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Research Article

ASSESSMENT OF DIFFERENT TOMATO VARIETIES AND CHEMOTHERAPEUTIC COMPOUNDS FOR CONTROLLING ROOT ROT DISEASE CAUSED BY *RHIZOCTONIA SOLANI*

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Abstract

Solanum lycopersicum L., commonly known as tomato, is a vital vegetable crop with significant nutritional value but is susceptible to various pathogens causing diseases. Among all of these, *Rhizoctonia solani*-induced root rot is a highly destructive disease affecting tomato production and quality. This study aimed to identify resistant tomato varieties and effective chemical strategies for root rot management. Ten tomato varieties and advanced lines were evaluated under field conditions, and disease incidence was recorded. Nadir (11.80%) and Sundar (18.43%) exhibited resistance with the lowest disease incidence, while To-1057 f1 (25.87%), Ahmar (32.13%), and Litth-879-2 f3 (36.77%) showed moderate resistance. Moderately susceptible varieties included Rio-grandi (40.67%), Salar (47.90%), and Naqeeb (52.83%), while FS-2187 (75.90%) and Litth-861-4-17 (80.93%) were susceptible and highly susceptible, respectively. Additionally, six fungicides were tested under *in-vitro* and greenhouse conditions, with Dolomite, Nativo, and Evicine exhibiting the best performance. Further evaluation of these fungicides in field conditions confirmed Evicine effectiveness in reducing disease incidence. These findings emphasize the need for future research and development of resistant tomato cultivars and recommend the use of Evicine as an effective treatment against *Rhizoctonia solani* for disease management.

Keywords: *Rhizoctonia solani*, root rot, fungicides, *Solanum lycopersicum* L, resistant, screening, advanced line.
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1. INTRODUCTION

The tomato (Solanum lycopersicum L.), a well-known plant in the Solanaceae family, is a native of Central and South America. The second-largest vegetable crop in the world, it has gained prominence in cultivation in all tomato-growing regions due to its exceptional nutritional value and commercial significance (Abdussamee et al., 2014; Gao et al., 2010). The production of tomatoes increased dramatically in 2021, reaching 5,167 thousand hectares and producing a surprising 189 million tons of this essential crop. Pakistan made a major contribution, planting tomatoes on 168 thousands hectare and harvesting 802 thousands ton (FAOSTAT, 2021).

The tomato plays a key part in a variety of food products, from juice to soups, from canned treats to powerful sauces (Romdhane et al, 2023; Wu and Nelson, 2023). This is because the tomato's organic germplasm is enhanced by the presence of phenols, flavonoids, vitamin C, tocopherols, and antioxidants. Tomatoes encounter numerous difficulties, including over 200 diseases caused by worms, fungi, viruses, and bacteria. Among them, fungal diseases have shown themselves to be particularly potent foes, causing annual losses in the billions of dollars (Sultana et al., 2011).

R. solani, a notorious pathogen, stands out among the numerous fungi that pose a threat because it is the cause of the sneaky root rot disease that threatens agricultural output by up to 45% and has a significant negative effect on global food security (Hao et al., 2022; Yang et al., 2022). Recent discoveries have made it clear that there are over 200 species of host plants that R. solani can infect and cause specific indications. The symptoms of this root rot disease range from the pre-emergence stage, which is characterized by seed decay, to the post-emergence stage, which is characterized by the appearance of brown to reddish lesions on roots and stems that are located just below the soil line. Plant decline is the result of the spread of these lesions, with symptoms including leaf yellowing, wilting, and stunted growth that ultimately cause plant death (Sahar et al., 2016). Fairly moist soils and temperature around 23°C are ideal components for the development of disease (Zer et al., 2021).

In spite of its complication, root-rot requires the combination of chemical, biological and cultural approaches for the effective control. Dumping straw, soil management, Crop rotation, and the use of aseptic propagative materials are prominent examples of these methods. Moreover, chemicals are crucial for the wide-ranging control of disease along with the prevention and reduction of contaminations (Haque et al., 2021). In the quest for effective management solutions, investigations has discovered the prospective of resistant cultivars and fungicides. Such as, the fungicide Sedaxane has arose as a practical choice for seed treatment counter to R. solani (Panozzo et al., 2023). Parallel effects on root rot (R. solani) transmission and plant growth were perceived with thiamine and zinc sulphate, emphasizing the prospective of chemicals to shield tomatoes (Kheyri & Taheri, 2021). The seeds treatment of tomato to prevent R. solani directed to innovations in crop setting up, flowering consistency, seed weight, yield, and over-all plant growth (Jayaweera and Ray, 2023). Similarly, the effectiveness of chemical control in opposing R. solani is apparent, where the mixing of Carbendazim displayed substantial hang-up of the fungal pathogen, emphasizing the potential of chemical tactics in minimizing its impact (Paliwal et al., 2023). As a result, the usefulness of chemical control in contradiction of R. solani is shown in studies, where fungicides for instance Rizolex–T, Maxim-XL, and Moncut unveiled substantial inhibitory effects against the fungal pathogen. Jointly, these studies highlight the importance and potential of chemical control strategies in handling R. solani, posing a valuable method for disease control and crop protection (Aboelmagd et al., 2021).

Furthermore, the use of resistant-varieties is considered as a suitable, economical, durable and finest method which diminish the disease incidence and offer source of resistance (Kiptoo et al, 2021; Sales et al., 2011). However, in cases where resistant varieties are unavailable, farmers are compelled to use susceptible varieties. When diseases like R. solani occur suddenly and reach epidemic levels, chemical control becomes the only option. Therefore, this study aimed to evaluate various chemicals and resistant cultivars for their potential in combating R. solani under both laboratory and field conditions.

2. Materials and Methods

2.1. Isolation, purification and identification of pathogen

Samples were collected from established nursery of tomato plant that exhibited the typical symptoms of disease. For the isolation of pathogens, diseased samples were cut into 0.5 cm pieces along with healthy and infected part, surface sterilized with 1% sodium hypochlorite solution, placed on a potato dextrose agar (PDA) medium plate (amended with antibiotics), and incubated at 27 °C. After 24–48 hours of incubation, emerging colonies of fungus were identified as *R.*

solani on the basis of morphological and cultural features. Sub culturing was done using the single spore method (Brown, 1924) to acquire pure culture. Then a pathogenicity test was done to confirm Koch's postulates.

2.2. Evaluation of various tomato germplasm for resistance against Rhizoctonia *solani*

During 2020-2021, a disease-free nursery was established in the experimental area at the Department of Plant Pathology, University of Agriculture Faisalabad, to find out the resistant cultivars of tomato against root rot disease. The seedlings of 10 varieties/advanced lines obtained from the Vegetable Research Institute of AARI (Ayub Agriculture Research Institute) were used for this experiment. Each variety's nursery seedlings were grown on beds with three replications, following a Randomized Complete Block Design (RCBD). The spacing between plants (P x P) and rows (R x R) was maintained at 30cm and 70cm, respectively. All necessary agronomic practices were implemented to create a favorable environment for the crop. During the growing season, the plants were subjected to natural disease outbreaks. The disease rating scale developed by Abdeljalil et al., (2016) was utilized to assess the incidence of root rot in tomatoes, ranging from 0 to 5. A rating of 0 indicated no symptoms, 1 represented reddish-brown lesions on the root and stem near the soil line, 2 denoted the enlargement of lesions, 3 indicated sunken and girdling lesions on the root and stem, 4 represented necrosis and yellowing of leaves, and 5 indicated plant death. The disease incidence for each variety/advanced line was calculated using a specific formula.

Disease incidence (%) = (No. of infected plants/ Total no. of plants) × 100

Table: 1. Fungicides along with active ingredients and mode of action

Sr.#	Product	Active Ingredient	Mode of Action	References
1	Evicine	Sulphur	Disrupts transfer of electrons and cause formation of H_2S	(Möth et al., 2023)
2	Nativo	Tebuconazole 50%+ Trifloxystrobin 25% w/w WG (75 WG)	Disrupts fungal cell wall, Inhibits fungal respiration, preventing the growth and reproduction of plant pathogenic fungi.	(Shi et al., 2020)
3	Ridomil Gold	Mefenoxam	Hyper-systemic uptake and translocation properties	(Fentahun et al., 2023)
4	Dolomite	Magnesium & Calcium	Soil Treatment, pH Balance, Reduces Acidity	(Berg et al., 2020)
5	Ellectus	Azoxystrobin + Benzovindiflupyr	Inhibit fungal growth by targeting the succinate dehydrogenase mechanism of the citric acid cycle.	(Grichar and Meador, 2023)
6	Benlate	Benomyl+methyl1-2-benzimidazolecarbamate	Inhibits fungal growth by disrupting tubulin polymerization.	(Uddin et al., 2023)

2.3. Exploring the antifungal efficacy of chemicals against *Rhizoctonia solani*

Six different chemicals/fungicides, namely Evicine, Nativo, R-Gold, Dolomite, Ellectus, and Benlate (Table: 1.), were evaluated under *in vitro* conditions using the poisoned food technique (Falck, 1907). Stock solutions of each chemical were prepared, and three concentrations (0.5%, 0.75% and 1%) were created by adding them to distilled water. Filter paper discs were autoclaved and dipped in the different concentration solutions, then placed on *R. solani* culture plates. Each plate was replicated three times using a completely randomized design (CRD). The plates were then incubated at 27˚C for 24-96 hours. Control plates without any chemical/fungicides were also used for each concentration. The inhibition of the pathogen was measured using a Vernier Caliper in millimeters after 48, 72, and 96 hours.

2.4. Investigating the efficacy of chemical agents against Tomato root rot in greenhouse and field environments

The ability of five different chemicals to prevent the root rot disease in tomato plants (Nativo, R-Gold, Dolomite, Ellectus, and Evicine) was examined. In a greenhouse setting, this evaluation was carried out under carefully monitored circumstances. Complete randomization was used for the experiment's design (CRD). Three different chemical concentrations (0.5%, 0.75% and 1%) were applied to three separate replications through soil drenching at the commencement of disease symptoms. At one, two, and three week intervals, the effect of these treatments on the plants were thoroughly recorded. Three chemicals i.e. Dolomite, Nativo, and Evicine were then chosen for additional testing in the field based on the encouraging results shown in the greenhouse trial. These seedlings were transplanted in accordance with predetermined guidelines, assuring ideal plant-to-plant and row-to-row spacing. To reduce potential sources of bias in the field experiment, a Randomized Completely Block Design (RCBD) was used. To create a setting that would support reliable appraisal, all crucial cultural practices were strictly implemented. Each chosen chemical was applied via soil drenching once disease symptoms were visible, using the same three concentration levels, with a 15-day gap between applications. The incidence of disease was carefully monitored at predetermined intervals of 7 and 14 days after each application in both the greenhouse and field environment.

2.5. Statistical Analysis

The experimentally collected data was precisely documented and statistically analyzed using a Completely Randomized Design (CRD) for chemical evaluation in both laboratory and greenhouse conditions. On the other hand, the field experiment used a Randomized Completely Block Design (RCBD), and all statistical analyses were done with SAS statistical software (SAS Institute, 1990).

3. Results:

3.1. Evaluating varietal responses to root rot disease

Starting with lowest disease incidence Nadir had an 11.80% disease incidence and a resistance rating of 1, while Sundar had

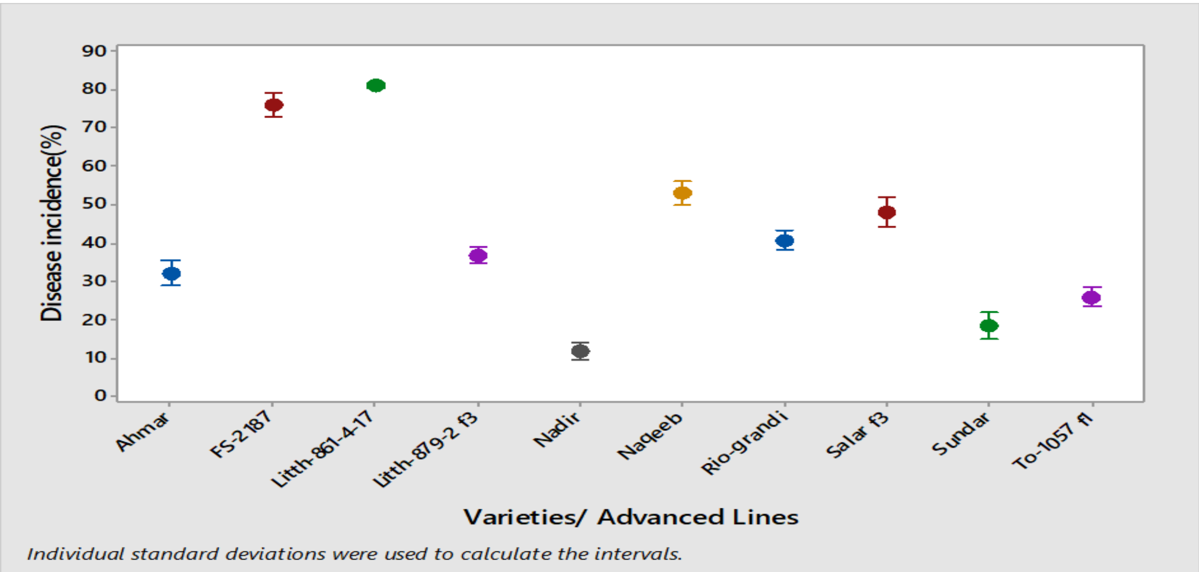

Figure 1. Response of different varieties/advanced lines against root rot disease under field conditions.

18.43% disease incidence with the same resistance rating. To-1057 f1 had a disease incidence of 25.87% followed by Ahmar (32.17%) and Litth-879-2 f3 (36.77%) with a rating of 2, suggesting a moderately resistant (MR) response. Moving on to higher disease ratings, Rio-grandi exhibited a disease incidence of 40.07%, followed by Salar (47.90%) and Naqeeb (52.83%) and a rating of 3, indicating a moderately susceptible (MS) response. Towards the higher end of the disease spectrum, FS-2187 had a disease incidence of 75.90% and a rating of 4, indicating a susceptible (S) response. Lastly, Litth-861-4-17 had the highest disease incidence of 80.93% and a rating of 5, representing a highly susceptible (HS) response Figure 1.

3.2. Management of root rot through chemicals under lab conditions

Results of this study revealed significant variations in fungal growth inhibition among the different treatments and concentrations tested. Evicine treatment demonstrated the most effective control, with the lowest fungal growth measurement of 18.24 mm. Dolomite, Nativo, R-Gold, Ellectus, and Benlate also demonstrated varying degrees of mycelial growth inhibition (24.1 mm, 32.34 mm, 36.78 mm, 44.73 mm, 48.80 mm, respectively) as shown in Figure 2.

Moreover, the treatment × concentration interaction underscored the effect of diverse concentrations on mycelial growth. Benlate exhibited the durable inhibitory effect, with mycelial growth measurements of 25.70, 16.64, and 12.40 mm at 0.5%, 0.75%, and 1% concentrations respectively. R-Gold, Ellectus, Dolomite, Nativo, and Evicine also confirmed inhibition depending on concentration, as revealed in Figure 3. Moreover, the treatment × duration interaction highlighted the influence of time on mycelial growth. Benlate demonstrated the quickest inhibition, with mycelial growth figures (20.71 mm, 17.91 mm, and 16.12 mm) after 24, 48, and 72 hours, respectively. R-Gold, Ellectus, Dolomite, Evicine and Nativo also established time dependent inhibition, as represented in Figure 4 and 5.

3.3. Management of root rot disease through chemicals under greenhouse condition:

The analysis of the different chemicals used in this study revealed variations in disease incidence among the treatments. Evicine demonstrated the lowest disease incidence at 19.15%, followed by Dolomite (22.72%), Nativo (23.28%), R-Gold (28.50%), Ellectus (34.01%), and the Control group (80.26%), as illustrated in Figure 6. The interaction between treatment and

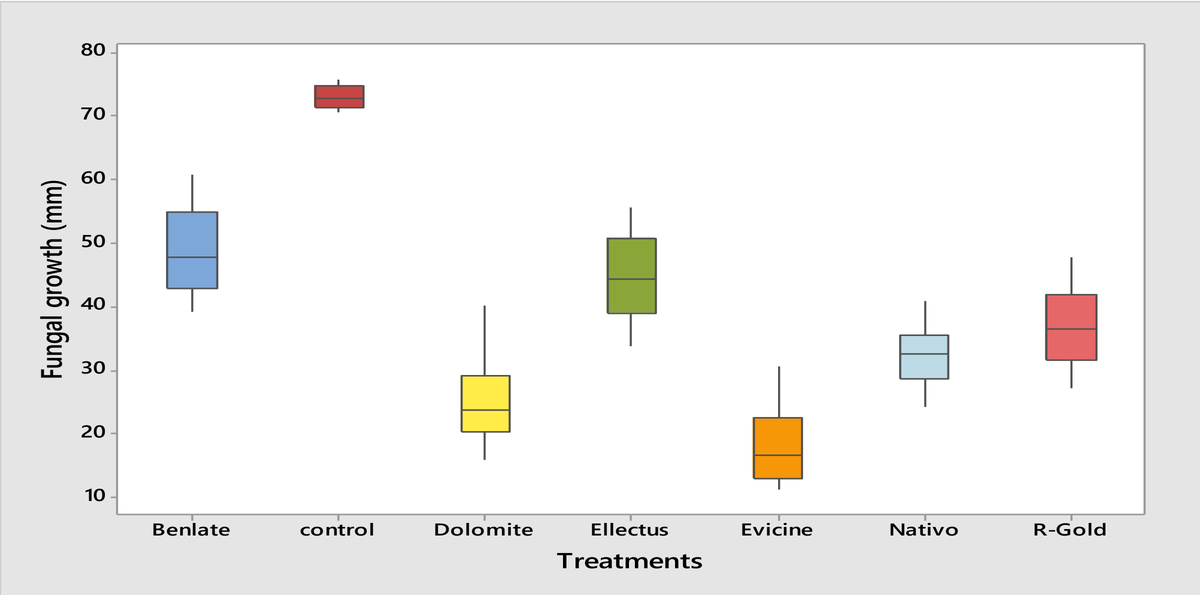

Figure 2. Response of different chemicals on fungal growth of Rhizoctonia solani under lab Conditions

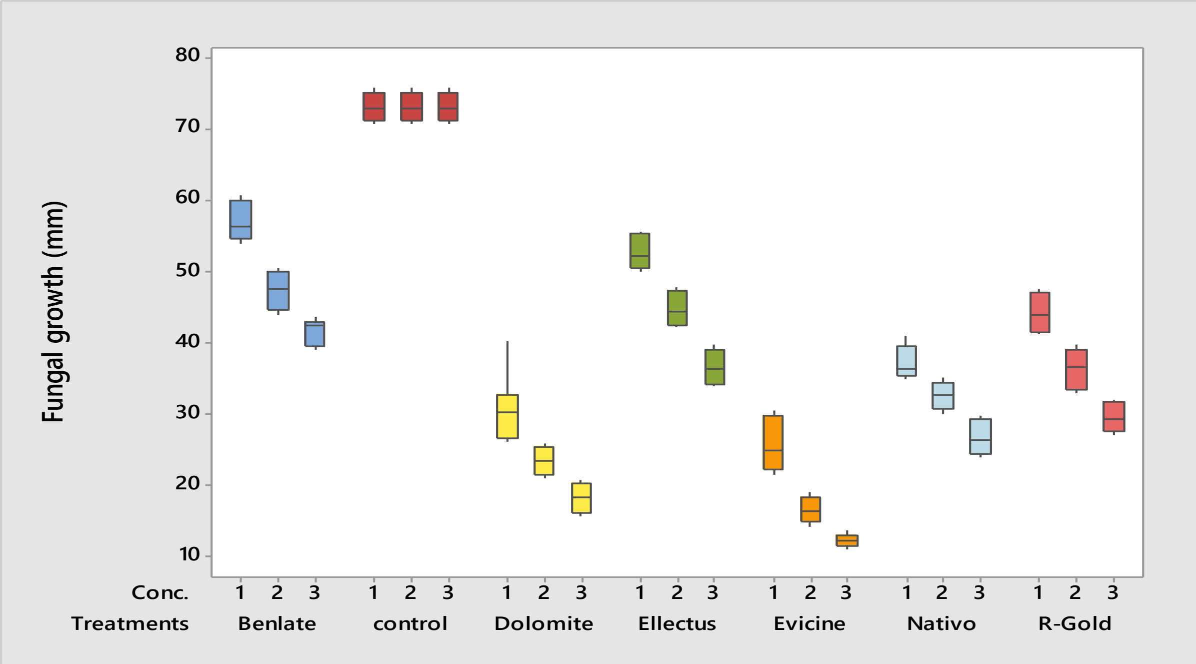

Figure 3. Response of different chemicals at different concentrations on fungal growth of Rhizoctonia solani under lab conditions.

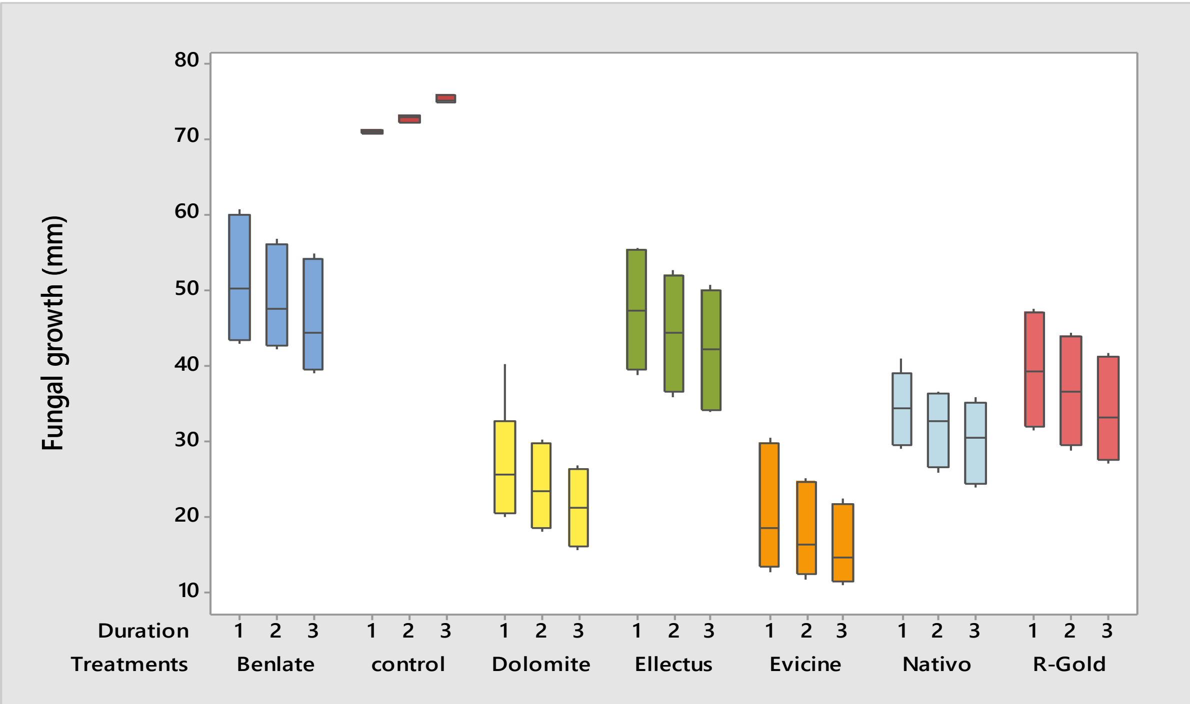

Figure 4. Response of different chemicals and duration on fungal growth of Rhizoctonia solani under lab conditions.

concentration further highlighted the impact of different concentrations on disease incidence. Evicine consistently exhibited the lowest disease incidence at all three concentrations, with measurements of 28.91%, 19.91%, and 8.64% at 0.5%, 0.75%, and 1% concentration, respectively. Dolomite, Nativo, R-Gold, and Ellectus also showed concentration-dependent reductions in disease incidence, as compared to the Control group, as shown in Figure 7. Additionally, the interaction between treatment and soil drenching demonstrated the influence of different soil

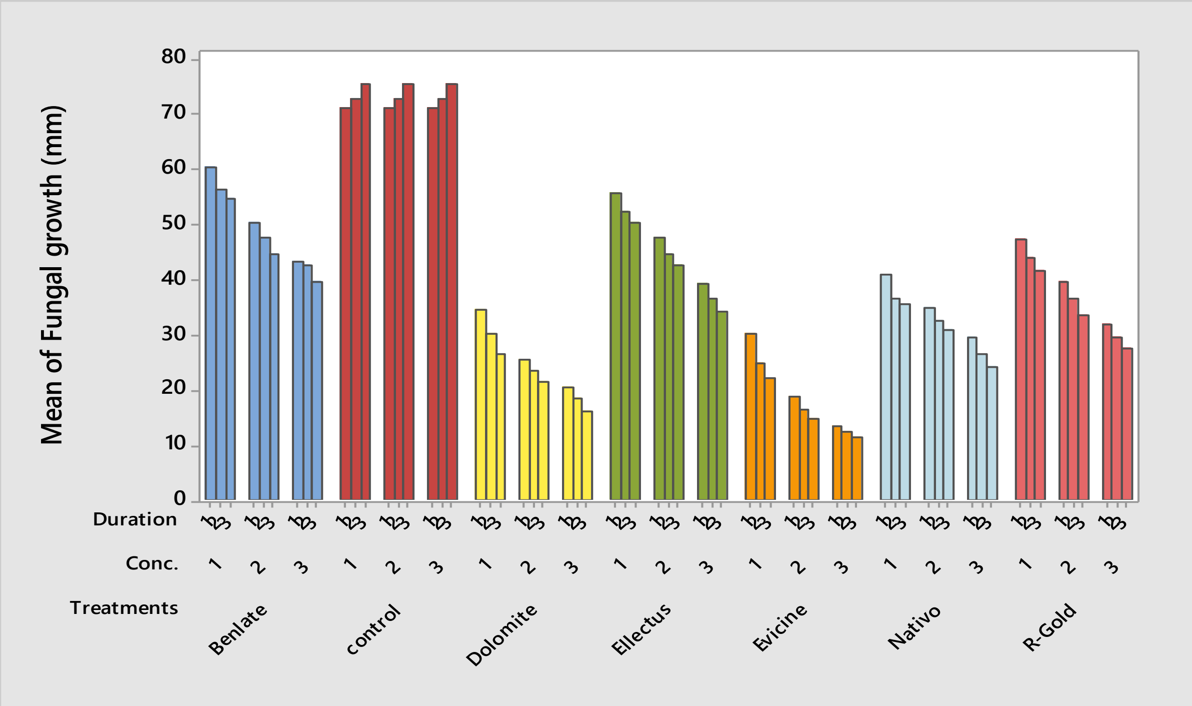

Figure 5. Response of different chemicals at different concentration on fungal growth of *Rhizoctonia solani* under lab conditions.

drenching applications on disease incidence. Evicine consistently showed the lowest disease incidence (22.41%, 18.33%, 16.72%) compared to the Control group (84.00%) after the 1st, 2nd, and 3rd soil drenching applications, indicating its effectiveness in reducing disease incidence through this method, as depicted in Figure 8.

3.4. Management of Rhizoctonia root rot through chemicals under field condition

The evaluation of various chemicals revealed variations in disease incidence, with Evicine exhibiting the lowest disease incidence at 16.70%, followed by Dolomite (22.77%) and Nativo (27.77%), as depicted in Figure 9. Furthermore, the interaction

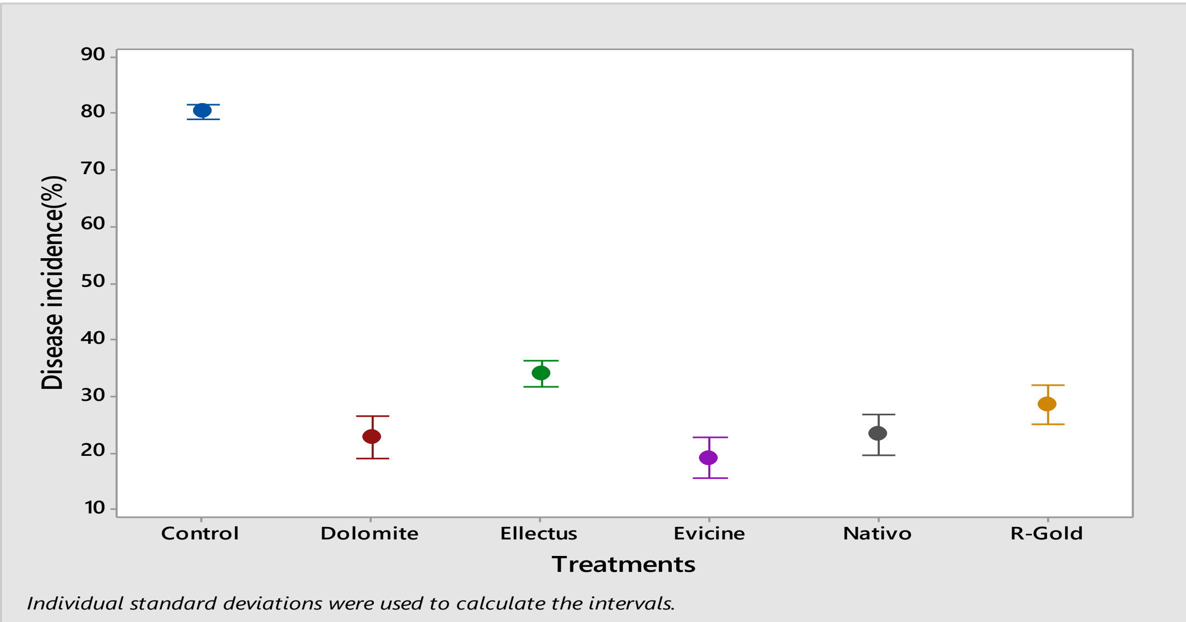

Figure 6. Response of different chemicals on disease incidence of Rhizoctonia solani under greenhouse conditions.

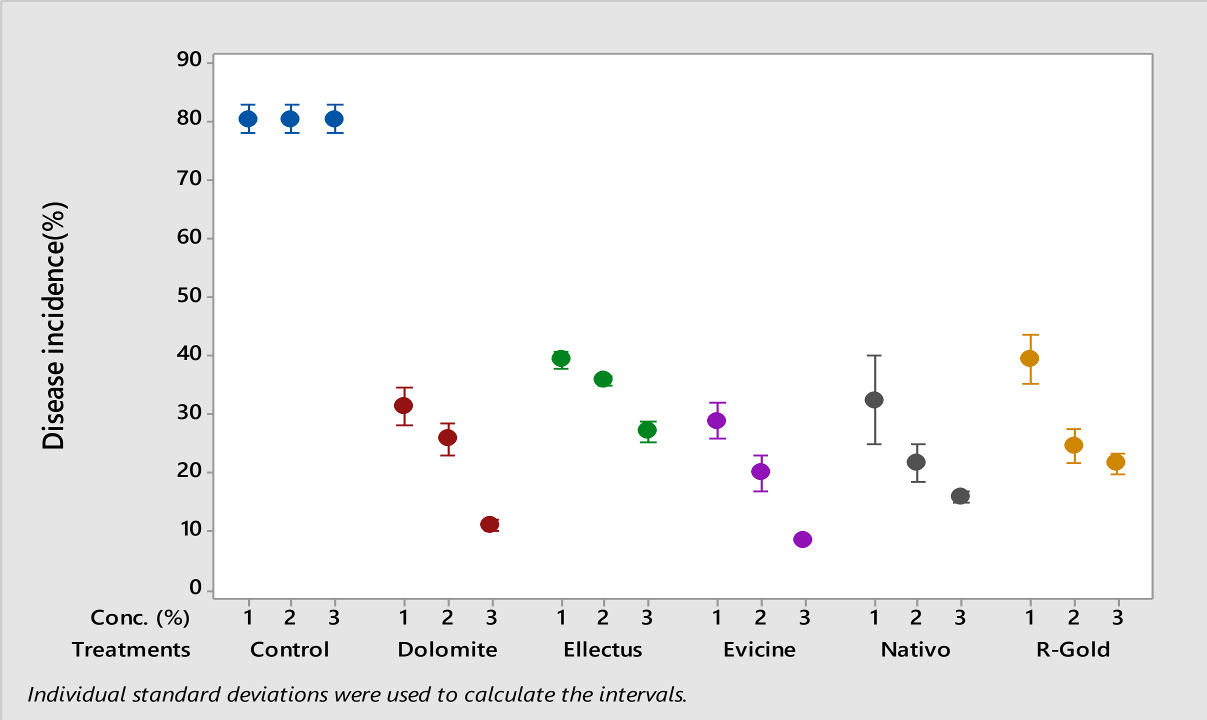

Figure 7. Response of different chemicals at different concentrations on disease incidence of Rhizoctonia solani under greenhouse conditions.

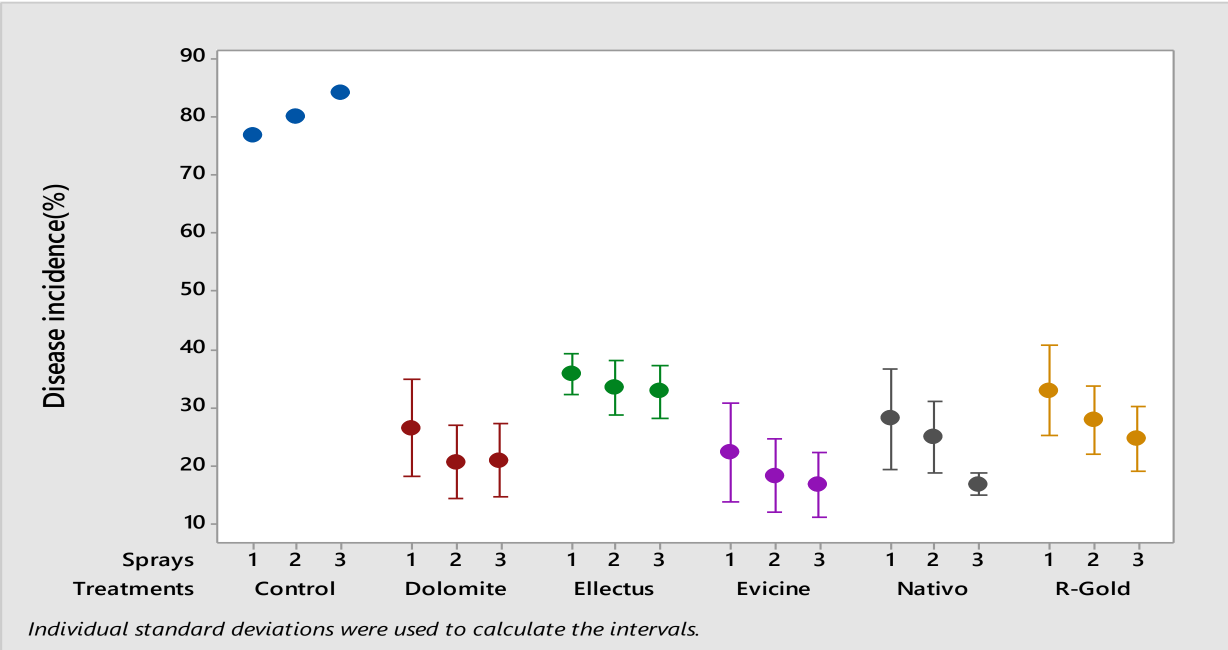

Figure 8. Response of different chemicals and Soil drenching on disease incidence of *Rhizoctonia solani* under greenhouse conditions.

between treatment and concentration demonstrated the impact of different concentrations on disease incidence. Evicine consistently displayed the lowest disease incidence (24.56%, 18.133%, and 7.40%) across the three concentrations of 0.5%, 0.75%, and 1%. Dolomite and Nativo also exhibited concentration-dependent reductions in disease incidence, with measurements of 32.24%, 25.17%, 10.90% and 35.94%, 32.05%, 13.67%, respectively, at the corresponding concentrations, as illustrated in Figure 10. Additionally, the interaction between treatment and soil drenching highlighted the influence of different soil drenching applications on disease incidence. Evicine consistently demonstrated the minimum disease incidence (20.50%, 15.60%, 14.00%) compared to the control group (74.20%, 80.00%, 86.00%) after the 1st, 2nd, and 3rd soil drenching applications, indicating its effectiveness in reducing disease incidence through this method, as shown in Figure 11.

4. DISCUSSION

Rhizoctonia root rot poses a significant threat to tomato production, as it is known as the most destructive disease affecting tomatoes (Maeda et al., 2023). In this study, various tomato genotypes were tested to assess their resistance to this devastating disease. The results revealed valuable information about the performance of different genotypes,

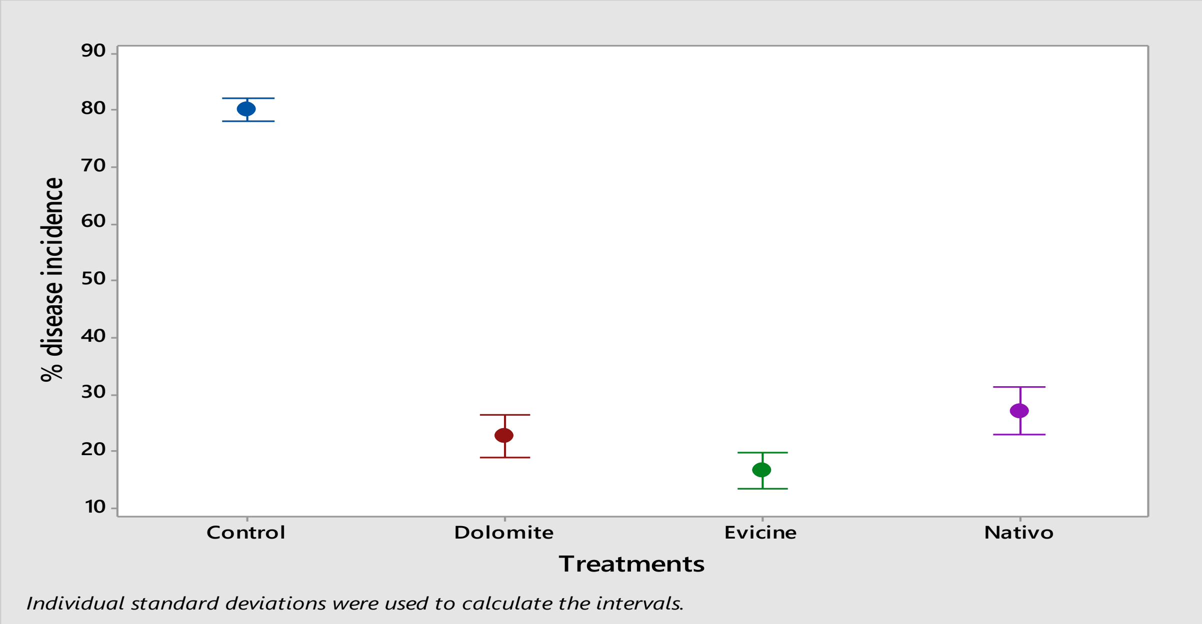

Figure 9. Response of different chemicals on disease incidence of Rhizoctonia solani under field

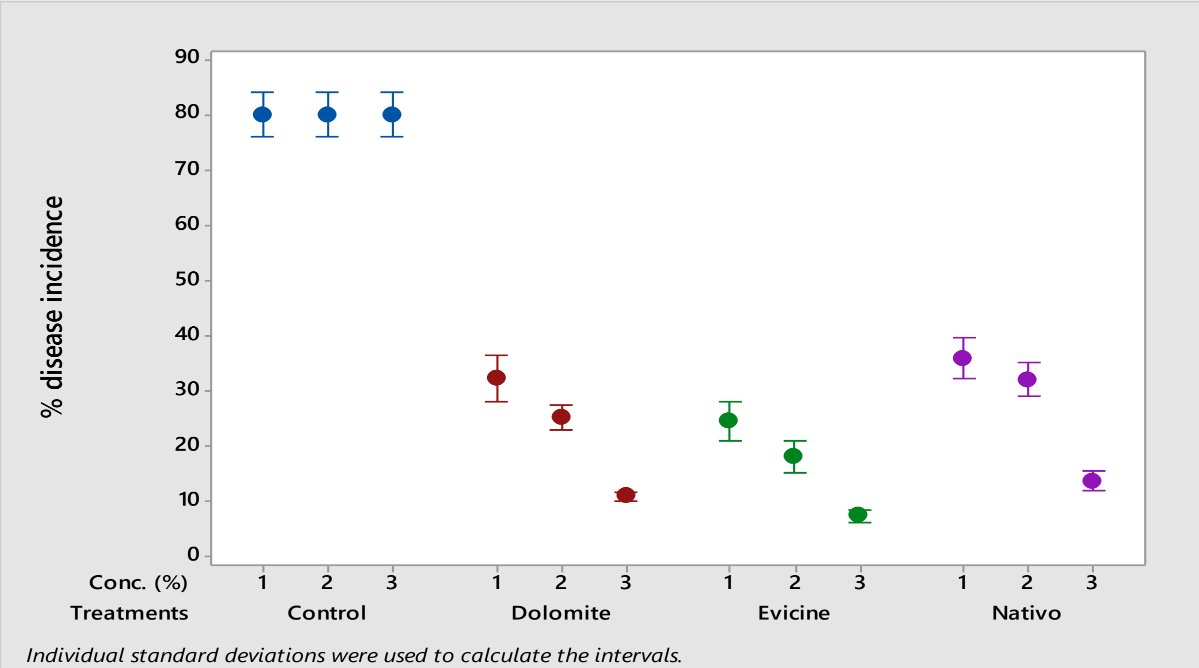

Figure 10. Response of different chemicals at different concentrations on disease incidence of *Rhizoctonia solani* under field conditions.

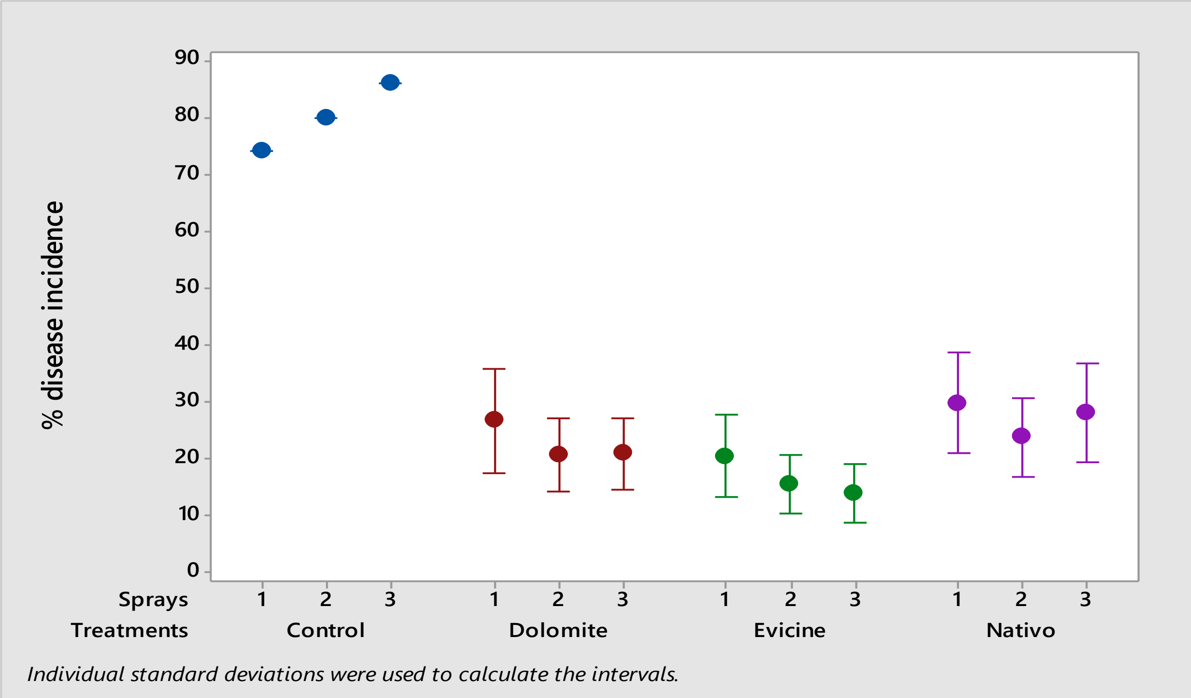

Figure 11. Response of different chemicals and Soil drenching on disease incidence of *Rhizoctonia solani* under field conditions

shedding light on their susceptibility or resistance to Rhizoctonia root rot. Among the tested varieties and advanced lines, Nadir and Sundar stood out as the most promising cultivars, exhibiting the lowest disease incidence of 11.80% and 18.43%, respectively. These genotypes were rated as resistant (R), indicating that they possess robust mechanisms to fend off the disease. On the contrary, Litth-861-4-17 showed the highest disease incidence at 80.93% and was rated as highly susceptible (HS), signifying its vulnerability to the disease. Other varieties, such as To-1057 f1, Ahmar, and Litth-879-2 f3, displayed moderate resistance (MR), while Rio-grandi, Salar, and Naqeeb demonstrated moderate susceptibility (MS). FS-2187, unfortunately, was classified as susceptible (S). To supplement these findings, we can refer to related studies conducted by experts in the field. Castoldi et al. (2016) conducted a greenhouse study focusing on screening tomato genotypes for resistance to *R. solani*. Although the specific genotypes evaluated in their study were not mentioned, their research aligns with our results, as the genotypes Nadir and Sundar displayed susceptibility to *R. solani*, consistent with Castoldi et al.'s findings.

In another study by Kubota et al. (2017), the researchers evaluated the field resistance of various tomato cultivars against *Rhizoctonia* crown rot, their research contributes to our understanding of tomato cultivar resistance against *R. solani*. Our results suggest that the genotypes To-1057 f1, Ahmar, and Litth-879-2 f3 demonstrate moderate resistance, which could potentially correspond to the resistant cultivars identified by Kubota et al. (2017). Similarly, Park et al. (2017) conducted a study screening tomato rootstocks for resistance to a specific strain of *R. solani*, AG2-2IIIB. Despite the fact that our experimental outcomes didn't remark the specific *R. solani* strain, we can conclude that the genotypes experienced in Park et al.'s research displayed resistance to the pathogen. Our results designate that Salar, Rio-grandi and Naqeeb confirmed modest susceptibility, telling that the resistant root-stock varieties recognized by Park et al. (2017) might not contain these specific genotypes. Moreover, Gao et al. (2018) executed a screening experiment to evaluate the resistance of different tomato cultivars against *R. solani* under field conditions. Their experimental discoveries offer valuable understandings into the resistance of tomato cultivars in contradiction of the pathogen. Our outcomes bring into line with Gao et al.,'s research, demonstrating that Salar, Naqeeb and Rio-grandi exhibited modest susceptibility to *R. solani*, strengthening the perception that these cultivars might be susceptible to the pathogen under open field conditions.

Adding to the previous investigations, Li et al. evaluated the resistance of tomato genotypes against both *R. solani* and *Pseudomonas syringae* pv. Comparing our test results with their findings, we observe that FS-2187 and Litth-861-4-17 displayed the highest levels of resistance to *R. solani*. These genotypes hold promise for resistance breeding programs targeting the pathogen, (Li et al., 2020). This study highlights the threat of Rhizoctonia root rot in tomato production and offers valuable insights into the performance of different genotypes in greenhouse and field conditions. Incorporating previous research strengthens our findings and establishes connections with prior studies in the field. The present study evaluated the *In-vitro* impact of different chemical treatments on the colony growth of *R. solani*. Among the tested treatments, Benlate and Ellectus emerged as the best performers, exhibiting significant inhibition of fungal growth. These findings align with research conducted by Derbalah et al, (2022) that investigated eco-friendly control agents against *R. solani*, where the best-performing treatment was carvone. Unquestionably, the versatility of Azoxystrobin (Ellectus) in combatting fungal infections in tomato and rose highlights its broad-spectrum antifungal efficacy (Qaisar et al., 2023). The results from both studies highlight the potential effectiveness of chemical treatments like Benlate and Ellectus, as well as Carvone and Recado in managing *R. solani*.

Furthermore, in a distinct research by Abdel-Rahman et al. (2023), Moncut was recognized as the utmost effective fungicide counter to *R. solani*. This discovery is consistent by our outcomes, where Benlate established the maximum inhibitory consequence on the growth of *R. solani*, tracked by Ellectus and R-Gold. These outcomes propose that Benlate and Moncut display potential for controlling *R. solani*. The shared indication from these investigations emphasize the status of choosing suitable fungicides, such as Moncut and Benlate, to successfully inhibit *R. solani* infections. Additionally, in the investigations directed by Abdussamee et al. (2014), Difenoconazole exhibited the maximum inhibition of pathogenic growth, whereas in our findings, Benlate and Ellectus appeared as the best performing fungicides, showing substantial inhibition of *R. solani*. These results propose that Benlate, Difenoconazole, and Ellectus embrace potential as effective fungicides in lieu of controlling *R. solani*. The management of *R. solani* was assessed in both glasshouse and field conditions. In the glasshouse, numerous fungicides including Dolomite, Evicine, R-Gold, Ellectus and Nativo showed major control of the disease, with Evicine by way of the supreme effective with the lowermost disease-incidence at 19.156%.

These results were consistent with the outcomes achieved by Gouda et al. (2021), who assessed fungicides in rice crops. They found that tebuconazole + trifoxystrobin, Carbendazim, propiconazole and hexaconazole were also effective in minimizing disease caused by *R. solani*. As well, El-Kholy et al. (2021) explored the effectiveness of fungicides in controlling root rot disease of common-bean caused by *R. solani*. Their findings exposed that fungicides i.e. Maxim XL 3.5% FS, Rizolex-T 50% WP and Tendro 40% FS successfully lowered damping-off and rotted roots, leading to enhanced plant survival. These results align with our research findings, emphasizing the prospective of these fungicides in handling root rot in common bean crops caused by *R. solani*. Likewise, Rahman et al. (2020) assessed the worth of fungicides, including RhizoGuard 45% EC, Fungimax 500 SC and SolaniTrol 25% WP, against root rot induced by *R. solani*. Their investigation correspondingly established momentous decreases in disease incidence and severity, further highlighting the success of these fungicides in controlling diseases associated with *R. solani*.

5. Conclusion

The research conducted on managing *R. solani* on tomato crops has generated valued perceptions. Capable tomato genotypes like Nadir and Sundar have established resistance to root-rot disease, whereas chemical treatments such as Benlate and Ellectus have exposed their ability to inhibit fungal growth. Fungicides like Dolomite, Nativo and Evicine have confirmed efficacy in both glasshouse and field conditions. Imminent research should focus on the mechanism of genetic-resistant, monitoring fungicide usefulness and resistance, discovering constructive microorganism-interactions, and examining alternative control ways and means. These exertions will add to improved disease controlling and sustainable agriculture.

6. Conflict of Interest

The authors declared that there is no conflict of interest.

7. Acknowledgement:

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8. Author's Contribution:

All authors contributed equally.

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