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# Intervention of Bacterial Leaf Spot of Bell Pepper through Neem Mediated Copper and Zinc Hybrid Nanoparticles

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Bacterial leaf spot caused by *Xanthomonas campestris* pv. *vesicatoria* is a key pathological problem faced by bell pepper crop. The extensive use of chemical treatments has heightened environmental toxicity. Green-synthesized nanoparticles have been developed for managing plant diseases intervention while minimizing environmental harm. An experiment was carried out with three replications for each treatment, testing different concentrations (0.25%, 0.50%, 0.75%) of green-synthesized nanoparticles of Copper (Cu), Zinc (Zn), and a combination of Copper and Zinc (Cu+Zn) under laboratory conditions using a Complete Randomized Design (CRD) to manage *Xanthomonas campestris* pv. *vesicatoria*. The Cu+Zn nanoparticles exhibited the largest inhibition zone (13.733 mm), followed by Zn nanoparticles (7.9378 mm) and Cu nanoparticles (7.0048 mm), all compared to the control. Similarly, a greenhouse experiment with three replications of each treatment and concentration, which demonstrated significant results in lab conditions, was conducted. The Cu+Zn nanoparticles resulted in the lowest disease incidence (17.937 %), followed by Zn nanoparticles (22.966 %) and Cu nanoparticles (27.768 %), compared to the control. Additionally, a field trial was performed using a RCBD with three replicates per treatment and a single concentration that had shown the best results in lab conditions. In field conditions, Cu nanoparticles showed the highest disease incidence (31.374 %), followed by Zn nanoparticles (27.442 %) and Cu+Zn nanoparticles (22.637 %) in comparison to the control group. Recent outcomes suggest that neem-mediated copper-zinc hybrid nanoparticles are an effective tool for combating bacterial leaf spot in bell peppers.
**Keywords:** *Capsicum annuum*, *Xanthomonas compestris* pv. *vesicatoria*, *Azadirechta indica*, Nanoparticles. Cu-Zn.

## INTRODUCTION

Bell pepper (*Capsicum annuum* L.), a significant vegetable crop in Pakistan, is part of the Solanaceae family and the genus Capsicum (Jabeen *et al*., 2014). Globally, it is cultivated on an area of 2.6 million hectares with 36.13 million tons production (FAOSTAT, 2020). In Pakistan, bell peppers are cultivated on 47.35 thousand hectares, producing 127 thousand tons (Hussain *et al*., 2022). Its contribution is 1.5% of the GDP of Pakistan (GOP, 2020). Bell pepper is a good nutritional source having bioactive compounds, like vitamins (A, B, C, D, E, and K) and minerals (potassium, sodium, magnesium, calcium, and phosphorus) (Thuphairo *et al*., 2019). It is also a rich source of flavonoids and phenolic compounds that are beneficial for human health, and helpful in controlling various diseases like diabetes, osteoporosis, and cancer (Baenas *et al*., 2019). It is used for making pickles paste, and sauce (Buendía *et al*., 2020) and also used as a coloring agent for food and cosmetics (Rozylo, 2020).

Bell pepper is affected by biotic (bacterial leaf spot, bacterial wilt, gray mold, powdery mildew, and anthracnose) and abiotic (drought, blossom end rot, sunscald, fruit cracks, and salinity) stresses (Mishra *et al*., 2018). One of the most important biotic factor is the bacterial leaf spot of bell pepper caused by *Xanthomonas campestris* pv. *vesicatoria,* causing 23 to 44 % yield losses (Osdaghi *et al*., 2021). In the 1920s it was first described in the USA (Indiana) and South Africa. It is a gram negative, rod shaped and seed borne pathogen (Potnis *et al*., 2015). Some epidemiological factors like excessive irrigation, high relative humidity, high plant

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density, and temperature are also sportive in the developmental phases of the pathogen (Young *et al*., 1978; Abrahamian *et al*., 2021). The pathogen is transmitted through seeds and can also spread via contaminated seeds (Potnis *et al*., 2015), rain splashes, and heavy dew (McAvoy *et al*., 2021). Xanthomonas infects the host plant in two stages: the epiphytic phase, where bacteria enter through natural openings and wounds, and the endophytic phase, where bacteria multiply within the host tissue (An *et al*., 2020). On both leaves and fruits, symptoms include water-soaked spots that change from green to dark brown as the disease progresses. Yellow halos often surround these spots or lesions, which vary in size. Some cultivars may have numerous tiny lesions in size range of 0.25 - 0.5 cm covering over 80 % of the leaf area, while others may have few but larger lesions (greater than 0.5 cm) (Diab *et al*., 1982).

Managing bacterial leaf spot in bell peppers involves various strategies, including biocontrol agents, plant defense activators, resistant varieties, and synthetic chemicals. Farmers often prioritize using resistant varieties, but this becomes challenging over time due to pathogen variability (Scortichini *et al*., 2013). Management of pepper bacterial spot involves both preventive and curative measures. Cultural practices, such as disinfecting soil in seedling production, planting disease-free seeds, maintaining low temperature and moisture in protected areas, removing plant debris, and employing appropriate agro-technical measures, are crucial for disease prevention. However, these measures often fall short, particularly under weather conditions favorable for pathogen spread, leading to severe epidemics (Sevic *et al*., 2019). Growers prefer to use synthetic chemicals for the management of plant diseases but excessive application of synthetic chemicals poses a negative impact on human health and the environment. Moreover, these chemicals also affect the beneficial organisms (Rhizobium and Trichoderma) that sustain soil fertility (Chhipa, 2019). To overcome such adversity in soil and environment, scientists have applied nanotechnology in agriculture. Nanotechnology is an emerging technology that has shown promising results in maintaining soil fertility and the environment. Various nanoparticles have shown great potential for pest management (Sarkar *et al*., 2021). Nanoparticles have been synthesized through physical, chemical, and biological pathways. Biological synthesis of NPs is the most innovative method than the other two as it involves the synthesis of NPs from plant extracts and microorganisms. Nanoparticles have multiple mechanisms of action against plant pathogens. NPs diffuse into the bacterial cell leading to the breakdown of the bacterial cell membrane and producing reactive oxygen species that interact with bacterial DNA and protein that leads to cell death (Nisar *et al*., 2019). The efficacy of Cu nanoparticles against plant disease management has been investigated and CuNPs can offer an alternative to bactericide and probably slow down the development of bacterial resistance (Ntasiou *et al*., 2021). Therefore, keeping in consideration, the significance of the issue, the recent study was planned to manage bacterial leaf spots of bell pepper through green synthesized nanoparticles of zinc and copper synthesized from neem leaves.

## MATERIALS AND METHODS

***Isolation and Bacterial identification*:** NA (Nutrient agar) media was used to isolate the bacterial pathogen. In a 500 mL media bottle, 13.3 g of nutrient broth and 20 g of agar were added. Subsequently, D.W (distilled water) was added to reach a total volume of 500 mL. Media was sterilized at 121°C and 15 PSI pressure for 15 minutes in an autoclave (RTA85). After that, the autoclave was turned off and when temperature comes down to 60-65˚C, Nilstate was introduced to prevent fungal growth. Then media was poured into sterilized Petri plates (9cm) and allowed for solidification.

To prepare the samples, diseased portions of leaves with healthy ones were sliced into small portion (2-3 mm) with the help of sterilized scissors. After that, leaves were surface disinfection with 1% NaOCl for half minute, followed by three consecutive rinses with distilled water to eliminate any NaOCl residues. After drying with sterilized filter paper (Sharif *et al*., 2021), the samples were placed onto NA media plates using sterilized forceps. These plates were wrapped with paraffin tape and incubated at 28°C for 24 hours (Heraeus) to observe bacterial growth. Subsequent to incubation, bacteria were purified via the streaking method. A sterilized loop was used to select a single colony and streak it onto new NA media plates. Then plates were covered for incubation at 28°C. The protocol was repeated 2-3 times for multiplication and obtains a purified bacterial culture (Ranjan *et al*., 2021). Bacterial was identified on the bases of morphological traits such as shape, color, growth, and colony pattern, alongside biochemical tests including Gram staining, catalase, and oxidase testing (Sharma and Singh 2019).

***Trial for the confirmation of pathogen* (*Pathogenicity test)*:** A trial was conducted to confirm the presence of the pathogen, adhering to Koch's postulates. The bacterial suspension was prepared @ $1 \times 10^8$ CFU/mL with the help of a spectrophotometer (Hitachi U-2001, model 121003) (Tahir et al., 2016; Mushtaq *et al*., 2019). For this purpose, peat moss was filled in the seedling tray (200 holes) then seeds were sown in this tray. After 45 days when seedlings were established then it was transferred to pots (11 inches). For this purpose, sterilized soil (10% formalin stock solution i.e. 1 part of formalin with 9 parts of water) was filled in pots. After that, a susceptible variety of bell pepper was sown into these pots. Once the bell pepper plants were established, suspension of bacteria was applied early in the morning, a time when the maximum number of stomata are open, using a sterilized syringe. When symptoms appeared after 4-5 days, pathogen was isolated from infected samples and was compared with symptoms of parental plants to full fill Koch's Postulates.

***Preparation of Cu-Zn green synthesized hybrid nanoparticles*:** To prepare nanoparticles, neem leaves were collected and dried under shade for 4-5 days and were sun-dried for 1-2 days. After that, these leaves were placed in a dry oven (101-1AB) at 65°C for 4 hours to remove the remaining moisture. These leaves were ground by using pestle and mortar and Muslin cloth was used to get fine powder. 20 g powder of plant extract was added into a beaker having 100mL methanol and after rapping with aluminum foil it was placed in darkness for 1 day. Then it was passed through Whatman filter paper No. 41 (12.5 cm size). 17 g Copper sulfate (CuSO4 and 17 g ZnO were mixed separately to filtrate. Then, it was placed on a magnetic stirrer (C-MAG HS 4) for 15 minutes at 70°C. After this, it was placed on ultrasonic cleaner (VEVOR) at 60°C for bond breaking. Then, it was placed in a water bath at 65°C for 15 minutes. Sulfate and oxide were evaporated and copper and zinc nanoparticles were prepared. For obtaining these nanoparticles in powder form solution was placed in a furnace oven (Mon-0067) (Atiq *et al*., 2022). Hybrid nanoparticles of Cu and Zn were prepared by mixing. These NPs were used at variable doses *in lab, green house and field* conditions.

**Lab scale *appraisement of Neem based NPs towards Xanthomonas compestris* pv. *vesicatoria:*** Green mediated nanoparticles of Cu and Zn extracted from neem leaves were assessed against *Xantomonas compestris* pv. *vesicatoria* under *in vitro* conditions through the inhibition zone method. One liter NA media was prepared. To prepare three concentrations of NPs (0.25, 0.50 and 0.75 %), 0.25, 0.50, and 0.75 g of (Zn and Cu) powder were added in a conical flask containing 100mL of distilled water separately. Media was poured into Petri plates (9 cm) and allowed for solidification. The bacterial culture was spread in the Petri dish using a sterilized inoculating loop under a laminar airflow cabinet (RTVL-1312). Sterilized filter paper was cut into circular discs of 1Cm diameter and these sterilized discs were dipped into nanoparticles (Zn and Cu) solution alone and in combination (Zn+Cu) with prepared concentrations of 0.25, 0.50, and 0.75 %. After removing of excessive moisture, the filter paper was kept in the center of plates containing culture of pathogen. In the control group, filter paper segments were immersed in distilled water. The plates were then sealed with wrapping tape and placed in an incubator at a temperature range of 25-30°C (Heraeus) for 48-72 hrs. The experimental was performed under (CRD) with three replicates per treatment. Measurements were taken at 1, 2 and3 days intervals. The growth inhibition zone of pathogen was assessed utilizing digital Vernier caliper (VCL-150).

***Appraisement of green synthesized NPs in the greenhouse*:** For in greenhouse evaluation of green synthesized (Cu and Zn) NPs, one moderately susceptible variety of bell pepper was grown. For this purpose, peat moss was filled in the seedling tray (200 holes) then seeds of susceptible variety were sown in these tray. After 45 days when seedlings were established them it was transferred into pots in the Research Area Department of Plant Pathology, UAF. Then 2 days 2-day-old *Xcv* culture was taken for the preparation of bacterial suspension @ 1×108 CFU/mL by using a spectrophotometer (Hitachi U-2001, model 121003UV/Vis). By using a syringe inoculation technique bacterial suspension was injected into the midrib at the lower surface of plant leaves early in the morning (when a maximum number of stomata will be opened) (Atiq *et al*., 2022). After three days, the most effective concentration of each treatment identified under lab conditions was applied in greenhouse conditions using the spray method under field conditions (Yang *et al*., 1998). Distilled water was used for the control treatment. The trial was conducted through a CRD with three replicates per treatment. Data were noted at 3 intervals of 5 days using the following formula (Prasad *et al*., 2021).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

***Field scale assessment of green synthesized NPs*:** For in-field evaluation of green synthesized (Cu and Zn) NPs, one moderately susceptible variety of bell pepper was grown. For this purpose, peat moss was filled in the seedling tray (200 holes) then seeds of susceptible variety were sown in these tray. After 45 days, once the seedlings were established, they were transplanted into the field with a spacing of 1.0 feet between plants and 1.5 feet between rows in the Research Area of the Department of Plant Pathology at UAF. A bacterial suspension was prepared using a 2-day-old *Xcv* culture, achieving a concentration of 1×10^8 CFU/mL, measured with a spectrophotometer (Hitachi U-2001, model 121003UV/Vis). This suspension was then injected into the midrib on the underside of plant leaves during the early morning, coinciding with the peak opening of stomata, employing a syringe inoculation technique (Atiq *et al*., 2022). After three days, the most effective concentration of each treatment identified under lab conditions was evaluated in the field using a spray method (Yang *et al*., 1998). Distilled water was used for the control treatment. The trial was conducted through (RCBD) with 3 replications per treatment. Data were recorded at weekly intervals over three weeks using the following formula (Prasad *et al*., 2021).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

## RESULTS

Morphological features of *Xanthomonas campestris* pv. *vesicatoria* expressed Yellow, mucoid, circular and convex shaped growth on NA plates. While, biochemical tests showed that Gram staining, Oxidase test expressed –ve response and Catalase test exhibited +ve response.

**Table 1. Morphological features for the identification of *Xanthomonas campestris* pv. *vesicatoria***

| Morphological features | Observations |
|---|---|
| Shape | Circular |
| Color | Yellow color |
| Elevation | Convex |

**Table 2. Biochemical tests for the identification of *Xanthomonas campestris* pv. *vesicatoria***

| Biochemical Tests | Response |
|---|---|
| Gram staining | -ve |
| Catalase test | +ve |
| Oxidase test | -ve |

***Lab scale appraisement of different nanoparticles against Xanthomonas compestris pv. vesicatoria*:** Cu + Zn nanoparticles exhibited the greatest inhibition zone, measuring 13.733 mm, followed by Zinc nanoparticles with 7.9378 mm, and Copper nanoparticles with 7.0048 mm, in comparison to the control group, as depicted in Table 3 and Figure 1. When examining the interaction effect of treatments and concentrations (T × C), the maximum inhibition zones were achieved with Cu+Zn NPs (17.12, 13.62, 10.45 mm), followed by ZnNPs (8.34, 7.94, 7.52 mm) and CuNPs (7.76, 7.19, 6.05 mm) at concentrations of 0.75 %, 0.50 %, and 0.25 %, respectively, as depicted in Table 4 and Figure 2.

**Table 3. Lab scale evaluation of nanoparticles against *Xanthomonas compestris* pv. *Vesicatoria.***

| **Treatments** | **Inhibition zone** (mm) |
|---|---|
| Cu+Zn NPs | 13.733 a |
| ZnNPs | 7.938 b |
| CuNPs | 7.005 c |
| Control (Distilled water) | 0.000 |
| LSD | 0.471 |

Values within a column sharing the same letters indicate no significant difference, as established by the LSD test ($P \leq 0.05$).

**Table 4. Interaction effect of treatment and concentration on the inhibition zone of Pathogen.**

| Treatments | Inhibition zone (mm) Concentration (%) | | |
|---|---|---|---|
| | 0.25 | 0.50 | 0.75 |
| Cu + ZnNPs | 10.450 c | 13.626 b | 17.122 a |
| ZnNPs | 7.526 ef | 7.943 de | 8.344 d |
| CuNPs | 6.057 g | 7.197 g | 7.761 def |
| Control | 0.000 h | 0.000 h | 0.000 h |
| LSD | | 1.0469 | |

Values within a column sharing the same letters indicate no significant difference, as established by the LSD test ($P \leq 0.05$).

Furthermore, the interaction effect of treatments and days (T × D) indicated that the smallest inhibition zones were shown by Cu NPs (6.5467, 6.9667, 7.5011 mm), followed by Zn NPs (7.3989, 7.9311, 8.4833 mm) and Cu+Zn NPs (12.511, 13.928, 14.759 mm) over 1, 2, and 3 days, respectively, in comparison to untreated group.

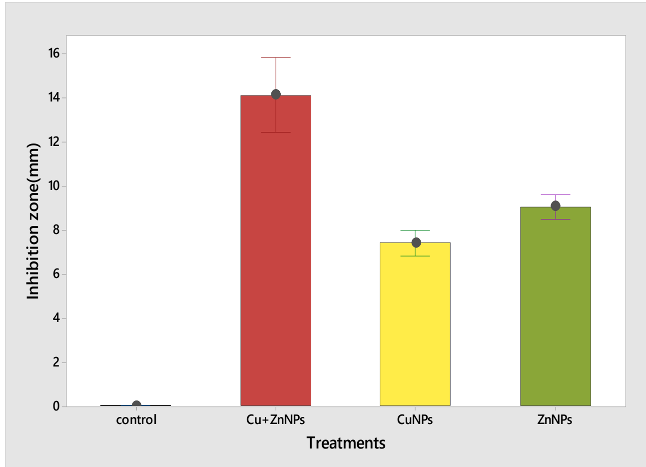


**Figure 1. Lab Scale evaluation of nanoparticles against *Xanthomonas compestris* pv. *vesicatoria***

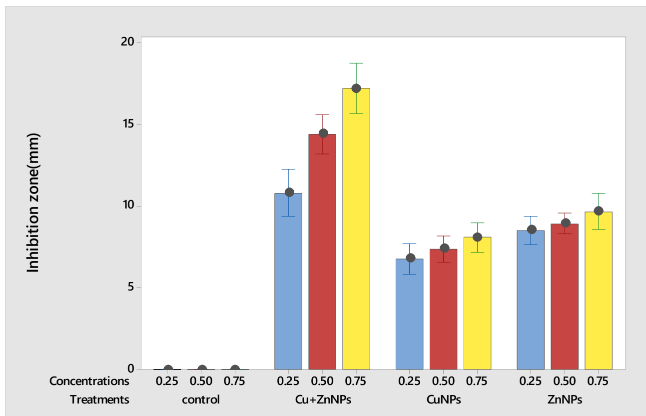


**Figure 2. Interaction effect of treatment and concentration on the inhibition zone of Pathogen.**

**Table 5. Interaction effect of treatment and days on the inhibition zone of Pathoogen.**

| Treatments | Inhibition zone (mm) Concentration (%) | | |
|---|---|---|---|
| | 0.25 | 0.50 | 0.75 |
| Cu + ZnNPs | 12.511 c | 13.928 b | 14.759 a |
| ZnNPs | 7.399 ef | 7.931 de | 8.483 d |
| CuNPs | 6.547 g | 6.967 fg | 7.501 ef |
| Control | 0.000 h | 0.000 h | 0.000 h |
| LSD | | 1.0469 | |

Values within a column sharing the same letters indicate no significant difference, as established by the LSD test ($P \leq 0.05$).

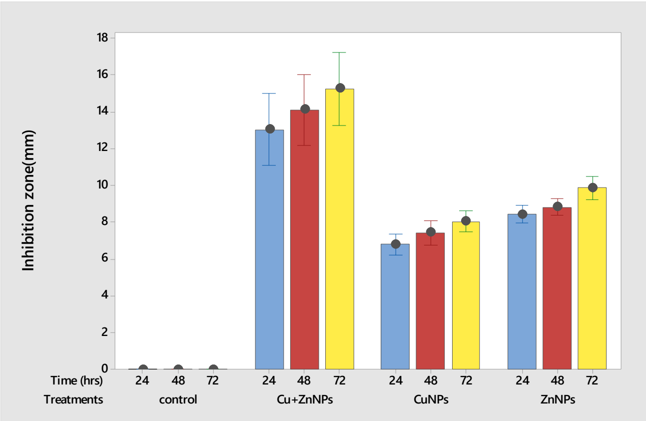


**Figure 3. Interaction effect of Treatments and Days on the Inhibition Zone of Pathogen.**

***Greenhouse scale appraisement of different nanoparticles against Pathogen*:** Among all treatments, the lowest disease incidence was observed with Cu+Zn nanoparticles (17.937 %), followed by Zn nanoparticles (22.966 %) and Cu nanoparticles (27.584 %) in comparison to the control. Interaction effect of treatments and days (T×D) expressed that highest disease incidence was recorded with Cu nanoparticles (34.503 %, 26.443 %, and 21.807 %) followed by Zn nanoparticles (27.113 %, 22.540 %, and 19.243 %) and Cu+Zn nanoparticles (23.643 %, 17.327 %, and 12.840 %) at interval of 5, 10 and 15 days, respectively, compared to untreated group.

**Table 6. Greenhouse scale apprasiment of nanoparticles towards bacterial leaf spot of bell pepper.**

| Treatments | Disease incidence(%) |
|---|---|
| Cu + ZnNPs | 17.937 d |
| ZnNPs | 22.966 c |
| CuNPs | 27.584 b |
| Control | 47.768 a |
| LSD | 2.592 |

Values within a column sharing the same letters indicate no significant difference, as established by the LSD test (P ≤ 0.05).

**Table 7. Interaction effect of T×D on the disease incidence of bacterial leaf spot of bell pepper under greenhouse scale.**

| Treatments | Inhibition zone (mm) Days | | |
|---|---|---|---|
| | 5 | 10 | 15 |
| Cu + ZnNPs | 23.643d ef | 17.327 gh | 12.840 h |
| ZnNPs | 27.113 d | 22.540 ef | 19.143 fg |
| CuNPs | 34.503 c | 26.433 de | 21.807 fg |
| Control | 45.630 b | 47.360 ab | 50.313 a |
| LSD | 4.4897 | | |

Values within a column sharing the same letters indicate no significant difference, as established by the LSD test (P ≤ 0.05).

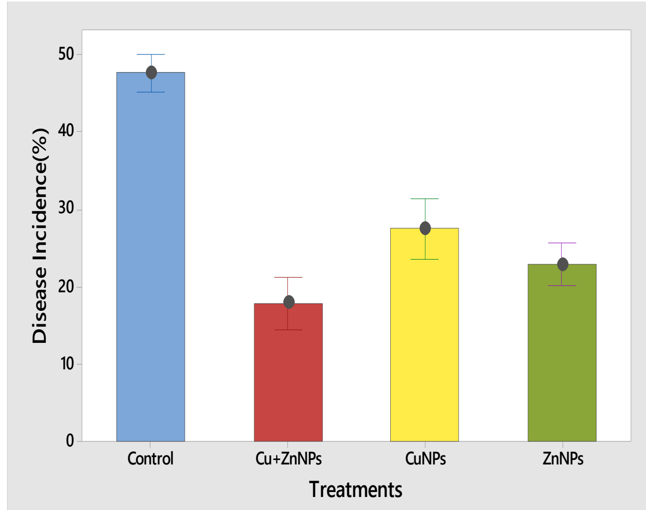


**Figure 4. Greenhouse scale assessment of nanoparticles against bacterial leaf spot of bell pepper.**

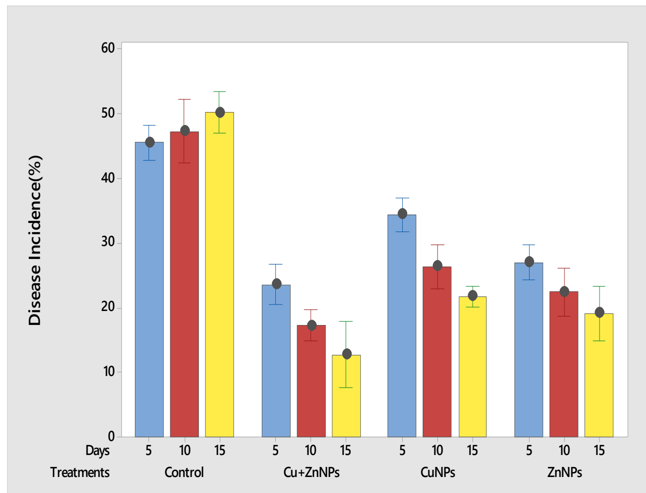


**Figure 5. Interaction effect of treatments and days on the disease incidence of bacterial leaf spot of bell pepper under greenhouse scale.**

***Field scale appraisement of different nanoparticles against bacterial leaf spot of bell pepper*:** Among all treatments, the highest disease incidence was observed with Cu nanoparticles (31.374 %), followed by Zn nanoparticles (27.442 %) and Cu+Zn nanoparticles (22.637 %) compared to the control. The interaction effect of treatments and days that lowest disease incidence was recorded with Cu+Zn nanoparticles (28.067 %, 23.767 %, and 16.077 %), followed by Zn nanoparticles (31.843%, 26.930%, and 23.553%) and Cu nanoparticles (36.587 %, 32.127 %, and 25.410%) at interval of seven days 7, 14 and 21 days, respectively, in compared to untreated group.

**Table 8. Field scale apparaisment of NPs towards bacterial leaf spot of bell pepper.**

| Treatments | Disease incidence (%) |
|---|---|
| Cu + ZnNPs | 22.637 c |
| Zn NPs | 27.442 b |
| Cu NPs | 31.374 b |
| Control | 55.374 a |
| LSD | 4.212 |

Values within a column sharing the same letters indicate no significant difference, as established by the LSD test (P ≤ 0.05).

**Table 9. Interaction effect of T×D on the incidence of bacterial leaf spot of bell pepper.**

| Treatments | Inhibition zone (mm) | | |
|---|---|---|---|
| | Days | | |
| | 5 | 10 | 15 |
| Cu + ZnNPs | 28.067 de | 23.767 ef | 16.077 f |
| ZnNPs | 31.843 cd | 26.930d e | 23.553 e |
| CuNPs | 36.587 c | 32.127 cd | 25.410 de |
| Control | 49.497 b | 55.127 ab | 61.500 a |
| LSD | | 7.2954 | |

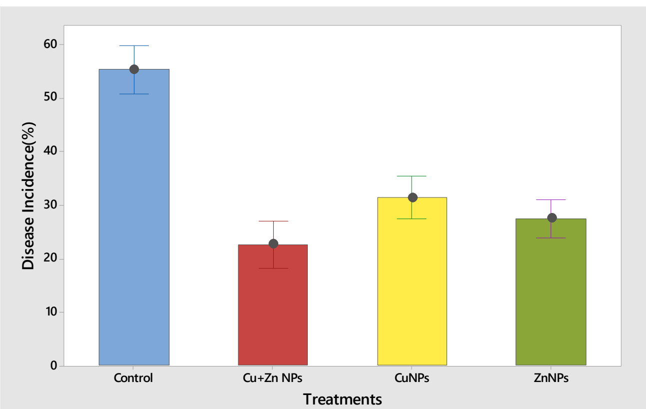


**Figure 6. Field scale evaluation of Nanoparticles against bacterial leaf spot of bell pepper.**

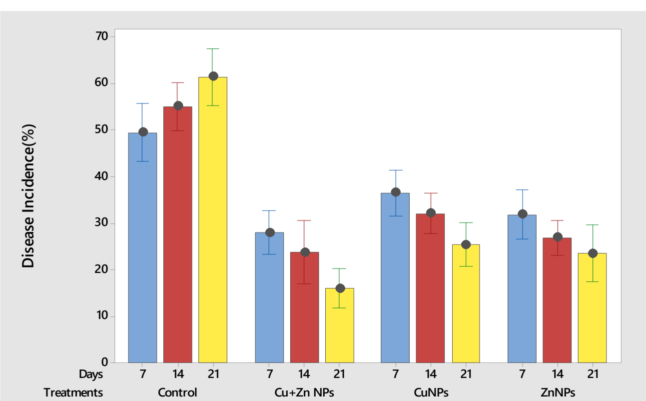


**Figure 7. Interaction effect of T×D on the D.I of bacterial leaf spot of bell pepper.**

## DISCUSSION

The morphological features of *Xanthomonas campestris* pv. *vesicatoria* showed mucoid, circular and yellow colony color pattern. Present outcomes are in line with Arshad et al. (2013) who reported the morphological characteristics of *Xanthomonas oryzae* pv. *oryzae*. And the biochemical features of *Xanthomonas campestris* pv. *vesicatoria* showed that Gram staining and Oxidase tests showed (-ve) response while, Catalase tests showed (+ve) response. Recent findings are encouraged by Arshad et al. (2013) who study and reported the biochemical features of *Xanthomonas oryzae* pv. *oryzae*.

BLS poses a significant and challenging threat to bell pepper crops. Typically, synthetic chemicals are employed to manage plant diseases, but their indiscriminate use leads to environmental pollution, posing risks to both human health and animal well-being. Continuous and excessive application of these chemicals against *Xcv* contributes to pathogen resistance, further complicating the management of bacterial leaf spot. Different management tools were used in the past to overcome the incidence of pathogens. Farmer's first preference is the use of chemicals to manage plant diseases due to cost-effectiveness and quick response but chemicals have some drawbacks as they pose a negative impact on human beings and the environment. Therefore, scientists and researchers are finding an eco-friendly approach to manage plant diseases. Nowadays researcher uses green synthesized nanoparticles to manage plant diseases. Nanotechnology has immense potential for disease management because it can show promising results in maintaining soil fertility and the environment (Rajwade *et al*., 2020). In the green mediated NPs approach, several biotic entities mitigated massive detrimental consequences of physical and chemical methods (Usman *et al*., 2019). The technique of biosynthesis of ZnO nanoparticles from neem leaves was investigated. Several researchers revealed the use of these green culinary strategies because it is cheap having no harmful impacts on the ecosystem. The stability and reduction of zinc ions in zinc oxide NPs were investigated. The extraction of Neem leaves is promising for particle size balance. The medical value result demonstrates significant antibacterial activity (Abel *et al*., 2021). Current investigation was done to probe green synthesized Cu and Zinc nanoparticles against *Xanthomonas compestris* pv. *vesicatoria* causing BLS of bell pepper. Results indicated that the combination of these nanoparticles expressed maximum inhibition zone and least disease incidence under greenhouse and field conditions. Findings of the current study are advocated by Atiq *et al*. (2023) who assessed that green synthesized NPs control the growth of *X. campestris* pv. *vesicatoria*. Recent outcomes of our study are in agreement with previous research conducted by Moradian *et al*. (2018), who investigated the impact of NPs on the expression of hrpE and their antibacterial activities against

*Xanthomonas campestris*. Additionally, our results are consistent with the findings of Varympopi *et al*. (2022), who demonstrated that green-based nanoparticles significantly suppressed the growth of pathogenic bacterium. Furthermore, our conclusions are supported by the work of Awad-Allah *et al*. (2021), who conducted a greenhouse pot trial to assess the efficacy of green mediated NPs towards BLS.

Nanoparticles reduce biotic stress by destabilizing bacterial cells, increasing membrane permeability, and reducing leakage of cellular content (Seong and Lee, 2007) nanoparticles activate antioxidants defense mechanisms by regulating the generation of biochemicals (SOD, POD and CAT , TPC and TFC ) in plants under biotic stress (Salachana *et al*., 2019). In response to nanoparticle-induced oxidative stress, the antioxidative defense system becomes activated (Sharma *et al*., 2019). An increased level of ROS leads to Oxidative stress (Sies *et al*., 2017). The mechanism for reactive oxygen species generation differs for each nanoparticle and the precise cellular mechanisms for ROS generation is still unclear and have to be clarified (Manke *et al*., 2013).

Current findings underscore the promising efficacy of CuNPs and ZnNPs, offering a sustainable and eco-friendly solution for disease management. However, it is pivotal to acknowledge the limitations inherent in recent study, such as the specific conditions under which the nanoparticles were tested and potential variations in efficacy *in vitro*, green house and *in vivo* conditions . Moving forward, future research directions may involve exploring the synergistic effects of combining nanoparticles with other organic compounds or elucidating the precise mechanisms by which they exert their antimicrobial activity. Additionally, long-term field trials and environmental impact assessments are warranted to further validate the practicality and sustainability of this approach.

***Conclusion***: The current investigation has concluded that Copper- Zinc hybrid nanoparticles stand out as the most effective treatment among all the tested nanoparticles. Findings revealed that Cu+Zn NPs demonstrated the highest inhibition zone under laboratory conditions, while these hybrid nanoparticles exhibited the lowest disease incidence in both greenhouse and field conditions, followed by ZnNPs and CuNPs, when compared to the control.

***The future implication of recent revelation for grower***: Growers can cope with bacterial leaf spot of bell pepper; a serious threat to pepper crops by using currently investigated fruitful management tools.

***Author's contributions statement***: Ali, F., M. Atiq, N.A. Rajput designed, completed the experiments; I. Ahmad, M.A. Latif, M.A. Aslam prepared the draft; M.J. Matloob, M. Mehtab, A. Ijaz, M. Qasim reviewed and finalized the draft.

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***Ethical statement***: This article does not contain any studies regarding human or animal.

***Availability of data and material***: We declare that the submitted manuscript is our work, which has not been published before and is not currently being considered for publication elsewhere?

***Code availability***: Not applicable.

***Consent to participate***: All authors participated in this research study.

***Consent for publication***: All authors submitted consent to publish this research.

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