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DETERMINATION OF BACTERICIDAL POTENTIAL OF GREEN BASED SILVER AND ZINC NANOPARTICLES AGAINST BACTERIAL CANKER OF TOMATO

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ABSTRACT

Bacterial canker of tomato is the most damaging one and is responsible for causing 70% yield losses each year. Green synthesis of nanoparticles has ushered in a new research field known as green nanotechnology and has emerged as vital tool for the management of plant diseases. In comparison to other conventional techniques, green synthesis of various nanoparticles was found to be eco-friendly, low-cost and profitable resulting in more stable synthesized materials. In present research, zinc and silver nanoparticles were synthesized and their antibacterial potential was evaluated alone and in combination against *Clavibacter michiganensis* subsp. *michiganensis* under lab and field conditions. In lab experiment, combination of Zinc and Silver NPs proved to be the most effective showing maximum inhibition zone (27.449 mm) followed by Zinc (19.50 mm), and Silver (16.21 mm). Similarly, in the field experiment combination of Ag+Zn NPs proved effective with minimum disease incidence (6.10%) followed by Zinc (10.15%) and Silver NPs (22.40%) as compared to the control.

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INTRODUCTION

Tomato is an important horticultural crop which comprises of several vitamins (A, E, K), minerals, and phenolic compounds. Phenolic compounds are effective against stress related diseases (Quinet et al., 2019). Globally, tomato is cultivated on an area of 5 million hectares with 186 million tons of production while, in Pakistan, it is cultivated on an area of 57 thousand hectares with 0.594 million tons of production (FAO, 2020). Among all constraints faced by tomato crop, bacterial canker caused by *Clavibacter michiganensis subsp. Michiganensis,* is the most important one, as it causes 70% yield losses each year. It was first reported in Michigan, USA (Siddique et al., 2020).

Various management strategies are used to manage this disease such as use of resistant varieties, chemical and biological control. However, because of unavailability of resistant varieties and development of resistance, farmer community preferred chemicals for disease management but successive use of these chemicals make the pathogens resistant against these chemicals and have negative impact on environment and have health hazard effects. So it is the most pressing need to find some alternatives to chemicals. Biological control is an efficient method for controlling plant diseases, but all the bio-control agents have different survival mechanisms and most of them cannot survive in new environment (Bashir et al., 2020). In this scenario, the use of nanoparticles (NPs) becomes the most effective strategy to manage plant diseases due to their small size, fast action, low dosage, long shelf life, and do not harm the crops. These NPs have high antimicrobial potential that boost up plant defense mechanism against diseases (Nazir et al., 2019; Khan et al., 2021; Singh et al., 2021).

Nanoparticles frequently have distinct physiochemical features that set them apart from their macro scale counterparts. These characteristics as well as their prospective applications are reflected in their optical, mechanical, chemical, magnetic, and electrical characteristics. NPs have the potential to increase crop yield (Kimber et al., 2018). NPs can increase crop output, by suppressing plant diseases, eliminating undesired weeds and insects. Nanoparticles are synthesized by biological, physical and chemical methods. Biological method of nanoparticles synthesis includes the use of microorganisms and plants (whole plant, plant extracts). In green chemistry, whole plant or plant parts are used to synthesize nanoparticles which is a cost effective, environment friendly method with less damage because no heavy metal is used in the synthesis (Singh et al., 2021). Plant extracts are used in green nanotechnology to create innovative nanoparticles with the desired properties needed for biosensors, biomedicine, cosmetics, and nano-biotechnology as well as for electrochemical, catalytic, antibacterial, electronics, sensing, and other applications (Khan et al., 2022).

Aqueous extracts of *Larrea tridentata* was used to synthesize silver nanoparticles against *C. michiganensis subsp. michiganensis* that proved effective with lowest disease incidence (*Méndez-Andrade et al., 2022).* Plant extracts of Thymus syriacus was used to form ZnO nanoparticles and their antibacterial activity was checked against plant pathogenic bacteria (Sahin et al., 2022). Results indicated that these NPs were effective against plant pathogenic bacteria especially C. michiganensis subsp. michiganensis. Antimicrobial activity of three types of copper nanoparticles, nanowires and nanosheets was tested against bacterial canker of kiwi fruit (Ren et al., 2022). The maximum reduction in diseases was shown by copper-based nanoparticles. Due to their efficacy, the present study was designed to evaluate the antibacterial activity of green synthesized nanoparticles against tomato bacterial canker.

MATERIALS AND METHODS

Green synthesis of AgNPs and ZnNps

One kilogram of *Moringa olefrea* leaves were taken from Forestry Research Area of University of Agriculture Faisalabad. These leaves were used to synthesize nanoparticles which were sun-dried for three days after being shade-dried for a week. After that, these leaves were placed in an oven to dry for four hours at a temperature of 65°C. After that, leaves were grinded into fine powder by using a pestle and mortar. The powder was sieved by using muslin cloth. Then 100 mL of methanol was mixed in 20 g powder in a beaker which was left in the dark for 24 hours. The filter paper was used to filter the mixture. The filtrate was mixed with 17 g each of zinc oxide (ZnO) and silver nitrate ($AgNO_3$) separately. Each solution was then placed at 70°C separately for 15 min on a magnetic stirrer. It was then placed on an ultrasonic cleaning for four hours in the Physics Department and then at 80°C in a furnace oven for 24 h. Then material was oven-dried at 65°C for 4 h to eliminate the surplus moisture. After evaporating moisture, remaining material was grinded with the help of pestle and mortar. After this, resultant fine powder evaluated at three different concentrations i.e. 0.25, 0.5, and 0.75% under *in vivo* and *in vitro* conditions (Atiq et al., 2021).

Collection of diseased specimen

Tomato canker-infected leaves were collected from the Research Area of Institute of Horticultural Sciences, University of Agriculture, Faisalabad. These samples were kept in a brown bag and brought to the Phytobacteriology lab. These samples were then washed with tap water and dried in open air. Safety precautions were taken during this process.

Media Preparation

The Nutrient Agar media was prepared for bacterial isolation. Twenty eight grams of synthetic nutrient agar was mixed in 1000 mL of distilled water in a glass bottle. Then the bottle was wrapped and autoclaved at 121°C and 15 PSI for 15 min.

In vitro management of *Clavibacter michiganensis* subsp. *michiganensis* through green based nanoparticles by using inhibition zone technique

Clavibacter michiganensis subsp*. Michiganensis* (*Cmm)* was examined for response towards green-based nanoparticles by using inhibition zone technique. Zinc and silver nanoparticles were applied at three different concentrations alone and in combination with one control treatment. Distilled water was used as a control treatment. In flasks containing 100 mL of distilled water, the concentrations of 0.25%, 0.5%, and 0.75% were prepared by adding 0.25 g, 0.5 g, and 0.75 g, of nanoparticles respectively. Each concentration was replicated three times. Nutrient Agar media was poured into Petri dishes and the bacteria were streaked in a Zigzag manner. Blotter paper discs of 1 cm were cut and sterilized. These discs were subsequently dipped in the nanoparticle concentrations and placed at the center of Petri plates containing streaked media. One plate was treated as control by placing the disc dipped in distilled water placed in the middle of plate. After that, the plates were wrapped, labeled, and put in an incubator set at 30°C. The inhibitory zone was examined after every 24 h (Atiq et al., 2021).

Management of bacterial canker of tomato through green based silver and zinc nanoparticles

For the management of *Clavibacter michiganensis* subsp. *michiganensis* causing bacterial canker under field condition, 3 concentrations of the green based nanoparticles were prepared which were applied in the field by using spray method. Zinc and Silver Nps alone and in combination were used in the field with one control treatment against bacterial canker of tomato. Three concentrations (0.25%, 0.5% and 0.75%) with three replications were used in the field. The concentrations were prepared by adding 0.25 g, 0.5 g and 0.75 g in 100 mL of water respectively under RCBD. Data were recorded after 7 days intervals by using disease rating scale. The disease rating scale was given from 0-4 in which 0 showed no wilting, 1 showed 1-20% wilting, 2 showed 20-40% wilting, 3 showed 40-60% wilting and 4 showed more than 60% symptoms (Girish and Umesha, 2005). Disease incidence was recorded by using the following formula;

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total no.of healthy plants}} \times 100$$

Data analysis

Least Significant Difference was applied using Statistic 8.1 to the recorded data to determine the significant differences between treatments.

RESULTS

Effect of nanoparticles against *Clavibacter michiganensis* subsp. *michiganensis* under lab conditions

Among all the treatments of nanoparticles, combination of Zinc and Silver Nps showed the maximum inhibition zone (27.44 mm) followed by ZnNPs (19.5 mm) and AgNPs (16.21 mm) as compared to the control (Figure 1). The interaction between treatments and concentrations (T×C) showed that the maximum inhibition zone was shown by the combination of Zinc and Silver Nps i.e. 24.10 mm at 0.25%, 27.99 mm at 0.5%, and 30.25 mm at 0.75%, followed by Zinc (17.55, 19.79, and 21.16 mm), and Silver Nps (12.26, 15.96, and 20.41) at 0.25%, 0.5% and 0.75% respectively as compared to the control (Figure 2). In the interaction between treatments and duration (T×D), silver Nps showed the minimum inhibition zone that was 13.41 mm after day 1, 16.78 mm after day 2, and 22.62 after day 3, Zinc Nps showed inhibition zone, 17.17 mm, 18.72 mm, and 22.62 mm and their combination (Ag+Zn Nps) showed the maximum inhibition zones of 25.14, 27.58, and 28.61 mm after 1, 2 and 3 days respectively as compared to the control (Figure 3).

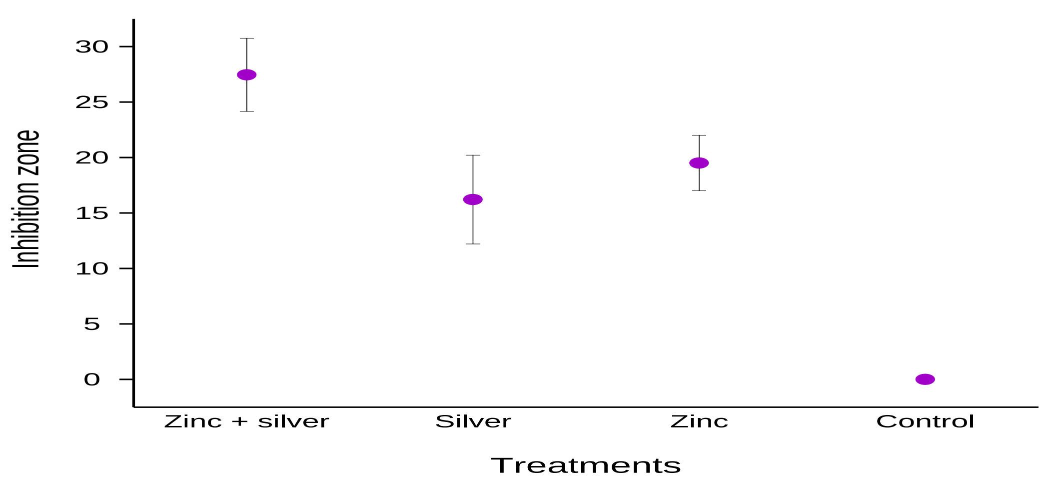

Figure 1. Evaluation of nanoparticles against *Clavibacter michiganensis* sub sp. *michiganensis* under lab conditions.

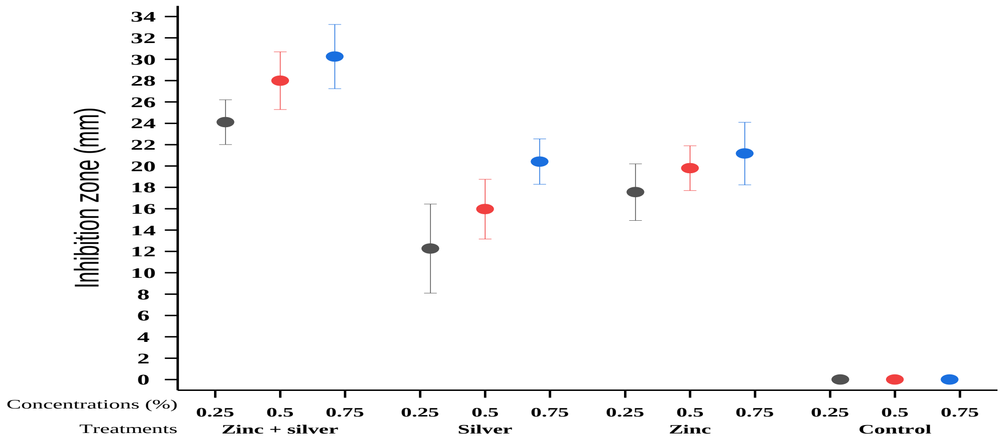

Figure 2. Evaluation of interaction between treatments and concentrations (T×C) against *Clavibacter michiganensis* subsp. *michiganensis* under lab conditions.

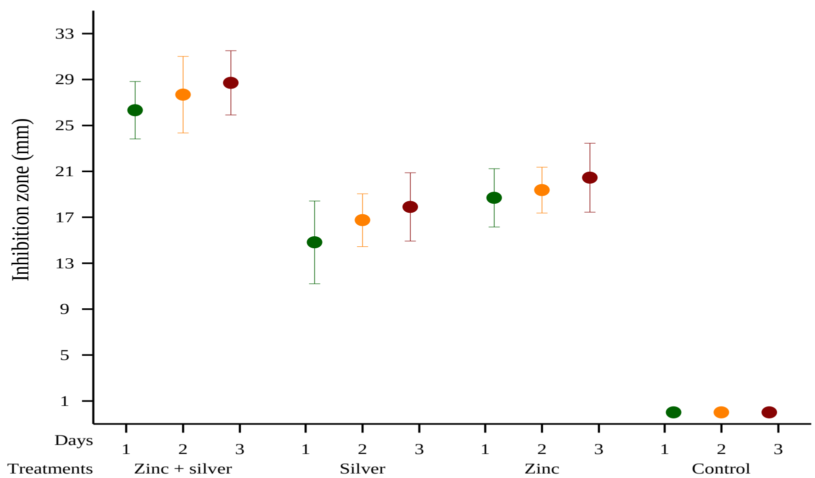

Figure 3. Evaluation of interaction between treatments and days (T×D) against *Clavibacter michiganensis* subsp. *michiganensis* under lab conditions.

***In vivo* evaluation of different nanoparticles against bacterial canker of tomato**

The combination of Zinc and Silver Nps showed the minimum disease incidence among all the treatments (6.10%) followed by Zinc Nps (10.10%) and Silver Nps (22.4%) as compared to the control (51.58%) (Figure 4). Among interaction between treatments × concentrations (T×C), combination of Zinc and Silver Nps showed the minimum disease incidences in the field (9.79, 5.84, and 2.67%) at (0.25, 0.5 and 0.75%) concentrations, followed by Zinc (20.19, 8.30, and 1.96%) and Silver Nps (28.37, 19.53, and 14.30%) as compared to the control respectively (Figure 5). Among interactions between treatments and days (T×D), the minimum disease incidence was showed by the combination of Zinc and Silver Nps (8.48, 6.57, and 3.25%), after 7, 14, and 21 days followed by Zinc (21.68, 8.68, and 6.10%) and Silver Nps (29.37, 21.47, and 16.36%) after 7, 14, and 21 days respectively as compared to the control (Figure 6).

DISCUSSION

Researchers are struggling to handle plant diseases in an environmentally sustainable manner. Nanoparticles are used in a variety of forms such as copper, gold, silver, and zinc nanoparticles. By limiting any chemical toxicity, green nanotechnology provides nano-tools for the conversion of biological systems to nanomaterial production (Nasrollahzadeh et al., 2019). Green synthesis productions of nanoparticles are environmentally friendly tools for the management of plant microorganisms (Jabbar et al., 2022; Shahbaz et al., 2022). Plant extract synthesis of nanoparticles is advantageous because it reduces the danger of future contamination by shortening the reaction time and by retaining cell integrity (Pakzad et al., 2019).

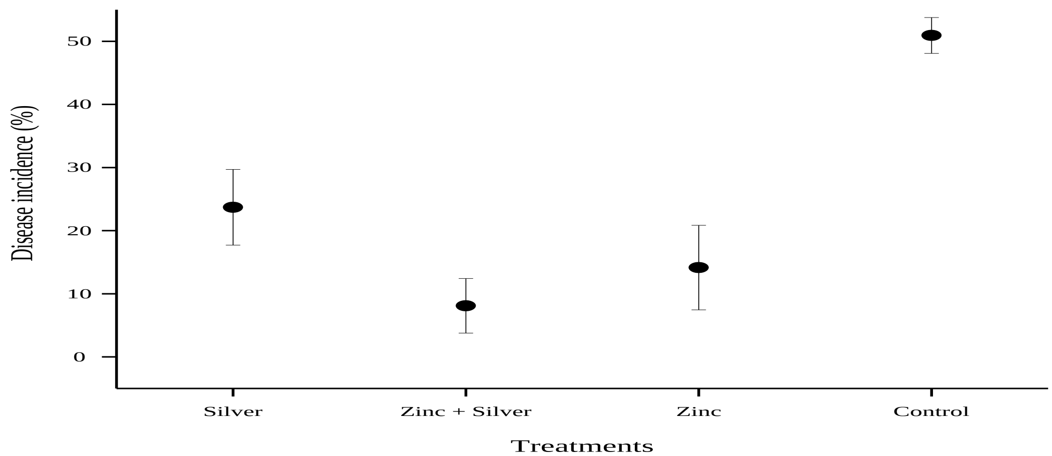

Figure 4. Evaluation of nanoparticles against bacterial canker of tomato under field conditions.

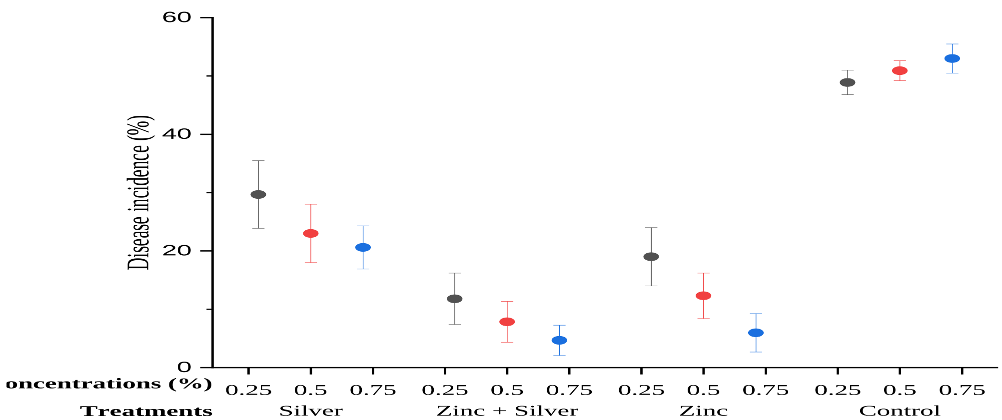

Figure 5. Evaluation of interaction between treatments and concentrations (T×C) against bacterial canker of tomato under field conditions.

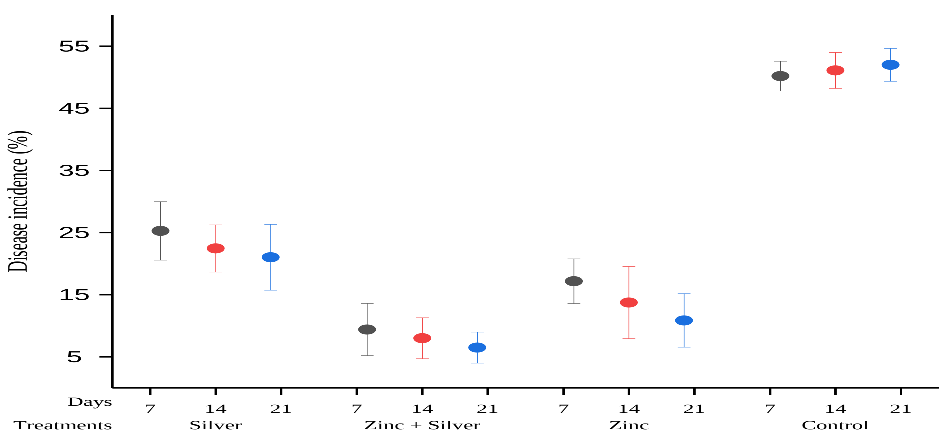

Figure 6. Evaluation of interaction between treatments and days (T×D) against bacterial canker of tomato under field conditions.

In the present study, zinc and silver nanoparticles were synthesized by using Moringa leaves and evaluated in the lab against *Cmm* causing bacterial canker of tomato. Among nanoparticles, combination of both Ag+Zn Nps showed the maximum inhibition zone under lab condition at 0.75% concentration. Nanoparticles were also applied in the field to control the incidence of bacterial canker. Disease incidence was measured on weekly basis and the maximum reduction in disease incidence was shown by the combination of Zinc and Silver nanoparticles at 0.75% concentration after 3 weeks. The ability of Moringa leaves to guard cells from free radical damage makes them a possible source of natural antioxidants. The results of the current study are supported by Noshad et al. (2019, 2020) who used silver nanoparticles against *Cmm* and concluded that for CAT, APX, POD, and total phenol and flavonoid concentrations in the leaves, AgNPs boosted enzyme activity. This bactericidal action and the establishment of an antioxidative defense mechanism may explain the slower symptom onset and decreased bacterial growth in AgNPs-treated plants.

ZnO-NPs are preferred over other nanostructured metal oxides due to their higher antibacterial capability and biocompatibility. The basic mechanism of ZnO-NPs' bactericidal nature comprised physical interaction between the bacterial cell wall and ZnO-NPs, free radical production, and reactive oxygen species (ROS) (Gharpure et al., 2022). Present study is in line with previous findings of Sahin et al. (2021) who used ZnO nanoparticles against *C. michiganensis* subsp. *michiganensis* and it was concluded that these particles exhibited significant antibacterial potential against specially gram negative bacteria. These results are also supported by a previous investigation of Rivas-Caceres et al. (2018) who used silver nanoparticles against *C. michiganensis* subsp. *michiganensis* with 6 different concentrations of nanoparticles. Best results were shown at a concentration of 84 micrograms.

CONCLUSIONS

It was concluded from this study that silver nanoparticles have great antimicrobial properties that are helpful in inhibiting the pathogen population. In the current study, bactericidal potential of green based silver and zinc nanoparticles against bacterial canker of tomato was evaluated. In both lab and field conditions, combination of zinc and silver nanoparticles proved the most effective treatment. Therefore, these hybrid nanoparticles may be tested for protection of several other crops against a wide range of diseases.

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AUTHORS' CONTRIBUTION

MA conceived the idea of research, MA conducted research and manuscript write up, NAR edited the manuscript, STS improved and edited the manuscript, AA performed the data analysis and interpretation, MU prepared green based nanoparticles, SI helped in preparation of green based nanoparticles, AN helped in conducting field trials, AMA helped in manuscript write up and AH collected and arranged the data.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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