OPEN ACCESS Plant Protection, 09 (03) 2025. 705-716 DOI:10.33804/pp.009.03.5842

Check for updates

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

Research Article

# Neem-Synthesized Metallic Nanoparticles as Biocontrol Agents against Phytophthora-Associated Citrus Gummosis

[a]Hadeed Ahmad, [a]Muhammad Atiq, [b]Muhammad Ahsan Khan, [a]Nasir Ahmed Rajput

[a] *Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan.*
[b] *Department of Entomology, University of Agriculture, Faisalabad, Pakistan.*

**ARTICLE INFO**

***Article history***
*Received: 11th July, 2025*
*Revised: 22nd September, 2025*
*Accepted: 23rd September, 2025*

***Keywords***
*Neem-mediated nanoparticles*
*Phytophthora gummosis*
*Citrus disease management*
*Biocontrol agents*
*Green nanotechnology*

**ABSTRACT**

Citrus gummosis, primarily caused by *Phytophthora nicotianae*, is one of the most destructive diseases in citrus cultivation. This study investigated the antifungal efficacy of neem-synthesized metallic nanoparticles (silver, zinc, and copper) as potential biocontrol agents against *P. nicotianae* under both *in vitro* and greenhouse conditions. Nanoparticles were synthesized using methanolic neem leaf extract and subsequently purified and characterized. *In vitro* assays demonstrated significant inhibition of mycelial growth, with zinc nanoparticles (ZnNPs) showing the highest antifungal activity, followed by silver nanoparticles (AgNPs) and copper nanoparticles (CuNPs), in a dose- and time-dependent manner. Greenhouse trials using foliar sprays and soil drenching revealed that the combined application of ZnNPs and AgNPs resulted in the lowest disease incidence and smallest lesion size. The most effective disease suppression was achieved at a 0.75% concentration. Furthermore, nanoparticle treatments significantly enhanced root and shoot growth parameters, with the ZnNPs + AgNPs combination exhibiting the most pronounced effects. These findings highlight the dual role of neem-mediated metallic nanoparticles in suppressing citrus gummosis and promoting plant growth, providing a sustainable alternative to conventional chemical fungicides.

*Corresponding Author: Nasir Ahmed Rajput*
*Email: nasir.ahmed@uaf.edu.pk*


## INTRODUCTION

Citrus is a major fruit crop belonging to the subfamily Aurantioideae of the family Rutaceae and order Sapindales, comprising 162 genera and 2,085 species (Groppo et al., 2022; Hazarika, 2023; Richa et al., 2023). Globally, citrus provides significant nutritional and medicinal benefits. The principal cultivated varieties include sweet oranges, mandarins, grapefruits, kumquats, lemons, and limes (Jabeen et al., 2023). Citrus ranks among the most palatable fruits and occupies a distinctive position within the plant kingdom. Its global production is estimated at 161.8 million tons from 10.22 million hectares, whereas Pakistan contributes 2.32 million tons from 0.199 million hectares (FAOSTAT, 2021). During 2022-23, Pakistan produced 2.37 million tons, of which 0.53 million tons, valued at 52,458 million rupees, were exported (GOP, 2023). Punjab province accounts for more than 95% of national production, with Bhalwal in Sargodha district being especially renowned for Kinnow cultivation due to favorable soil and climatic conditions (Naseem et al., 2016; Ameer et al., 2023; Naheed et al., 2023).

Citrus fruits are consumed worldwide for their nutritional and medicinal properties, particularly their role in enhancing immunity (Luo et al., 2023). They are hydrating and rich in minerals (K, P, Mn, Ca, Zn, and Na), vitamins (A, E, and B-complex), vitamin C, and natural antioxidants including flavonoids, phenolics, and carotenoids (Uthman and Garba, 2023). These bioactive compounds reduce the risk of chronic diseases such as diabetes, cardiovascular disorders, stroke, and cancers (Lv et al., 2015; Cheng and Zhou, 2023). Moreover, citrus fruits are high in dietary fiber and possess antibacterial, anti-inflammatory, and low-glycemic properties (Al-Snafi, 2016; Minami et al., 2023).

Despite its global and national trade significance, citrus production in Pakistan faces several constraints. Yields remain suboptimal due to poor orchard management, limited adoption of modern technologies, and heavy pest and disease pressure (Tariq et al., 2021). Fruit quality losses often occur during the first 8-12 weeks after fruit set, primarily from insect damage and disease infections (Hasan et al., 2021). Among the diseases, gummosis caused by *Phytophthora* spp. is particularly destructive, responsible for 10-30% global yield losses (Mounde et al., 2008; Choudhary et al., 2021). In Pakistan, *P. nicotianae* and *P. citrophthora* have been documented as the major causal agents (Iqbal et al., 2020; Rajput et al., 2020). Disease development is favored by extended periods of surface wetness (≥18 h) and temperatures ranging between 25-30 °C (Timmer et al., 2000; Savita and Nagpal, 2012). Typical symptoms include gum exudation, bark splitting, root rot, chlorosis, wilting, dieback, and eventual tree mortality (Chaudhary et al., 2020; Belisle et al., 2023).

Accurate and rapid detection of *Phytophthora* spp. is critical for effective management. Molecular approaches such as PCR, real-time PCR, nested PCR, and loop-mediated isothermal amplification (LAMP) offer greater sensitivity and specificity compared with conventional diagnostic methods (Van Gent-Pelzer et al., 2007; Meyer et al., 2012; Mostowfizadeh, 2012; Hou et al., 2013; Le and Vu, 2017; Panno et al., 2020; Uysal and Kurt, 2020; Driciru et al., 2021; Ahmad et al., 2024). Furthermore, genetic characterization provides valuable insights for breeding resistant cultivars and implementing targeted control strategies (Hariharan and Prasannath, 2021). Current management options include resistant cultivars, improved cultural practices, biological control agents, chemical fungicides, and emerging nanotechnology-based approaches (Nazarov et al., 2020; Sawake et al., 2022). Resistant cultivars are regarded as the most sustainable strategy, while fungicides and medicinal plants such as neem serve as eco-friendly supplementary tools (Rajput et al., 2011, 2018; Iqbal et al., 2020).

Nanotechnology, a rapidly evolving multidisciplinary field, has gained prominence as an innovative tool in agriculture, offering environmentally friendly alternatives to synthetic chemicals (Fatima and Mushtaq, 2023). It involves the synthesis, characterization, and application of nanoparticles within the 1-100 nm range (Hano and Abbasi, 2021). In plant disease management, green-synthesized nanoparticles derived from plants have emerged as promising antifungal agents. They enable the targeted delivery of pesticides, fungicides, and other bioactive compounds, thereby minimizing excessive chemical inputs while ensuring sustainable crop protection, yield stability, and food security (Manna et al., 2017; Nguyen et al., 2018). Nanoparticles with antifungal activity can serve as potential substitutes for conventional fungicides and may revolutionize strategies for managing fungal pathogens in citrus and other crops (Sawake et al., 2022). In this study, neem-mediated metallic nanoparticles were evaluated for their effectiveness in managing citrus gummosis caused by *P. nicotianae*.

## Materials and Methods

### Culture purification and plant material

The *P. nicotianae* isolate (NAR81-Pn.S35) used in this study was obtained from the Plant Pathogen Interaction Laboratory, University of Agriculture Faisalabad (UAF), Pakistan. The isolate was maintained on V8 agar medium and incubated at 25 ± 2°C under dark conditions to ensure optimal growth and virulence (Rajput et al., 2015; Ali et al., 2024). Fresh, healthy leaves of neem (*Azadirachta indica*) were collected from the Botanical Garden, UAF (31°25'45.0"N, 73°04'17.3"E), and subsequently used for nanoparticle synthesis.

### Preparation of neem-synthesized metallic nanoparticles

Neem leaf extract was prepared following the method of Atiq et al. (2022) with slight modifications. Briefly, freshly collected leaves were thoroughly washed with distilled water, shade-dried for 10-14 days, and further oven-dried (Memmert, Germany) at 65°C for 4 h. The dried leaves were ground into a fine powder, and 20 g of the powder was mixed with 100 ml of methanol in a beaker, covered with aluminum foil, and incubated overnight in the dark. The mixture was then stirred for 10-15 min at 70°C and filtered through Whatman No. 41 filter paper.

For nanoparticle synthesis, a 0.5 M solution of each metal salt, silver nitrate (17 g), copper sulfate (17 g), and zinc sulfate (17 g), was individually mixed with the plant extract filtrate under continuous stirring for 10 min to initiate nucleation. The reaction mixture was subjected to sonication using an LX100UC ultrasonic cleaner at 65°C for 60 min, followed by drying in a Dry-Line 56 oven to obtain fine nanoparticle powders.

Purification of the synthesized nanoparticles was carried out by centrifugation at 10,000-15,000 rpm for 10-20 min, after which the supernatant was discarded. The nanoparticle pellets were thoroughly washed with distilled water to remove residual metabolites and unbound metal ions. Finally, the purified nanoparticles were collected and stored for further experimental use.

### Characterization of nanoparticles

#### X-ray diffraction (XRD)

XRD analysis was performed to determine the crystalline structure, elemental composition, and average particle size of the synthesized silver (AgNPs), copper (CuNPs), and zinc nanoparticles (ZnNPs).

#### Scanning electron microscopy (SEM)

SEM was used to examine the surface morphology, shape, and structural characteristics of AgNPs, CuNPs, and ZnNPs.

### Evaluation of nanoparticles against *P. nicotianae* under *in vitro* conditions

The antifungal efficacy of ZnNPs, CuNPs, and AgNPs was evaluated against *P. nicotianae* isolate (NAR81-Pn.S35) using the poisoned food technique. Three concentrations (0.25%, 0.5%, and 0.75%) were prepared by incorporating 0.25 g, 0.5 g, and 0.75 g of each nanoparticle type into 100 ml of V8 agar medium. The medium was poured into 90 mm Petri plates and inoculated at the center with a 5 mm mycelial disc of *P. nicotianae*. Plates were incubated at 25 ± 2°C, and radial mycelial growth (mm) was measured daily until full growth was achieved in the control treatment. Nanoparticle concentration, incubation period, and mycelial growth were considered as experimental factors for statistical analysis. The experiment was laid out in a Completely Randomized Design (CRD) with three replications per treatment.

### Evaluation of nanoparticles against citrus gummosis disease under greenhouse conditions

A total of forty-four citrus plants (~1.5 years old) were obtained from Qadir Bakhash Nursery Farm (31°28'18.9"N, 73°12'16.5"E) and transplanted individually into pots containing sterilized soil. Greenhouse conditions were maintained at 25-28°C and 60-70% relative humidity. Based on laboratory screening results, the most effective nanoparticles (AgNPs, ZnNPs) and their combination (AgNPs + ZnNPs) were selected for greenhouse evaluation. Their efficacy in reducing disease incidence and severity was assessed in two independent trials, each following CRD with three replications per treatment.

In Trial 1, sixteen plants were inoculated by wounding the stem 5 cm above the soil surface using the I-cut method, and each wound was inoculated with a 5 mm culture disc of *P. nicotianae*, while six control plants received sterile V8 agar discs. The wounds were wrapped with moist sterile cotton and sealed with parafilm. Nanoparticle treatments at 0.5% and 0.75% concentrations were applied as foliar sprays, whereas sterilized water served as the control. Disease incidence (%) and lesion length (cm) were recorded weekly. In Trial 2, the remaining sixteen plants were inoculated by applying 100 ml of *P. nicotianae* spore suspension to the root zone, followed by the application of nanoparticle treatments (0.5% and 0.75%) as foliar sprays, with sterilized water as the control. Disease incidence (%) was recorded weekly, and at the end of the experiment, root length, shoot length, root weight, and shoot weight were measured to assess overall plant health.

### Statistical analysis

All laboratory and greenhouse experiments were conducted using CRD. Data were recorded for all parameters and subjected to statistical analysis using Statistix 8.1 software (Ahmad et al., 2019). Treatment means were compared using appropriate post-hoc tests, and differences were considered statistically significant at $P < 0.05$.

## Results

### Characterization of AgNPs, CuNPs, and ZnNPs

SEM was used to investigate the surface morphology of the nanoparticles. The results revealed that AgNPs were predominantly spherical, ZnNPs exhibited both spherical and hexagonal shapes, while CuNPs were mainly spherical. The nanoparticle suspensions showed distinct visual appearances: AgNPs were brownish-yellow, ZnNPs ranged from white to off-white, and CuNPs appeared reddish-brown. XRD analysis confirmed the crystalline nature of the nanoparticles, with average crystallite sizes calculated as 21.34 nm for AgNPs, 22.25 nm for ZnNPs, and 17.68 nm for CuNPs (Table 1).

Table 1. Characterization of Ag, Zn, and Cu nanoparticles.

| Characteristics | AgNPs | ZnNPs | CuNPs |
|---|---|---|---|
| Average particle size | 21.34 nm | 23.22 nm | 16.88 nm |
| Appearance | Brownish-yellow | White/off-white | Reddish-brown |
| Shape | Spherical | Spherical and Hexagonal | Spherical |

**Assessment of the efficacy of nanoparticles against *P. nicotianae* under *in vitro* conditions**

The results revealed significant differences ($P < 0.05$) in the mean radial mycelial growth of *P. nicotianae* among the nanoparticle treatments. Maximum growth inhibition was observed with ZnNPs (11.3 mm), followed by AgNPs (13.5 mm), whereas the control exhibited the highest mean growth (24.5 mm) (Figure 1A).

The Treatment × Concentration (T × C) interaction demonstrated that the highest inhibition was achieved with ZnNPs at concentrations of 0.25%, 0.5%, and 0.75% (13.9, 11.6, and 8.4 mm, respectively), followed by AgNPs (15.9, 13.5, and 11.0 mm) and CuNPs (19.0, 16.8, and 14.6 mm), compared with the control (Figure 2B).

Similarly, the Treatment × Time (T × T) interaction indicated progressive inhibition over 48, 72, and 96 h. ZnNPs consistently produced the smallest colony diameters (8.4, 11.4, and 14.1 mm), followed by AgNPs (10.7, 13.4, and 16.4 mm) and CuNPs (14.0, 16.7, and 19.7 mm), relative to the control (Figure 1C).

**Evaluation of the efficacy of nanoparticles (spray application) against citrus gummosis under greenhouse conditions based on disease incidence**

Disease incidence was assessed following the spray application of nanoparticles. Among all treatments, the combination of ZnNPs + AgNPs resulted in the lowest incidence (25.65%), followed by ZnNPs (31.20%) and AgNPs (36.77%), as compared with the control (Figure 2A). The Treatments × Weeks (T×W) interaction indicated a progressive reduction in disease incidence over time across all treatments. ZnNPs + AgNPs consistently exhibited the lowest incidence (32.85%, 25.90%, and 18.50% at 1, 2, and 3 weeks, respectively), followed by ZnNPs (38.81%, 31.90%, 24.50%) and AgNPs (43.89%, 36.90%, 29.50%). In contrast, the control group showed a steady increase, with incidence values of 45.31%, 53.50%, and 70.90% at the respective intervals (Figure 2B).

Tukey's HSD all-pairwise comparisons for Treatments × Concentrations (T×C) revealed that ZnNPs + AgNPs achieved the lowest incidence (31.19% and 20.31%) at 0.5% and 0.75% concentrations, respectively, followed by ZnNPs (37.23% and 26.25%) and AgNPs (42.19% and 31.34%). The control group consistently recorded the highest incidence, reaching 64.20% at both concentrations (Figure 2C).

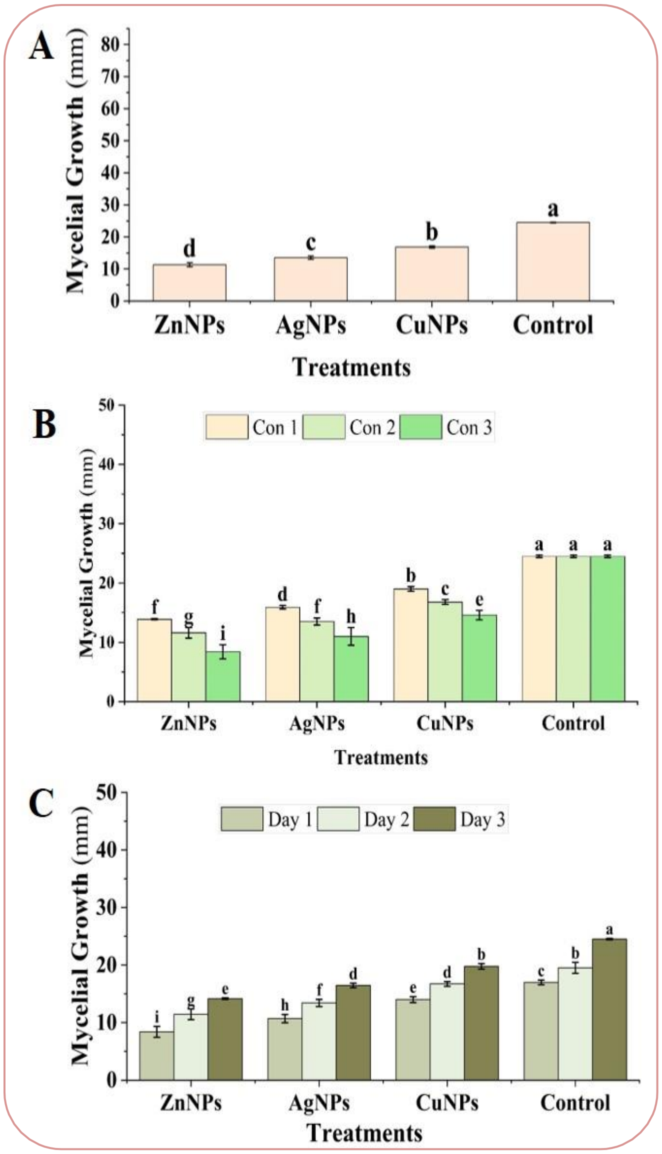


Figure 1. Effect of nanoparticles (A), their concentrations (B), and time intervals (C) on the *in vitro* mycelial growth of *P. nicotianae*.

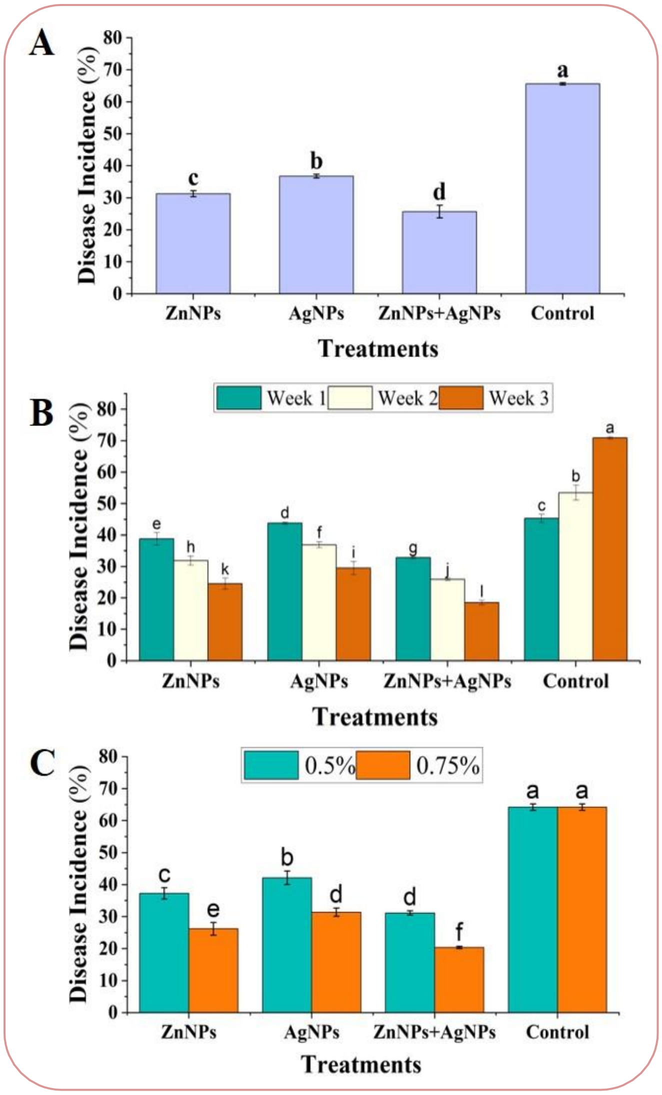


Figure 2. Effect of nanoparticle spray application on citrus gummosis incidence under greenhouse conditions: (A) overall effect, (B) treatment × week interaction, and (C) concentration × treatment interaction.

**Evaluation of the efficacy of nanoparticles (spray application) against citrus gummosis under greenhouse conditions based on lesion size**

Foliar application of Zn+Ag nanoparticles resulted in the greatest reduction in lesion size (71.22%), with a mean value of 19.56 mm, followed by ZnNPs (57.34%; 29.00 mm) and AgNPs (44.31%; 37.86 mm). All treatments were statistically different from the control (Figure 3A). Overall lesion size decreased with time, from 42.13 mm in week 1 to 35.36 mm in week 3. Higher concentrations enhanced efficacy, with 0.75% producing the lowest lesion size (35.85 mm) compared to 0.5% (41.34 mm).

Tukey's HSD comparisons indicated significant differences across treatments and weeks (T × W interaction). ZnNPs+AgNPs exhibited the greatest reduction, decreasing from 27.76 mm in week 1 to 12.08 mm in week 3, followed by ZnNPs (37.14 mm to 20.88 mm) and AgNPs (46.03 mm to 29.67 mm). In contrast, lesion size in the control group increased from 57.61 mm to 78.82 mm over the same period (Figures 3B and 3C).

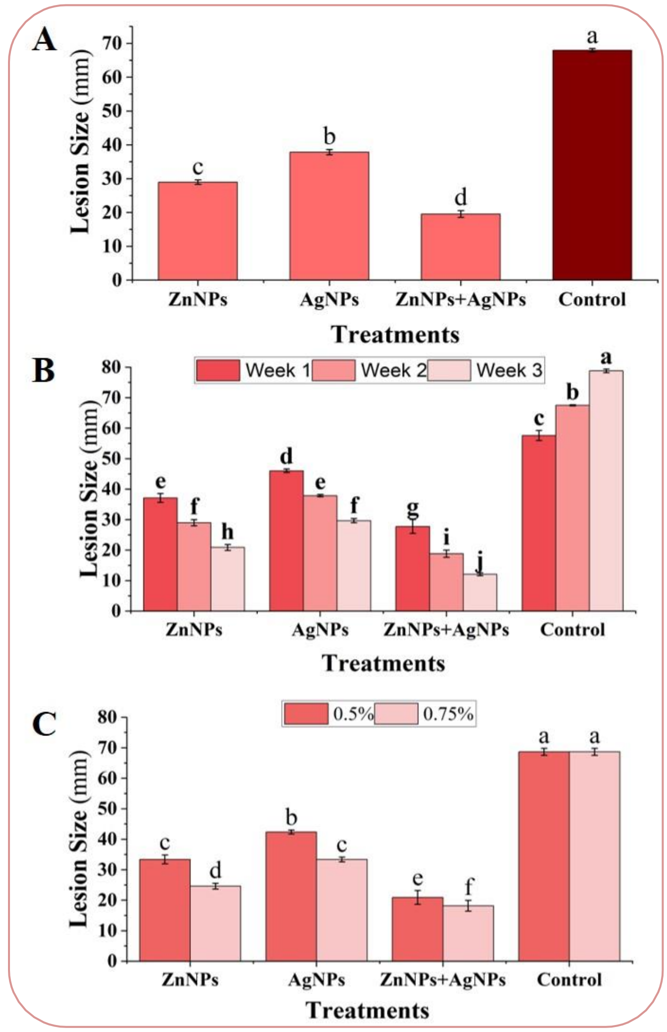


Figure 3. Lesion size on citrus stems exhibiting gummosis after nanoparticle spray application under greenhouse conditions: (A) overall effect, (B) treatment × week interaction, and (C) concentration × treatment interaction.

**Evaluation of the efficacy of nanoparticles (soil drenching) against citrus gummosis under greenhouse conditions based on disease incidence**

Soil drenching with Zn+Ag nanoparticles resulted in the lowest disease incidence (25.16%), followed by ZnNPs (29.09%) and AgNPs (35.25%), whereas the control exhibited the highest incidence (70.59%) (Figure 4A).

The interaction between treatments and weeks (T×W) revealed that Zn+Ag nanoparticles consistently produced the lowest disease incidence at 1, 2, and 3 weeks (33.87%, 24.20%, and 17.30%, respectively), followed by ZnNPs (36.33%, 29.17%, and 21.76%) and AgNPs (42.49%, 35.33%, and 27.93%). In contrast, the control group recorded the highest incidence values (65.93%, 70.91%, and 74.93%) at the corresponding intervals (Figure 4B).

Similarly, Tukey's HSD test for treatment × concentration (T×C) interactions indicated that at 0.5%, Zn+Ag nanoparticles exhibited the lowest incidence (28.65%), followed by ZnNPs (31.57%) and AgNPs (36.53%), whereas the control remained the highest (70.50%). At 0.75%, Zn+Ag nanoparticles again recorded the lowest incidence (21.60%), followed by ZnNPs (26.60%) and AgNPs (33.90%) (Figure 4C).

Overall, increasing nanoparticle concentration significantly enhanced disease suppression, with Zn+Ag nanoparticles demonstrating superior efficacy in reducing disease incidence across all treatments and evaluation intervals (Figure 4D).

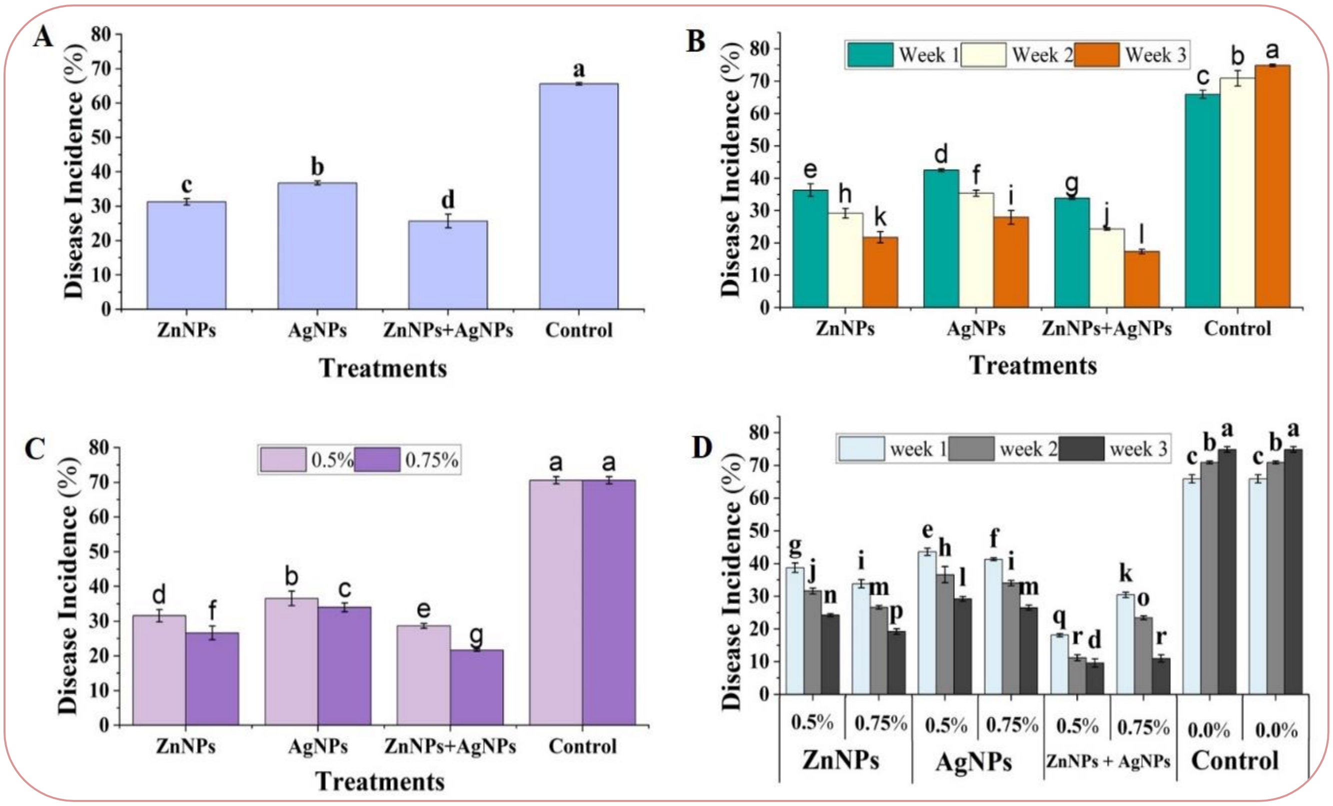


Figure 4. Effect of nanoparticle soil drenching on the incidence of citrus gummosis under greenhouse conditions: (A) overall effect, (B) treatment × week interaction, (C) concentration × treatment interaction, and (D) treatment × week × concentration interaction.

**Evaluation of the effect of nanoparticle treatments (soil drenching) against citrus gummosis on root and shoot development under greenhouse conditions**

Root and shoot development were evaluated following soil drenching with nanoparticle treatments. Tukey's HSD all-pairwise comparisons test confirmed significant differences (P<0.05) among treatments, with the control group exhibiting the lowest values for all measured parameters. Specifically, the control recorded the lowest mean shoot weight (10.9 g), shoot length (18.4 cm), root weight (9.8 g), and root length (13.7 cm). In contrast, the combined treatment of ZnNPs + AgNPs resulted in the highest values for both root and shoot traits, followed by individual applications of ZnNPs and AgNPs. Moreover, the 0.75% concentration generally enhanced root and shoot growth compared to the 0.5% concentration (Figure 5). These findings highlight the dual role of nanoparticle treatments in suppressing citrus gummosis while simultaneously enhancing plant growth and vigor under greenhouse conditions.

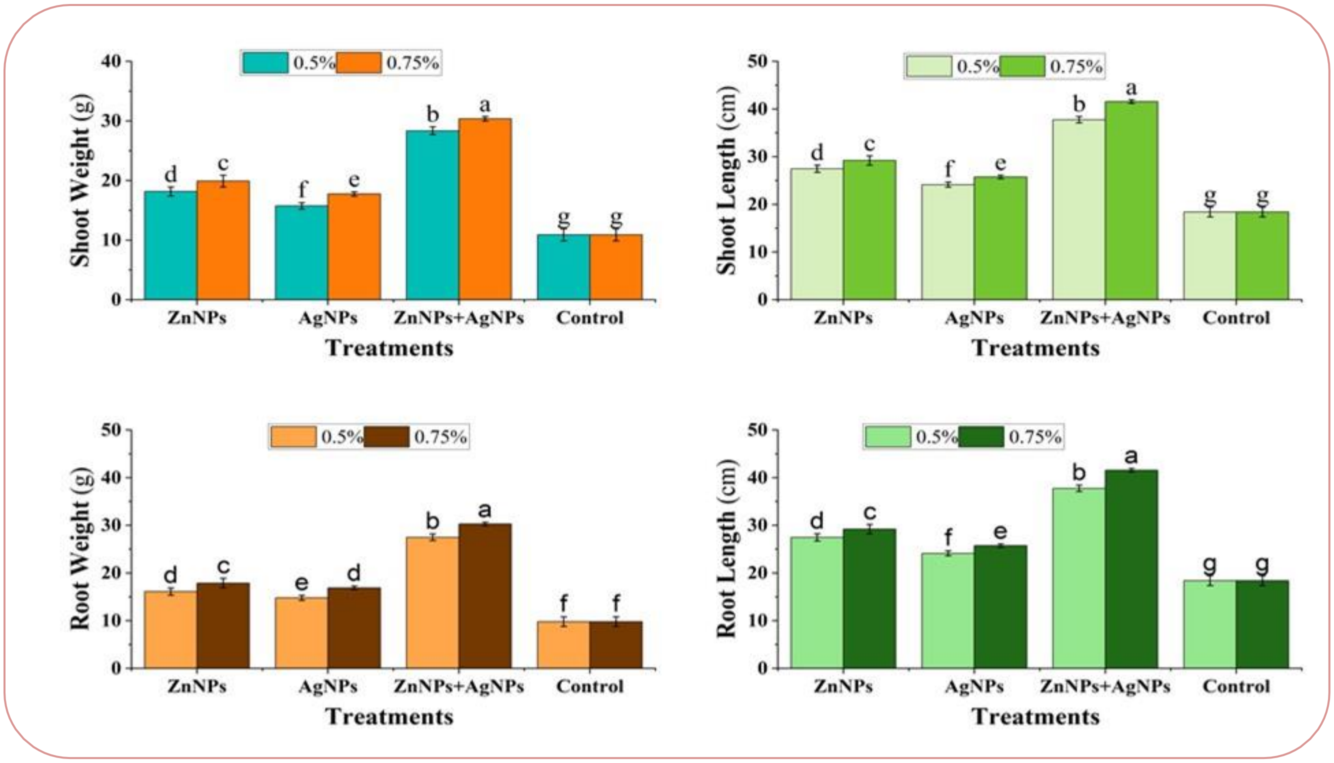


Figure 5. Effect of nanoparticle soil drenching on root and shoot development against citrus gummosis under greenhouse conditions.

## Discussion

Citrus gummosis caused by *Phytophthora* species is one of the most serious threats to citrus production worldwide (Graham and Feichtenberger, 2015). It is estimated that up to 46% of citrus trees are damaged annually due to gummosis (Savita et al., 2012). *Phytophthora* primarily affects the root system and induces leaf yellowing, which disrupts the plant's physiological processes (Badnakhe et al., 2015). Lesions formed on the trunk and roots by *Phytophthora* spp. result in substantial yield losses, foot and root rot, brown fruit rot, canopy discoloration, and premature leaf yellowing (Zhang et al., 2019). Other major symptoms include slow decline, twig dieback, gum exudation, early flowering, and reduced fruit size (Naqvi, 2004).

*Phytophthora* spp. occurs in all citrus-growing regions of the world (Mounde et al., 2008) and is responsible for billions of dollars in annual losses. Gummosis is a devastating disease that can ultimately lead to the complete decline of orchards (Mekonen et al., 2015). Naqvi and Singh (2002) reported that the pathogen spreads rapidly and, being soil-borne, is difficult to detect and manage.

The management of citrus gummosis remains a major challenge because of the pathogen's ecological plasticity and adaptability. *Phytophthora* species are well known for their ability to infect a wide range of hosts and produce diverse disease syndromes. Their capacity to form long-lived structures such as chlamydospores and oospores, along with lesion exudation, further complicates management (Hwang and Ko, 1978; Turkensteen et al., 2000; Panabières et al., 2016; Scanu et al., 2015, 2021; La Spada et al., 2022). Addressing these challenges requires a detailed understanding of pathogen biology under fluctuating environmental conditions and the development of targeted, sustainable control strategies.

Excessive reliance on conventional fungicides for *Phytophthora* management has led to the emergence of resistant strains and caused environmental contamination, thereby emphasizing the urgent need for eco-friendly alternatives. Nanotechnology is emerging as a transformative tool in agriculture, particularly in plant disease management, by enabling precise and efficient delivery of agrochemicals at the nanoscale (Hano and Abbasi, 2021). Green-synthesized nanoparticles (NPs) derived from plant extracts offer a sustainable and environmentally friendly alternative to chemical fungicides, minimizing ecological risks while enhancing disease suppression (Manna et al., 2017; Nguyen et al., 2018). This approach is particularly promising for managing *Phytophthora*-induced gummosis in citrus (Sawake et al., 2022).

Nanoparticles such as copper (CuNPs), zinc (ZnNPs), and silver (AgNPs) act as "nano-fungicides" with unique properties, including high surface area, small size, and superior antimicrobial activity, offering enhanced efficacy compared to conventional fungicides (Kim et al., 2011; Prema and Thangapandiyan, 2013; Shaffiey et al., 2014). They also help reduce toxicity, cost, and resistance development (Pal et al., 2007).

In this study, we investigated the efficacy of biogenic ZnNPs, CuNPs, and AgNPs against *P. nicotianae* and other pathogenic species. The combination of ZnNPs + AgNPs exhibited the strongest inhibitory effect on mycelial growth, achieving a 69.1% reduction compared to the control. Under greenhouse conditions, this combination again proved most effective, reducing disease incidence by 64.9% and lesion size by 71.2% through foliar spraying and soil drenching. Moreover, nanoparticles enhanced plant growth parameters such as shoot and root length and biomass.

Field trials confirmed these greenhouse findings: ZnNPs + AgNPs significantly reduced both disease incidence and lesion size while promoting plant growth. These results demonstrate that biogenic nanoparticles, particularly the ZnNPs + AgNPs combination, represent a promising and sustainable approach for managing citrus gummosis and improving crop health.

ZnNPs in particular showed notable antimicrobial efficacy. Compared to chemically engineered nanoparticles, biogenic ZnNPs possess lower toxicity and enhanced biological activity (Pimprikar et al., 2009; Kaur et al., 2012; Rehman et al., 2024). Consistent with previous reports, our results confirmed that ZnNPs significantly suppressed pathogen growth and disease development while exhibiting minimal phytotoxicity.

CuNPs also demonstrated strong antifungal activity against *P. nicotianae*, inhibiting hyphal growth, spore germination, and sporangium formation. Their effects were associated with fungal cell damage, elevated reactive oxygen species (ROS), and increased superoxide dismutase (SOD) activity, in agreement with earlier studies (Hao et al., 2017, 2019; Chen et al., 2022). Disease incidence was reduced by 33.7% without phytotoxic effects, underscoring CuNPs' utility as potent nanofungicides (Heinlaan et al., 2008; Sathiyabama and Manikandan, 2018).

AgNP uptake varied depending on application method, with foliar treatments achieving higher accumulation in stems compared to root uptake, corroborating previous findings (Cocozza et al., 2019; Avellan et al., 2021). Although AgNPs displayed significant antimicrobial activity, their potential accumulation and biotransformation in edible tissues raise biosafety concerns that warrant further investigation (Huang et al., 2022).

Overall, the use of green-synthesized nanoparticles in disease management aligns with global goals for sustainable agriculture. By utilizing plant-derived extracts for nanoparticle synthesis, this approach minimizes environmental risks while providing effective pathogen control (Ikram et al., 2022; Umair Raza et al., 2023). Our findings demonstrate that ZnNPs, CuNPs, and AgNPs are effective eco-friendly alternatives to conventional fungicides, with strong potential for integration into citrus gummosis management programs. Future research should focus on optimizing nanoparticle formulations, assessing their long-term ecological impacts, and ensuring safe agricultural applications.

**Acknowledgments**

This research article is extracted from the Ph.D. dissertation of Hadeed Ahmad. The author is deeply grateful to the Plant Pathogens Interaction Lab, Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan, for providing laboratory space and facilities to conduct the experiments.

**Authors' Contributions**

NAR and MA conceived and designed the overall research framework and experimental plan; HA carried out the experiments with technical guidance and support; HA and MAK performed data analysis and interpretation. HA and MAK were also responsible for the provision of essential reagents, materials, and analytical tools required for the study; HA drafted the manuscript by with substantial contributions and critical revisions from NAR and MA. All authors read and approved the final version of the manuscript.

**Research Funding**

This work was financially supported by the Higher Education Commission of Pakistan under the National Research Program for Universities (Project No. 9422).

**Conflict of Interest**
The authors declare no conflict of interest.

**Sustainable Development Goals Targeted**
SDG 12: Responsible Consumption and Production
SDG 12: Responsible Consumption and Production
SDG 15: Life on Land

**References**
Ahmad, H., Rajput, N.A., Atiq, M., Kachelo, G.A., Usman, M., Tariq, H., Wahab. M., 2024. Detection of *Phytophthora nicotianae* induced citrus gummosis by the loop-mediated isothermal amplification. Pakistan Journal of Botany 56(5), 1741-1748.
Ahmad, I., Tanveer, M.U., Liaqat, M., Dole, J.M., 2019. Comparison of corm soaks with preharvest foliar application of moringa leaf extract for improving growth and yield of cut Freesia hybrida. Scientia Horticulturae 254, 21-25.
Ahmed, Y., Hubert. J., Fourrier-Jeandel, C., Dewdney, M.M., Aguayo J., Ioos, R., 2019. A set of conventional and multiplex real-time PCR assays for direct detection of *Elsinoe fawcettii*, *E. australis*, and *Pseudocercospora angolensis* in citrus fruits. Plant Disease 103(2), 345-356.
Al-Snafi, D.A.E., 2016. Nutritional value and pharmacological importance of citrus species grown in Iraq. IOSR Journal of Pharmacy 06(08), 76-108.
Ali, M.U., Rajput, N.A., Atiq, M., Atif, R.M., Crandall, S.G., 2024. Population dynamics and aggressiveness of fungal pathogens associated with chilli root rot. Pakistan Journal of Botany 56(1), 377-387.
Ameer, A., Mumtaz, S., Asghar, N., Hameed, M., Ahmad, F., Mahmood, A., Naqve, M., Naseer, M., Azeem, M., 2023. Structural and functional attributes of *Citrus reticulata* Blanco under diverse soil and environmental conditions. Pakistan Journal Botany 55(1), 357-366.
Atiq, M., Mazhar, H.M.R., Rajput, N.A., Ahmad, U., Hameed, A., Lodhi, A., 2022. Green synthesis of silver and copper nanoparticles from leaves of *Eucalyptus globulus* and assessment of its antibacterial potential towards *Xanthomonas citri* pv. *citri* causing citrus canker. Applied Ecology and Environmental Research 20(3), 2205-2213.
Avellan, A., Yun, J., Morais, B.P., Clement, E.T., Rodrigues, S.M., Lowry, G.V., 2021. Critical review: Role of inorganic nanoparticle properties on their foliar uptake and in planta translocation. Environmental Science and Technology 55(20), 13417-13431.
Badnakhe, M.R., Durbha. S., Adinarayana, J., 2015. Disease stress detection on citrus using a leaf optical model and field spectroscopy. Proceedings of SPIE, the International Society for Optical Engineering/Proceedings of SPIE 9637.
Belisle, R.J., Hao, W., Riley, N., Förster, H., Adaskaveg, J.E., 2023. Root absorption and limited mobility of mandipropamid as compared to oxathiapiprolin and mefenoxam after soil treatment of citrus plants for managing *Phytophthora* root rot. Plant Disease 107(4), 1107-1114.
Chaudhary, S., Laughlin, D.A., Setamou, M., da Graça, J.V., Kunta, M., Alabi, O.J., Crosby, K.M., Ong, K.L., Ancona, V., 2020. Incidence, severity, and characterization of *Phytophthora* foot rot of citrus in Texas and implications for disease management. Plant Disease 104(9), 2455-2461.
Chen, J.N., Wu, L.T., Kun, S.O.N.G., Zhu, Y.S., Wei, D.I.N.G., 2022. Nonphytotoxic copper oxide nanoparticles are powerful “nanoweapons” that trigger resistance in tobacco against the soil-borne fungal pathogen *Phytophthora nicotianae*. Journal of Integrative Agriculture 21(11), 3245-3262.
Cheng, Y.S., Zhou, Y., 2023. Impact of citrus fruit and hesperidin intake on multiple health outcomes: An umbrella review. Journal of Nutritional Oncology 8(1), 16-24.
Choudhary, A.K., Singh, N., Singh, D., 2021. Evaluation of the bioformulation of potent native strains of *Trichoderma* spp. against the foot rot/gummosis of kinnow mandarin. Egyptian Journal of Biology and Pest Control 31(1), 1-11.
Cocozza, C., Perone, A., Giordano, C., Salvatici, M.C., Pignattelli, S., Raio, A., Cherubini, P., 2019. Silver nanoparticles enter the tree stem faster through leaves than through roots. Tree Physiology 39(7), 1251-1261.
Driciru, P., Mugasa, C.M., Acidri, R., Adriko, J., 2021. Development of loop-mediated isothermal amplification (LAMP) assay for detection of *Pseudocercospora angolensis* in sweet orange. Biorxiv. 10.1101/2021.01.13.426516.
FAO (2021). World Food and Agriculture - Statistical Yearbook 2021. FAO.

Fatima, T., Mushtaq, A., 2023. Efficacy and challenges of carbon-based nanomaterials in water treatment: A review. International Journal of Chemical and Biochemical Sciences 23(1), 232-248.

GOP, 2023. Economic Survey. Government of Pakistan, Finance Division, Economic Advisor's Wing, Islamabad.

Graham, J., Feichtenberger, E., 2015. Citrus *Phytophthora* diseases: Management challenges and successes. Journal of Citrus Pathology 2, 12-24.

Groppo, M., Afonso, L.F., Pirani, J.R., 2022. A review of systematics studies in the citrus family (Rutaceae, Sapindales), with emphasis on American groups. Brazilian Journal of Botany 45, 181-200.

Hano, C., Abbasi, B.H., 2021. Plant-based green synthesis of nanoparticles: Production, characterization and applications. Biomolecule 12(1), 31.

Hao, Y., Cao, X.Q., Ma, C.X., Zhang, Z.T., Zhao, N., Ali, A., Hou, T.Q., Xiang, Z.Q., Zhuang, J., Wu, S.J., Xing, B.S., Zhang, Z., Rui, Y.K., 2017. Potential applications and antifungal activities of engineered nanomaterials against gray mold disease agent *Botrytis cinerea* on rose petals. Frontiers in Plant Science 8, 1332.

Hao, Y., Fang, P.H., Ma, C.X., White, J.C., Xiang, Z.Q., Wang, H.T., Zhang, Z., Rui, Y.K., Xing, B.S., 2019. Engineered nanomaterials inhibit *Podosphaera pannosa* infection on rose leaves by regulating phytohormones. Environmental Research 170, 1-6.

Hariharan, G., Prasannath, K., 2021. Recent advances in molecular diagnostics of fungal plant pathogens: a mini review. Frontiers in Cellular and Infection Microbiology 10, 600234.

Hasan, M.U., Saleem, B.A., Khan, S.A., Khalid, M.S., Hayat, F., Salik, R., 2021. Evaluating the Response of insecticides and fungicides for rind blemishes management in Kinnow mandarin (*Citrus nobilis Lour*× *Citrus deliciosa Tenora*) fruits caused by biotic factors. Journal of Horticultural Science & Technology 4, 102-108.

Hazarika, T.K., 2023. Citrus. In Fruit and Nut Crops. Singapore: Springer Nature Singapore. pp. 1-44.

Heinlaan, M., Ivask, A., Blinova, I., Dubourguier, H.C., Kahru, A., 2008. Toxicity of nanosized and bulk ZnO, CuO and TiO2 to bacteria *Vibrio fischeri* and *Crustaceans daphnia magna* and *Thamnocephalus platyurus*. Chemosphere 71(7), 1308-1316.

Hou, X., Huang, F., Zhang, T.Y., Li, H., 2013. Detection of *Elsinoe fawcettii* in planta through real-time PCR. Journal of Food, Agriculture and Environment, 11(4), 1085-1087.

Huang, D., Dang, F., Huang, Y., Chen, N., Zhou, D., 2022. Uptake, translocation, and transformation of silver nanoparticles in plants. Environmental Science: Nano 9(1), 12-39.

Hwang, S. C., Ko, W.H., 1978. Biology of chlamydospores, sporangia, and zoospores of *Phytophthora cinnamomi* in soil. Phytopathology 68(5), 10-1094.

Ikram, M., Raja, N.I., Mashwani, Z.U.R., Omar, A.A., Mohamed, A.H., Satti, S.H., Zohra E., 2022. Phytogenic selenium nanoparticles elicited the physiological, biochemical, and antioxidant defense system amelioration of Huanglongbing-infected 'Kinnow' mandarin Plants. Nanomaterials 12(3), 356.

Iqbal, Z., Ahmad, S., Asim, M., Rehman, M.A., Rehman, A., Raza, W., Raza, M., Bilal, M.S., Abid, H.U., 2020. Management of *Phytophthora* species associated with citrus decline in Pakistan. Management 5(1), 98-103.

Jabeen, S., Saif, R., Haq, R., Hayat, A., Naz, S., 2023. Whole-genome sequencing and variant discovery of *Citrus reticulata* "Kinnow" from Pakistan. Functional & Integrative Genomics 23(3), 227.

Kaur, G., Verma, R.K., Rai, D.K., Rai, S.B., 2012. Plasmon-enhanced luminescence of Sm complex using silver nanoparticles in polyvinyl alcohol. Journal of Luminescence 132(7), 1683-1687.

Kim, S.H., Lee, H., Ryu, D., Choi, S., Lee, D., 2011. Antimicrobial activity of silver nanoparticles again *Staphylococcus aureus* and *Escherichia coli*. Korean Journal of Microbiology and Biotechnology 39(1), 77-85.

La Spada, F., Cock, P.J., Randall, E., Pane, A., Cooke, D.E., Cacciola, S.O., 2022. DNA metabarcoding and isolation by baiting complement each other in revealing *Phytophthora* diversity in anthropized and natural ecosystems. Journal of Fungi 8(4), 330.

Le, D.T., Vu, N.T., 2017. Progress of loop-mediated isothermal amplification technique in molecular diagnosis of plant diseases. Applied Biological Chemistry 60 (2), 169-180.

Luo, J., Yuan, H., Mao, L., Wu, J., Jiang, S., Yang, Y., Fu, Y., Liu, L., Chen, S., Wang, W., 2023. The young fruit of *Citrus aurantium* L. or *Citrus sinensis* Osbeck as a natural health food: A deep insight into the scientific evidence of its health benefits. Arabian

Journal of Chemistry 2, 104681.

Lv, X., Zhao, S., Ning, Z., Zeng, H., Shu, Y., Tao, O., Xiao, C., Lu C., Liu, Y., 2015. Citrus fruits as a treasure trove of active natural metabolites that potentially provide benefits for human health. Chemistry Central Journal 9.

Manna, S., Ghosh, A., Rajak, R., Sarkar, A., Das, S., Laha, R., Paul, T., Paul, I., Ghosh, S., Mandal, S.M., 2017. Control of late blight of potato using plant micronutrients copper and zinc bimetallic nanoparticle. Advanced Science, Engineering and Medicine 9(11), 971-976.

Mekonen, M., Ayalew, A., Weldetsadik, K., Seid, A., 2015. Assessing and measuring of Citrus gummosis (*Phytophthora* spp.) in major citrus growing areas of Ethiopia. Journal of Horticulture 2(4), 1-4.

Meyer, L., Jacobs, R., Korsten, L., Truter, Korsten, M. L., 2012. Detection and molecular identification protocols for *Phyllosticta citricarpa* from citrus matter. South African Journal of Science 108, 1-6.

Minami, G.S., Lumbantoruan, E.C., Nuraini, R., Harianto, J.C., Fahrurroji, A., 2023. The potential of sweet orange (*Citrus sinensis*) in cardiovascular health: A literature review. JKKI: Jurnal Kedokteran dan Kesehatan Indonesia 2, 82-94.

Mostowfizadeh, G.R., 2012. Species-specific detection of *Phytophthora* by simple and nested-PCR. Iran. Journal of Plant Pathology 48, 69-80.

Mounde, L.G., A.W. Ateka., Wasilwa, E.M.L., Thuranira, E.G., 2008. Occurrence and distribution of citrus gummosis (*Phytophthora spp*.) in Kenya. pwani university.

Naheed, S., Tahira, R., Bashir, A., 2023. Growth and instability of export of selected fruits and vegetables in Pakistan. Pakistan Journal of Agricultural Research, 61(1), 83-91.

Naqvi, S.A.M.H., 2004. Diagnosis and management of certain important fungal diseases of citrus. In Diseases of Fruits and Vegetables Volume I: Diagnosis and Management (pp. 247-290). Dordrecht: Springer Netherlands.

Naqvi, S.A.M.H., S. Singh., 2002. Fungal diseases of citrus: Diagnosis and Management. Technical Bulletin 5, 61.

Naseem, S., Mahmood, S., Z. Ali., 2016. Occurrence of Citrus tristeza virus in Pakistan: a GIS based approach combining host distribution and disease reports. Pakistan Journal of Agricultural Sciences 53(3), 12-24.

Nazarov, P.A., Baleev D.N., Ivanova, M.I., Sokolova, L.M., Karakozova, M.V., 2020. Infectious plant diseases: Etiology, current status, problems and prospects in plant protection. Acta Naturae 12, 46.

Nguyen, V.T., Tran, K.V.Q., Tran, Q.N., 2018. Effect of oligochitosan-coated silver nanoparticles (OCAgNPs) on the growth and reproduction of three species *Phytophthora in vitro*. Archives of Phytopathology and Plant Protection 51, 227-240.

Pal, S., Tak, Y.K., Song, J.M., 2007. Dose the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli. Journal of Applied & Environmental Microbiology. 73, 1712-1720.

Panabieres, F., Ali, G.S., Allagui, M.B., Dalio, R.J., Gudmestad, N.C., Kuhn, M.L., Roy, S.G, Schena, L., Zampounis, A., 2016. *Phytophthora nicotianae* diseases worldwide: new knowledge of a long-recognised pathogen. Phytopathologia Mediterranea 55(1), 20-40.

Panno, S., Matic, S., Tiberini, A., Caruso, A.G., Bella, P., Torta, L., 2020. Loop mediated isothermal amplification: principles and applications in plant virology. Plants 9, 461.

Pimprikar, P. S., Josh, S.S, Kumar, A.R, Zinjarde, S.S., Kulkarni, S.K., 2009. Influence of biomass and gold salt concentration on nanoparticle synthesis by the tropical marine yeast Yarrowia lipolytica NCIM 3589. Colloids and Surfaces B: Biointerfaces. 74, 309-316.

Prema, P., Thangapandiyan S., 2013. *In vitro* antibacterial activity of gold nanoparticles capped with polysaccharide stabilizing agents. International Journal of Pharmaceutical Sciences Review and Research 5, 310-314.

Rajput, N.A., Pathan, M.A., Lodhi, A.M., Daolong, D., Rajput, S., 2011. Effect of neem (*Azadirachta indica*) products on seedling growth of shisham dieback. African Journal of Microbiology Research 5(27), 4937-4945.

Rajput, N.A., Zhang, M., Danyu, S., Liu, L., Zhang, Q., Yanyan, R., Sun, P., Daolong, D., 2015. Overexpression of a *Phytophthora* cytoplasmic CRN effector confers resistance to disease, salinity and drought in *Nicotiana benthamiana.* Plant and Cell Physiology 56(12), 2423-2435.

Rajput N. A., M. Atiq, N. Javed, Y. H. Ye, Z. Zhao, R. N. Syed, A. M. Lodhi, B. Khan, O. Iqbal and D. Dou., 2018. Antimicrobial effect of chinese medicinal plant crude extracts against *Rhizoctonia solani* and *Pythium aphanidermatum*. Fresenius Environmental Bulletin 27(06), 3941-3949.

Rajput, N.A., Atiq, M., Tariq, H., Saddique, W.M., Hameed, A., 2020. Citrus gummosis: A formidable challenge to citrus industry: A review. International Journal of Biosciences 16(5), 131-134.

Rehman, F. U., Paker, N. P., Rehman, S. U., Javed, M. T., Munis, M.F.H., Chaudhary, H.J., 2024. Zinc oxide nanoparticles: biogenesis and applications against phytopathogens. Journal of Plant Pathology 106(1), 45-65.

Richa, R., Kohli, D., Vishwakarma, D., Mishra, A., Kabdal, B., Kothakota, A., Richa, S., Sirohi, R., Kumar, R., Naik, B., 2023. Citrus fruit: Classification, value addition, nutritional and medicinal values, and relation with pandemic and hidden hunger. The Journal of Agriculture and Food Research 2, 100718.

Sathiyabama M., Manikandan A., 2018. Application of copperchitosan nanoparticles stimulate growth and induce resistance in finger millet (*Eleusine coracana* Gaertn.) plants against blast disease. Journal of Agricultural and Food Chemistry, 66, 1784-1790.

Savita, G.S.V., Nagpal, A., 2012. Citrus diseases caused by *Phytophthora* species. GERF Bulletin of Biosciences 3(1), 18-27.

Savita., Bhagat, A., Pati, P.K., Virk, G.S., Nagpal, A., 2012. An efficient micropropagation protocol for *Citrus jambhiri* Lush. and assessment of clonal fidelity employing anatomical studies and RAPD markers. In Vitro Cellular & Developmental Biology - Plant 48, 512-520.

Sawake, M.M., Moharil, M.P., Ingle, Y.V., Jadhav, P.V., Ingle, A.P., Khelurkar, V.C., Paithankar, D.H., Bathe, G.A., Gade, A.K., 2022. Management of *Phytophthora parasitica* causing gummosis in citrus using biogenic copper oxide nanoparticles. Journal of Applied Microbiology 132, 3142-3154.

Scanu, B., Jung, T., Masigol H., Linaldeddu, B.T., Jung, M.H., Brandano, A., Cacciola, S.O., 2021. *Phytophthora heterospora* sp. nov., a new pseudoconidia-producing sister species of *P. palmivora*. Journal of Fungi 7, 870.

Scanu, B., Linaldeddu, B.T., Deidda, A., Jung, T., 2015. Diversity of *Phytophthora* species from declining Mediterranean maquis vegetation, including two new species, *Phytophthora crassamura* and *P. ornamentata* sp. nov. PLoS One. 10, 0143234.

Shaffiey, S.F., Shaffiey, R., Ahmadi M., Azari, F., 2014. Synthesis and evaluation bactericidal properties of CuO nanoparticles against *Aromonas hydrophila*. Nanomedicine Journal 1, 198-204.

Tariq, H., Rajput, N.A., Atiq, M., Sahi, S.T., Rehman, A., Rashid, A., Khan, M.A., Hameed, A., Saddique, W.M., 2021. Resistance assessment of citrus varieties against gummosis disease caused by *Phytophthora nicotianae* under natural field conditions. Pakistan Journal of Agricultural Research 34, 824-829.

Timmer, L.W., Garnsey, S.M., Graham, J.H., 2000. Compendium of citrus diseases. APS. Florida, USA.

Turkensteen, L. J., Flier, W.G., Wanningen, R., Mulder, A., 2000. Production, survival and infectivity of oospores of *Phytophthora infestans*. Plant Pathology 49, 688-696.

Umair Raza, M., Abasi, F., Shahbaz, M., Ehsan, M., Seerat, W., Akram, A., Proćków J., 2023. Phytomediated silver nanoparticles (AgNPs) embellish antioxidant defense system, ameliorating HLB-diseased 'Kinnow'Mandarin plants. Molecules 28, 2044.

Uthman, A., Garba, Y., 2023. Citrus mineral nutrition and health benefits: a review. Citrus Research-Horticultural and Human Health Aspects. IntechOpen, London.

Uysal, A., Kurt S., 2020. Rapid diagnosis of citrus anthracnose caused by *Colletotrichum gloeosporioides* using a lamp (loop-mediated isothermal amplification) assay. Bitki Koruma Bülteni 60, 25-32.

Van Gent-Pelzer, M.P.E., Van Brouwershaven, I.R., Kox, L.F.F., Bonants, P.J.M., 2007. A TaqMan PCR method for routine diagnosis of the quarantine fungus *Guignardia citricarpa* on citrus fruit. The Journal of Phytopathology 155, 357-363.

Zhang, D., Liu., X., Ma, J., Yang, J., Zhang, W., Li, C., 2019. Genotypic differences and glutathione metabolism response in wheat exposed to copper. Environmental and Experimental Botany 157, 250-259.