoparticles against plant pathogenic *Rhizoctonia* species. American Journal of Life Science and Innovation 3(2), 63–71.

Mahdi, M.M., Al-Jbory, A.A.R.A., Asmaeel, S.M., 2024. The effect of using hydrogel and some biological and chemical treatments on controlling charcoal rot disease in soybeans under field experiment conditions. IOP Conference Series: Earth and Environmental Science 1371(3), 032039.

Mahmoud, R.K., 2024. Effect of ZnO-NPs on *Rhizoctonia solani* causing root and stem rot on broad bean (*Vicia faba* L.). SABRAO Journal of Breeding and Genetics 56(6), 2461.

Mohammed, A.T., Ahmed, W.K., Al-Jbory, A.A.A., 2025. Biological activity of ethanolic extract from a new local isolate of *Morchella rufobrunnea* against some fungal pathogens. Plant Protection 9(3), 633–638.

Sathiyabama, M., Manikandan, A., 2018. Application of copper-chitosan nanoparticles stimulate growth and induce resistance in finger millet (*Eleusine coracana* Gaertn.) plants against blast disease.

Journal of Agricultural and Food Chemistry 66(8), 1784–1790.

Wick, R.L., Bade, M.L., 1988. Protecting crops and wildlife with chitin and chitosan. In: Cutler, H.G. (Ed.), Biologically Active Natural Products, pp. 110–125. American Chemical Society.

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