OPEN ACCESS Plant Protection, 08 (04) 2024. 781-787 DOI:10.33804/pp.008.04.5528

Check for updates

Available Online at EScience Press
Plant Protection
ISSN: 2617-1287 (Online), 2617-1279 (Print)
http://esciencepress.net/journals/PP

Research Article

INTEGRATED MANAGEMENT OF TOMATO GRAY MOLD DISEASE USING SELECTIVE PLANT EXTRACTS AND RHIZOBACTERIA

[a]Rana Javaid Iqbal, [b]Ghulam Hussain Jatoi, [c]Aqeel Ahmad, [d]Ayesha Lutf, [e]Qasid Hussain, [f]Muhammad Nawaz Sukhera, [g]Muhammad Riaz Khan, [h]Saima Rafiq, [i]Tayyab Shehzad

[a] Rapid Soil Fertility Survey and Soil Testing Institute, Thokar Niaz Beg, Lahore, Pakistan.
[b] Department of Plant Pathology, Sindh Agriculture University, Tandojam, Pakistan.
[c] Department of Agronomy, University of Poonch, Rawalakot, Azad Jammu and Kashmir, Pakistan.
[d] Institute of Food Science and Nutrition, University of Sargodha, Sargodha, Pakistan.
[e] PARC-Agricultural Research institute, Jaffarbad, Pakistan.
[f] Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan.
[g] Department of Horticulture, University of Poonch, Rawalakot, Azad Jammu and Kashmir, Pakistan.
[h] Department of Food Science and Technology, University of Poonch, Rawalakot, Azad Jammu and Kashmir, Pakistan.
[i] Department of Plant Breeding and Genetics, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan.

ARTICLE INFO

Article history
Received: 13th October, 2024
Revised: 23rd December, 2024
Accepted: 29th December, 2024

Keywords
Azadirachta indica
Botrytis cinerea
Bacillus subtilis
Turmeric
Garlic
Integrated disease management

ABSTRACT

Botrytis cinerea is one of the most significant plant pathogens globally, causing extensive damage to fruits and vegetables. Botrycides remain a widely used method of plant protection; however, the introduction of plant-beneficial bacteria offers a promising and agroecologically sustainable alternative. The current study evaluated the effectiveness of plant extracts and rhizobacteria in controlling diseases caused by *B. cinerea* in tomatoes. Neem (*Azadirachta indica*), garlic (*Allium sativum*), and turmeric (*Curcuma longa*) extracts were tested alongside rhizobacterial strains (*Bacillus subtilis*, *Pseudomonas fluorescens*, and *Trichoderma harzianum*). *In vitro* experiments showed that, at a 15% extract concentration, neem and garlic inhibited fungal growth by 78% and 72%, respectively, while *B. subtilis* suppressed mycelial growth by 62%. Synergistic treatments, particularly neem combined with *B. subtilis*, exhibited the highest inhibition zone (22 mm) and an 85% synergy effect. Greenhouse trials demonstrated that neem extract combined with *B. subtilis* significantly reduced disease severity, achieving a disease severity index (DSI) of 1.3 and a 75% disease reduction compared to controls, which had a DSI of 4.8. Neem extract and *B. subtilis* alone reduced disease by 52.1% and 58.3%, respectively. The combination of neem extract and *B. subtilis* proved more effective in managing grey mold, promoting healthier plants, and providing a potential chemical-free alternative to fungicides. Further field trials are recommended to develop scalable, eco-friendly solutions for sustainable tomato disease management.

Corresponding Author: Tayyab Shehzad
Email: tshehzad173@gmail.com

INTRODUCTION

Tomato (*Solanum lycopersicum*) is one of the most widely cultivated and consumed vegetables worldwide, valued for its nutritional and economic importance

(FAO, 2022). However, the crop is highly vulnerable to various diseases, among which gray mold caused by *Botrytis cinerea* is one of the most destructive. This pathogen is notable for its broad host range and its ability to cause substantial yield losses under favorable environmental conditions (Williamson et al., 2007; AlKahtani et al., 2020).

The conventional management of gray mold predominantly relies on synthetic fungicides. Although effective, these chemicals pose significant environmental and health risks and can promote the emergence of fungicide-resistant strains of *B. cinerea* (Iqbal and Mukhtar, 2020; Mamiev et al., 2020; Almasaudi et al., 2022). As a result, there is growing interest in eco-friendly and sustainable disease management strategies.

B. cinerea is classified as a hemibiotrophic fungus due to its biotrophic phase, during which it colonizes living plant tissues and derives nutrients from viable plant cells. However, it is often categorized as a necrotrophic pathogen because it predominantly kills plant tissue to extract nutrients from dead cells (Agrios, 2005; Fillinger and Elad, 2016; Ahmed et al., 2018).

Plant extracts have gained significant attention as natural alternatives to synthetic fungicides (Hussain et al., 2011; Kayani et al., 2012; Mukhtar et al., 2013a; Iqbal et al., 2014; Saeed et al., 2021). Neem (*Azadirachta indica*), garlic (*Allium sativum*), and turmeric (*Curcuma longa*) are well-known for their antimicrobial properties. Neem extracts contain azadirachtin, a compound with notable antifungal activity (Bello and Sisterna, 2010; Goel et al., 2016; Bashir et al., 2020). Garlic extract is rich in allicin, a sulfur-containing compound with broad-spectrum antimicrobial effects (Gurvinder et al., 2023). Similarly, curcumin in turmeric exhibits potent antifungal properties, making it a promising candidate for plant disease control (Hajji-Hedfi et al., 2023).

Rhizobacteria, particularly *Bacillus subtilis* and *Pseudomonas fluorescens*, have demonstrated potential as biological control agents against various plant pathogens (Mukhtar et al., 2013b; Saeed et al., 2023; Aziz et al., 2024). These bacteria suppress pathogens through mechanisms such as producing antifungal compounds, competing for nutrients, and inducing systemic resistance in plants (Wang et al., 2009; Paz et al., 2018).

The combined use of plant extracts and rhizobacteria offers a synergistic effect, enhancing disease suppression while promoting plant growth. This integrated approach aligns with sustainable agricultural practices by reducing reliance on chemical inputs (Azeem et al., 2021).

The present study evaluates the efficacy of selected plant extracts and rhizobacteria, individually and in combination, for the integrated management of gray mold in tomato. By focusing on eco-friendly strategies, this research contributes to the advancement of sustainable agricultural practices.

MATERIALS AND METHODS

Plant materials and extracts

The selected plant materials included neem (*Azadirachta indica*), garlic (*Allium sativum*), and turmeric (*Curcuma longa*). Fresh neem leaves, garlic bulbs, and turmeric rhizomes were used to prepare the extracts. The botanical materials were thoroughly washed and ground into a fine paste to facilitate the preparation of aqueous extracts. These extracts were thoroughly stirred and left for 24 h to allow the active compounds to be extracted.

Pathogenic and rhizobacterial isolates

Identified pathogenic fungal isolates of *Botrytis cinerea* and rhizobacterial isolates, including *Bacillus subtilis* and *Pseudomonas fluorescens*, as well as *Trichoderma harzianum*, were obtained from the Department of Plant Pathology, Sindh Agriculture University, Tandojam. These isolates were used to evaluate their individual and synergistic effects on disease suppression.

In vitro efficacy of plant extracts

The antifungal properties of the plant extracts from neem, garlic, and turmeric were evaluated *in vitro* against *B. cinerea* using the poisoned food technique. Plates containing potato dextrose agar (PDA) were supplemented with each plant extract. A 5 mm mycelial disc of *B. cinerea* was inoculated onto the plates containing the treated media, while control plates without plant extracts were prepared for comparison. The plates were incubated at 25 ± 2°C for 5-7 days to monitor fungal growth. The percentage inhibition of mycelial growth relative to the control was calculated, providing a quantitative measure of the antifungal efficacy of the plant extracts.

Antagonistic potential of rhizobacteria

A dual culture assay was performed to evaluate the antifungal potential of *B. subtilis*, *P. fluorescens*, and *T. harzianum*. PDA plates were inoculated with a mycelial disc of *B. cinerea* and streaked with the rhizobacterial strains alongside the fungal disc. The inoculated plates were then incubated at 25 ± 2°C for 5-7 days to assess the zone of inhibition.

Synergistic effects of combined treatments

The synergistic effects of combined treatments involving plant extracts and rhizobacterial strains against *B. cinerea* were evaluated. Plant extract was incorporated into the media plates, and each rhizobacterial species was streaked 2 cm away from the central mycelial disc of *B. cinerea* on each plate. The plates were incubated at 25 ± 2°C for 5-7 days, and fungal growth was measured in terms of colony diameter.

Disease assessment under greenhouse conditions

Disease assessment was conducted to evaluate the effectiveness of the treatments against *B. cinerea* in tomato plants under greenhouse conditions. The disease severity index (DSI) was calculated 14 days after treatment application to quantify disease progression. Disease severity was rated on a scale of 0-5, where 0 indicated no symptoms and 5 represented severe infection. The DSI was computed using the following formula:

$$\mathrm{DSI}(\%) = \frac{\sum(\text{disease rating} \times \text{number of plants in each category})}{(\text{total plants} \times \text{maximum rating})} \times 100$$

Statistical analysis

The data were analyzed statistically using ANOVA and LSD tests (Steel et al., 1997) with the software Statistix 8 (version 8.1).

RESULTS

***In vitro* efficacy of plant extracts**

The results showed that all the tested plant extracts, namely neem, garlic, and turmeric, exhibited inhibition against mycelial growth; however, their efficacy varied. Neem extract demonstrated the highest inhibition rate of 78%, indicating its potent antifungal potential, followed by garlic extract with a 72% inhibition rate.

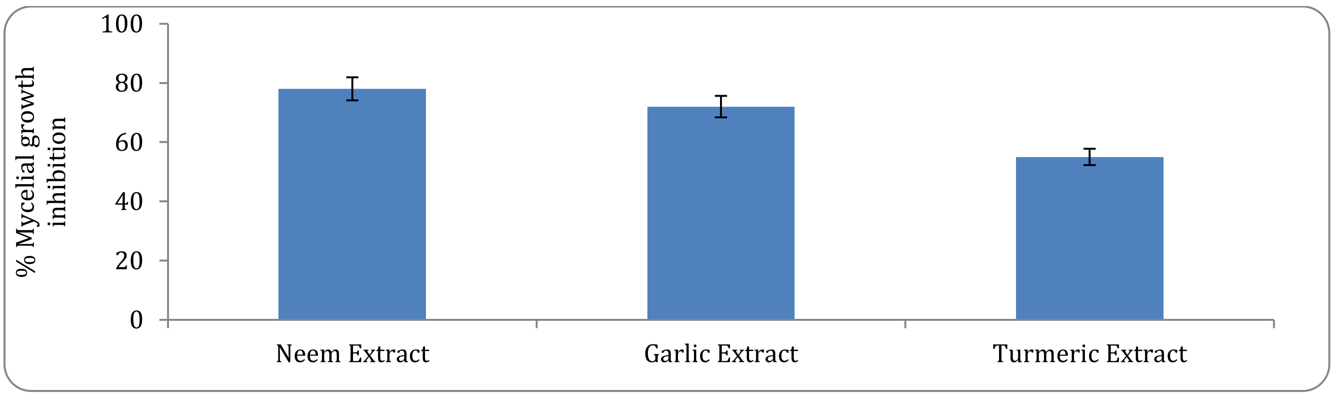

Figure 1. *In vitro* efficacy of plant extracts on mycelial growth of *Botrytis cinerea.*

Antagonistic potential of rhizobacteria

B. subtilis exhibited the highest mycelial growth inhibition, achieving a 62% reduction in fungal growth and demonstrating the strongest antagonistic effect. *P. fluorescens* also showed substantial inhibition, with a 54% reduction in mycelial growth, indicating a moderate antagonistic potential. In contrast, *T. harzianum* was the least effective, causing only a 48% reduction in mycelial growth (Figure 2). These findings suggest that *B. subtilis* is the most effective rhizobacterium for inhibiting the growth of *B. cinerea*.

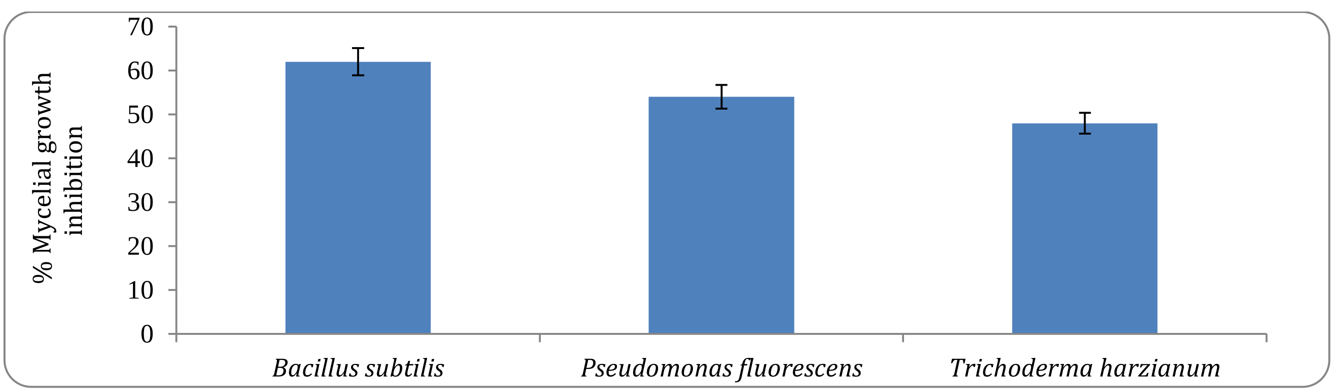

Figure 2. Antagonistic potential of rhizobacteria against *Botrytis cinerea.*

In vitro synergistic effects of combined treatments

The synergistic effects of combined treatments on fungal growth inhibition were evaluated under *in vitro* conditions. The results revealed that combinations of plant extracts and beneficial microorganisms exhibited enhanced antifungal activity compared to individual treatments. In particular, individual treatments with plant extracts demonstrated moderate to low inhibition of fungal growth. Among the plant extracts, neem extract was the most effective, producing an inhibition zone of 12 mm, which corresponded to a 50% synergistic effect. The beneficial microorganisms, *B. subtilis* and *P. fluorescens*, also showed notable antifungal activity, with inhibition zones of 15 mm and 11 mm, corresponding to synergistic effects of 60% and 45%, respectively. Notably, the combination of neem extract with *B. subtilis* exhibited the highest level of fungal growth inhibition, with an inhibition zone of 22 mm and an 85% synergistic effect (Figure 3).

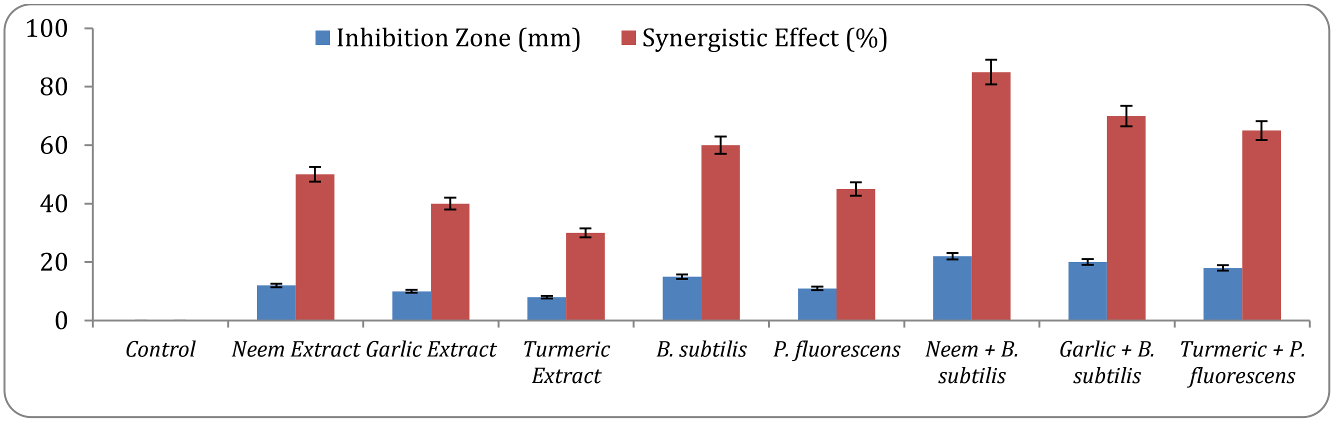

Figure 3. Combined treatment effects on disease inhibition.

Disease control under greenhouse conditions

The lowest disease severity was recorded in plants treated with the combined application of neem extract and *B. subtilis*, with DSI of 1.3 on a 0-5 scale, compared to 4.8 in the untreated controls. This integrated approach significantly improved plant health and growth, highlighting its potential as a sustainable alternative to chemical fungicides.

Figures 4 and 5 illustrate the disease severity and reduction percentages of the treatments evaluated under greenhouse conditions. The efficacy of treatments involving plant extracts varied, with neem extract being the most effective among them, achieving a DSI of 2.3 and a disease reduction of 52.1%. Among microbial treatments, *B. subtilis* was highly effective, with a DSI of 2.0 and a disease reduction of 58.3%, indicating substantial inhibition of fungal growth.

The most significant disease suppression was observed in the combination of neem extract and *B. subtilis*, which resulted in a DSI of 1.3 and a disease reduction of 75.0%, indicating near-complete control of the disease.

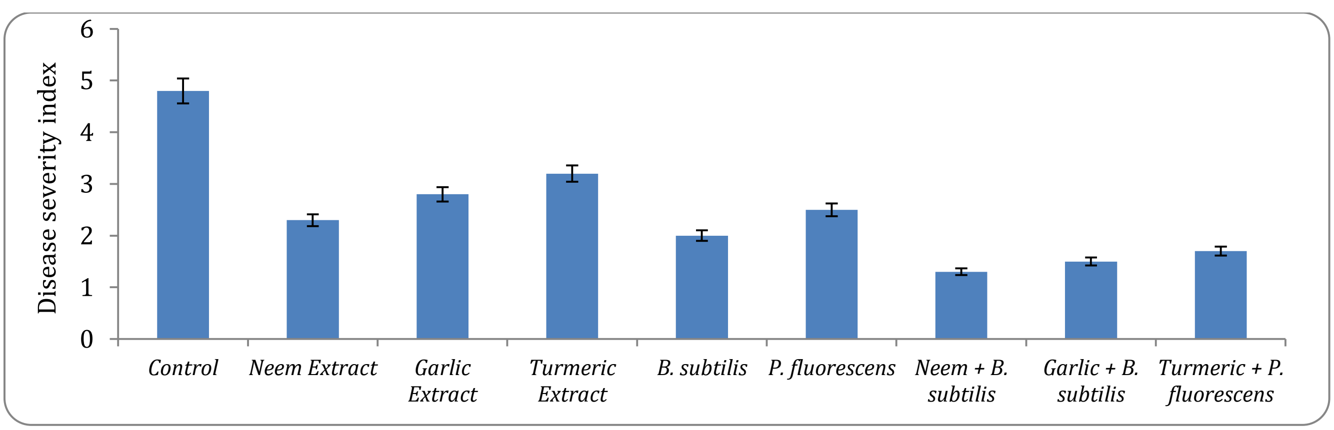

Figure 4: Evaluation of disease severity under greenhouse conditions.

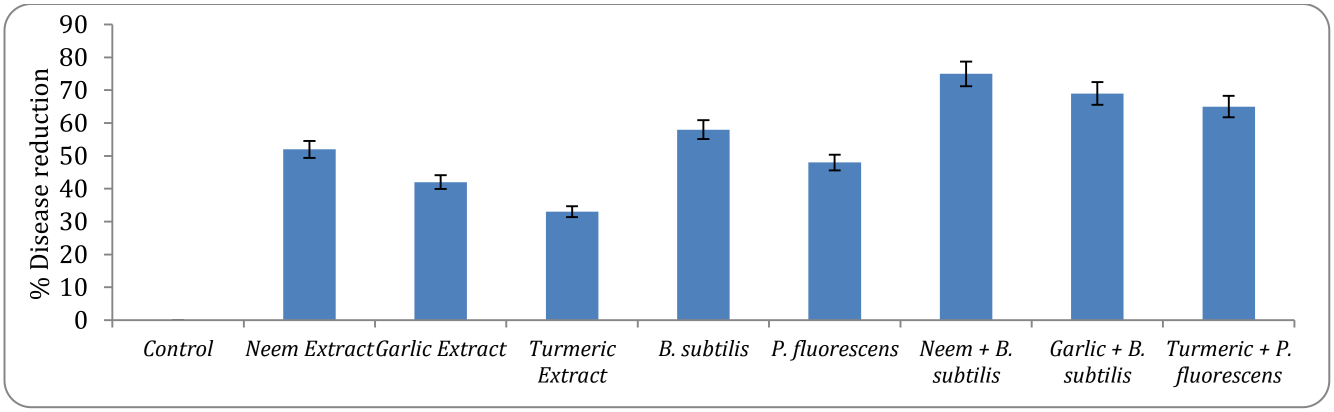

Figure 5. Evaluation of disease reduction under greenhouse conditions.

DISCUSSION

The results of the present study highlight the potential of integrating plant extracts and rhizobacteria as effective strategies for managing gray mold caused by *B. cinerea* in tomato plants. The *in vitro* analysis revealed that neem and garlic extracts were particularly effective, inhibiting fungal growth by 78% and 72%, respectively. These findings align with previous research demonstrating that azadirachtin, a key compound in neem, possesses antifungal properties, while allicin in garlic disrupts fungal cell membranes and inhibits spore germination (Wamani, 2020).

Moreover, the findings of this study are consistent with earlier research emphasizing the pathogenicity of *B. cinerea* on various horticultural crops, including bell peppers. For instance, Williamson et al. (2007) and Wamani (2020) identified *B. cinerea* as a widespread and highly aggressive pathogen capable of infecting a broad range of plant hosts, often causing significant postharvest losses due to gray mold development. Similarly, the results of this study corroborate these observations, as all inoculated fruits displayed distinct symptoms of rot and fungal growth characteristic of *B. cinerea* infection.

Similarly, the antagonistic activity of rhizobacteria was highest in *B. subtilis*, which reduced fungal growth by up to 62%. Its efficiency has been attributed to the production of antifungal compounds, competition for nutrients, and the systemic induction of resistance in host plants. These results are consistent with those previously reported, which highlighted the effectiveness of *B. subtilis* as a biocontrol agent against fungal pathogens (Karthika, 2020; Li et al., 2022).

The synergistic treatment, which combined neem extract with *B. subtilis*, resulted in an inhibition zone of 22 mm and an 85% synergistic effect *in vitro*. This suggests a potential for improved efficacy when combining plant extracts with rhizobacteria. Similar results were reported by Orozco-Mosqueda et al. (2023), who demonstrated that *Bacillus* species, known for their broad antifungal activities and plant growth-promoting properties, have been extensively studied. Moreover, Jiang et al. (2018) showed that two strains of *B. velezensis*, 5YN8 and DSN012, can significantly control pepper gray mold caused by *B. cinerea* and promote the growth of *Capsicum frutescens*. Their biocontrol mechanisms include the production of secondary metabolites and volatile organic compounds, which prevent fungal growth and spore formation. Furthermore, these strains induce plant immunity by upregulating genes such as NPR1, PR1, PIN2, TIN1, and peroxidase-related genes.

The results are consistent with prior research highlighting the pathogenic and economically detrimental impacts of *B. cinerea*. Although chemical fungicides are currently in use, their harmful environmental effects, coupled with the cultivation of resistant fungi, have spurred interest in alternative practices (Fillinger and Elad, 2016; Ally et al., 2023). As an eco-friendly and effective approach, plant-derived formulations combined with beneficial rhizobacteria can be used to protect plants.

CONCLUSION

Tomato gray mold disease was effectively controlled using an integrated approach of neem extract and *Bacillus subtilis*. This approach has great potential in promoting more environmentally friendly and sustainable agricultural practices, rather than relying on chemical fungicides. There is considerable evidence that integrating bioactive plant extracts with antifungal activity and rhizobacteria with antagonistic properties can significantly reduce disease incidence.

Future studies should focus on scaling these results to field conditions and investigating the long-term impacts of these treatments on soil health, crop yield, and microbial diversity. The development of commercial formulations of these bioactive combinations could significantly advance sustainable agriculture by reducing reliance on chemical fungicides.

AUTHORS' CONTRIBUTIONS

RJI, and GHJ designed the study; GHJ and TS conducted the experiments; GHJ, AA and AL collected and analyzed the data; MNS, and MRK provided technical assistance; SR and TS wrote the manuscript; GHJ, and RJI proofread the paper.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Agrios, G.N., 2005. Plant Pathology. Elsevier.

Ahmed, R., Gondal, A.S., Khan, M.T., Shahzaman, S., Hyder, S., 2018. First report of *Botrytis cinerea* causing gray mold disease on peach from Pakistan. International Journal of Phytopathology 7, 131-131.

ALKahtani, M.D., Fouda, A., Attia, K.A., Al-Otaibi, F., Eid, A.M., Ewais, E.E.-D., Hijri, M., St-Arnaud, M., Hassan, S.E.-D., Khan, N., 2020. Isolation and characterization of plant growth promoting endophytic bacteria from desert plants and their application as bioinoculants for sustainable agriculture. Agronomy 10, 1325.

Ally, N.M., Neetoo, H., Ranghoo-Sanmukhiya, V.M. and Coutinho, T.A., 2023. Greenhouse-grown tomatoes: microbial diseases and their control methods: a review. International Journal of Phytopathology 12(1), 99-127.

Almasaudi, N.M., Al-Qurashi, A.D., Elsayed, M.I., Abo-Elyousr, K.A., 2022. Essential oils of oregano and cinnamon as an alternative method for control of gray mold disease of table grapes caused by *Botrytis cinerea*. Journal of Plant Pathology 104, 317-328.

Azeem, W., Mukhtar, T., Hamid, T., 2021. Evaluation of *Trichoderma harzianum* and *Azadirachta indica* in the management of *Meloidogyne incognita* in Tomato. Pakistan Journal of Zoology 53(1), 119-125.

Aziz, S., Jamshed, S.A., Mukhtar, T., Irshad, G., Ijaz, S.S., Raja, M.U., 2024. Evaluation of *Bacillus* spp. as biocontrol agents against chili leaf spot caused by *Xanthomonas vesicatoria*. Journal of Plant Diseases and Protection 131, 987-997.

Bashir, A., Khan, M.T., Ahmed, R., Mehmood, B., Younas, M.T., Rehman, H.M. and Hussain, S., 2020. Efficiency of selected botanicals against (*Alternaria solani*) causing early blight disease on tomato in Azad Jammu and Kashmir. Pakistan Journal of Phytopathology 32(2), 179-186.

Bello, G., Sisterna, M., 2010. Use of plant extracts as natural fungicides in the management of seedborne diseases, Management of Fungal Plant Pathogens. CAB International, Wallingford, UK, pp. 51-66.

FAO. 2022. World Food and Agriculture Statistical year Book. Retrieved from Rome, Italy.

Fillinger, S., Elad, Y., 2016. *Botrytis*-the fungus, the pathogen and its management in agricultural systems. Springer.

Goel, N., Anukrati, K., Paul, P., 2016. Anti-phytopathogenic and SAR inducing properties of Neem: A review. Journal of Chemical and Pharmaceutical Sciences 9, 2547-2555.

Gurvinder, K., Negi, H.S., Ghosh, P., Sharma, S., Ojha, P.K., Singh, V., Chandel, S., 2023. Sensitivity of *Botrytis cinerea* isolate collected from gladiolus against selected fungicides, plant oils and botanicals in North India. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 51, 13360-13360.

Hajji-Hedfi, L., Rhouma, A., Hajlaoui, H., Hajlaoui, F., Rebouh, N.Y., 2023. Understanding the influence of applying two culture filtrates to control gray mold disease (*Botrytis cinerea*) in tomato. Agronomy 13, 1774.

Hussain, M.A., Mukhtar, T., Kayani, M.Z., 2011. Efficacy evaluation of *Azadirachta indica*, *Calotropis procera*, *Datura stramonium* and *Tagetes erecta* against root-knot nematodes *Meloidogyne incognita*. Pakistan Journal of Botany 43 (Special Issue), 197-204.

Iqbal, U., Mukhtar, T., 2020. Inhibitory effects of some fungicides against *Macrophomina phaseolina* causing charcoal rot. Pakistan Journal of Zoology 52(2), 709-715.

Iqbal, U., Mukhtar, T., Iqbal, S.M., 2014. *In vitro* and *in vivo* evaluation of antifungal activities of some antagonistic plants against charcoal rot causing fungus, *Macrophomina phaseolina*. Pakistan Journal of Agricultural Sciences 51(3), 689-694.

Jiang, C.H., Liao, M.J., Wang, H.K., Zheng, M.Z., Xu, J.J., Guo, J.H., 2018. *Bacillus velezensis*, a potential and efficient biocontrol agent in control of pepper gray mold caused by *Botrytis cinerea*. Biological Control 126, 147-157.

Karthika, S., Varghese, S., Jisha, M.S., 2020. Exploring the efficacy of antagonistic rhizobacteria as native biocontrol agents against tomato plant diseases. 3 Biotech 10(7), 320.

Kayani, M.Z., Mukhtar, T., Hussain, M.A., 2012. Evaluation of nematicidal effects of *Cannabis sativa* L. and *Zanthoxylum alatum* Roxb. against root-knot nematodes, *Meloidogyne incognita*. Crop Protection 39, 52-56.

Li, S., Xiao, Q., Yang, H., Huang, J., Li, Y., 2022. Characterization of a new *Bacillus velezensis* as a powerful biocontrol agent against tomato gray mold. Pesticide Biochemistry and Physiology 187, 105199.

Mamiev, M., Khakimov, A., Zuparov, M., Rakhmonov, U., 2020. Effectiveness of different fungicides in controlling *Botrytis* grey mould of tomato, IOP Conference Series: Earth and Environmental Science. IOP Publishing, p. 012112.

Mukhtar, T., Hussain, M.A., Kayani, M.Z., 2013b. Biocontrol potential of *Pasteuria penetrans*, *Pochonia chlamydosporia*, *Paecilomyces lilacinus* and *Trichoderma harzianum* against *Meloidogyne incognita* in okra. Phytopathologia Mediterranea 52(1), 66-76.

Mukhtar, T., Kayani, M.Z., Hussain, M.A., 2013a. Nematicidal activities of *Cannabis sativa* L. and *Zanthoxylum alatum* Roxb. against *Meloidogyne incognita*. Industrial Crops and Products 42, 447-453.

Orozco-Mosqueda, M.D.C., Kumar, A., Fadiji, A.E., Babalola, O.O., Puopolo, G., Santoyo, G., 2023. Agroecological management of the grey mould fungus *Botrytis cinerea* by plant growth-promoting bacteria. Plants 12(3), 637.

Paz, I.C.P., Santin, R.d.C.M., Guimarães, A.M., da Rosa, O.P.P., Quecine, M.C., e Silva, M.d.C.P., Azevedo, J.L., Matsumura, A.T.S., 2018. Biocontrol of *Botrytis cinerea* and *Calonectria gracilis* by eucalypts growth promoters *Bacillus* spp. Microbial Pathogenesis 121, 106-109.

Saeed, M., Mukhtar, T., Ahmed, R., Ahmad. T., Iqbal, M.A., 2023. Suppression of *Meloidogyne javanica* infection in peach (*Prunus persica* (L.) Batsch) using fungal biocontrol agents. Sustainability, 15(18), 13833.

Saeed, M., Mukhtar, T., Haq, M.I., Khan, M.A., 2021. Assessment of nematicidal potential of *Cannabis sativa* and *Azadirachta indica* in the management of root-knot nematode (*Meloidogyne javanica*) on peach. Pakistan Journal of Agricultural Sciences 58(5), 1555-1561.

Steel, R.G.D., Torrie, J.H., Dickey, D.A., 1997. Principles and procedures of statistics: A biological approach. McGraw-Hill.

Wamani, A.O., 2020. Screening of microbial antagonists and plant extracts against selected tomato pathogens and their potential in the management of bacterial wilt, Doctoral dissertation, University of Nairobi.

Wang, S., Hu, T., Jiao, Y., Wei, J., Cao, K., 2009. Isolation and characterization of *Bacillus subtilis* EB-28, an endophytic bacterium strain displaying biocontrol activity against *Botrytis cinerea* Pers. Frontiers of Agriculture in China 3, 247-252.

Williamson, B., Tudzynski, B., Tudzynski, P., Van Kan, J.A., 2007. *Botrytis cinerea*: The cause of grey mould disease. Molecular Plant Pathology 8, 561-580.