Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798314

ZINC OXIDE NANOPARTICLES FROM BRYOPHYTES: A PROMISING ANTIFUNGAL AGENT AGAINST *FUSARIUM OXYSPORUM* F.SP. *CAPSCI*

MUHAMMAD KHALEEL
Department of Plant Pathology, Faculty of Agricultural Sciences, University of Punjab, Lahore.

MUHAMMAD NASIR SUBHANI
Department of Plant Pathology, Faculty of Agricultural Sciences, University of Punjab, Lahore.

AHMED ALI SHAHID
Centre of Excellence in Molecular Biology, University of Punjab, Lahore.

SHABNUM SHAHEEN
Department of Botany, Lahore College for Women University, Lahore.

Abstract

Nano-formulated agricultural chemicals offer a unique approach to boosting agricultural productivity and controlling plant pathogens while minimizing adverse environmental impacts. Among the various nanoparticle classes, zinc oxide nanoparticles (ZnO-NPs) have garnered attention for their environmentally friendly properties and versatile applications. Recent years have witnessed a growing interest in the applications of ZnO-NPs for microbial control, particularly in combating multidrug-resistant microorganisms. Green synthesis methods for ZnO-NPs have gained prominence, offering an eco-friendly and cost-effective alternative to chemical and physical synthesis methods. Plant-mediated fabrication, in particular, has gained favor due to the ready availability of plant-derived materials, the presence of secondary metabolites, reduced culture maintenance time, and minimal risk of cross-contamination. This study explores the application of green-synthesized ZnO-NPs in combating Fusarium wilt, a devastating fungal disease affecting crops like chili plants. The research involves the collection and identification of bryophytes, the preparation of leaf extracts, and the collection of Fusarium isolates. ZnO-NPs are synthesized using bryophytes, and their inhibitory effect on Fusarium oxysporum f.sp. capsici is evaluated in vitro. The study also assesses the impact of ZnO-NPs on chili plant growth parameters before and after pathogen inoculation. The findings reveal that ZnO-NPs exhibit significant antifungal activity against Fusarium oxysporum f.sp. capsici. Moreover, these nanoparticles positively influence the growth parameters of chili plants, with notable improvements in root and shoot length, fresh and dry weights, and a reduction in disease incidence. These results underscore the potential of green-synthesized ZnO-NPs as a promising antifungal agent against soil-borne pathogens, offering environmentally friendly solutions to agricultural challenges and improved crop productivity.

Keywords: Nanotechnology, ZnO NPs, Fusarium Oxysporum, Antifungal, Green Synthesis, Bryophytes, Capsicum, Sustainable Agriculture.

INTRODUCTION

Richard Feynman introduced the concept of nanotechnology in 1959 during a talk titled "There is Plenty of Room at the Bottom." Recently, advanced technological approaches have been applied in agriculture to tackle the growing challenges of food security and sustainable production (Shang 2019). Strategies based on "nanoparticle technology" have shown remarkable results due to their unique characteristics (Sharma 2021). New

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798314

agricultural practices are a crucial research focus to ensure a steady food supply for the global population (Sighy 2020). Nanotechnology, an emerging interdisciplinary field, significantly impacts people's lives by potentially solving many scientific problems, proving its importance in various fields, including agriculture and related industries (Muradbeygi 2020).

Nanoscale materials, with properties like enhanced efficacy, lower eco-toxicity, and reduced inputs, offer a promising alternative for crop protection, providing numerous advantages over traditional methods (Wang 2020). Among the different types of nanoparticles (NPs), metal-oxide nanomaterials are considered safe for both the environment and humans. Previous studies have shown that Zinc-oxide nanoparticles (ZnONPs) are biocompatible and have antimicrobial properties against various pathogenic fungi and bacteria (Vasantharaj 2019). Today, nanotechnology plays a new role in agriculture by suppressing pathogen infections (bacterial, viral, and fungal), improving plant nutrition, and directly enhancing nutritional value and crop yield (Kah 2019). Furthermore, nanomaterials can influence plant cells and their developmental stages, including seed germination, root induction, cell metabolism, growth index, and biomass, as well as alter their redox levels (Baba 2019).

Zinc oxide nanoparticles (ZnO-NPs) have garnered significant attention owing to their remarkable properties and wide-ranging applications. They have been extensively utilized in biomolecular studies, diagnostics, and microelectronics (Singal). Notably, ZnO-NPs have demonstrated the capacity to effectively remove arsenic and sulfur from water, a feat that bulk zinc oxide cannot achieve. This capability stems from the significantly larger surface area of nanoparticles compared to their bulk counterparts (Singal). Consequently, ZnO-NPs remain a focal point of interest due to their intriguing characteristics and versatile applications. Furthermore, the environmentally friendly and eco-conscious synthesis of ZnO-NPs has been successfully achieved using accepted systems (Dheremendre). The eco-friendliness and potential applications of zinc oxide nanoparticles in fields like nanomedicine, biosensors, antibacterial treatments, antifungal agents, and photochemical processes have generated considerable enthusiasm among scientists. (Zhang P. et al., 2017; Zheng et al., 2017; Zhu L. et al., 2017). In recent years, the interest in utilizing ZnO nanoparticles (NPs) for controlling microbial infections has significantly increased, primarily due to the emergence of multidrug-resistant microorganisms (Kadiyala et al., 2018). The preference for green synthesis methods of ZnO NPs over chemical and physical approaches has gained prominence. This preference is driven by the eco-friendly and cost-effective nature of green synthesis, which eliminates the need for high temperatures, high pressures, and toxic chemicals, thus preventing the generation of hazardous substances (Basnet et al., 2018).Among the various green synthesis methods for ZnO NPs, plant-mediated biofabrication has gained particular popularity. This method stands out for several reasons. Firstly, it offers easy access to a large quantity of plant-derived materials. Secondly, plant-mediated synthesis involves the presence of secondary metabolites that contribute to the process. Additionally, this method reduces the time required for maintaining bacterial and fungal cultures. Furthermore, it minimizes the risk of cross-contamination when working with

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798314

different plant extracts (Ahmed et al., 2017; Vijayakumar et al., 2017). *Fusarium* wilt is a highly destructive fungal disease that significantly reduces the yield and nutritional value of various crops, especially chillies. It is caused by *Fusarium oxysporum* f. sp*. lycopersici* and affects chilli growth in both greenhouse and field conditions. While traditional methods such as using resistant varieties and applying fungicides are available to combat this disease, they are often environmentally unsustainable and not cost-effective.

METHODOLOGY

Collection and Identification of Bryophytes:

Bryophyte species were gathered and harvested in the mountainous regions of Islamabad and Murree. These gathered specimens were appropriately tagged, preserved, and then transported to the Institute of Agricultural Sciences at the University of the Punjab, Lahore, where they were stored at -4°C until the conclusion of the experimental trials.

Preparation of Leaf Extract

A bryophyte mixture was created by cleaning plant samples to eliminate extraneous materials and debris. The bryophyte samples were subsequently air-dried in the shade at a room temperature of 25°C. One gram of the dried material was finely ground using a mortar and pestle, and then it was diluted with 10 ml of sterilized water. The resulting suspension was stored in a refrigerator for eighteen hours, followed by centrifugation at 4°C for 30 minutes and autoclaving at 121°C for 15 minutes. The resulting filtrate was employed in the subsequent phase of the experiment.

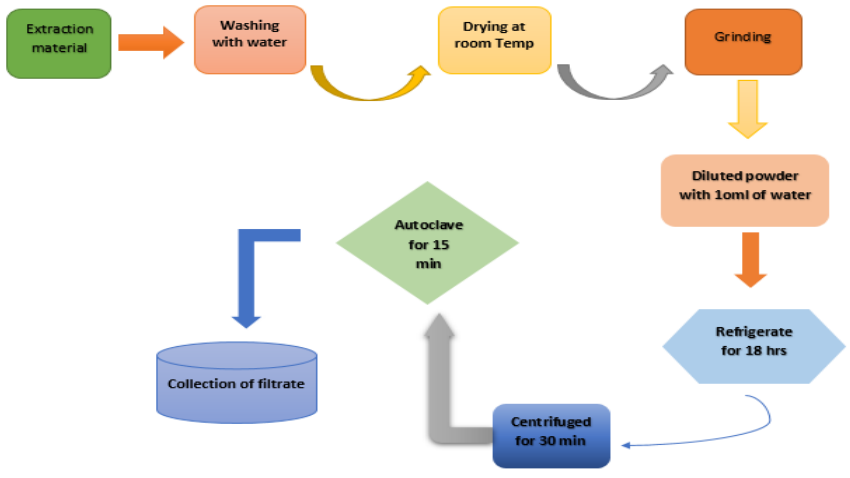

Figure 1: Schematic view of Preparation of Bryophytic Extract

Collection of Fusarium

Chilli plants were sourced from the Ayub Agriculture Research Institute (AARI) in Faisalabad, Pakistan. For this investigation, we employed "*Fusarium oxysporum* f. sp. *capsici* (IAGS-1322)," a highly challenging pathogen for Chilli plants.

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798314

This particular strain was provided by the Fungal Biotechnology Lab, situated within the Department of Plant Pathology, Faculty of Agricultural Sciences at the University of the Punjab in Lahore. It had been previously isolated from diseased Chilli fields and was selected due to its known virulence. The stock culture was cultivated and maintained on potato dextrose agar (PDA) slants at 4°C for long-term storage. To establish solid cultures of F. oxysporum, the stock cultures were sub-cultured onto Petri dishes containing PDA and incubated in the dark at 28°C for a period of 7 days. Distilled water was used as the solvent.

Zinc Oxide Nanoparticle Synthesis:

To synthesize ZnONPs, Bryophyte was utilized as the initial material. Initially, the leaves of Bryophytes were rinsed with distilled water (DI water) and subsequently air-dried at room temperature (25°C) for 2 hours. These dried leaves were then subjected to a muffle furnace at 500°C for 2 hours to obtain powdered leaves. Next, nano ball-milling was carried out for 4 hours at 3000 rpm using a pulverisette (23 mini mill, Fritsch) to produce a fine nanopowder. Four grams of the nano-milled powder were dissolved in 400 mL of DI water under vigorous stirring, forming what we'll call "solution A." To prepare a 0.01 M solution designated as "solution B," a BC (presumably Bryophyte) extract was slowly added drop by drop to solution A while stirring continuously. Solution B was then exposed to varying microwave powers, ranging from 100 to 1000 W, to facilitate the formation of stabilized iron-oxide powder. Careful control of the microwave's on and off times was maintained to prevent splattering due to localized heating within the microwave oven. The resulting product was separated through centrifugation and subsequently dried in a vacuum oven at 80°C for further characterization. A schematic representation of this process is depicted in Figure 1.

UV-visible spectral analysis was employed to monitor the progress of the reaction, and it revealed a distinct change in the color of the solution, turning it to a dark brown hue, signifying the completion of the reaction. This transformation was indicative of the bio-reduction of Zn ions within the experimental mixture. UV spectroscopy (UV-Vis) was utilized to analyze the solution in the wavelength range of 300 – 800nm. The resulting ZnO nanoparticles (NPs) were obtained through centrifugation at 14,000 rpm for ten minutes and subsequently air-dried for powder extraction.

Scanning electron microscopy (SEM) was conducted after air-drying the specimens to generate images that facilitated the study of the surface morphology and texture of the nanoparticles, as per the work of Abdallah Mohamed Elgorban et al. in 2016.

Transmission electron microscopy (TEM) was carried out with incremental voltage settings. Following the aeration of the liquid metallic (ZnO) NPs, drops were deposited onto copper-coated carbon grids and allowed to dry before loading them onto a sample holder. This testing procedure aimed to determine the various forms and sizes of ZnO NPs, following the method described by Abdallah Mohamed Elgorban et al. in 2016.

Fourier-Transform Infrared spectroscopy measurements (FT-IR) were conducted on the bio-transformed products obtained from the supernatant. These products were

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798314

dehydrated and blended with potassium bromide in a 1:100 ratio. FT-IR spectra of the samples were recorded using a diffuse reflectance mode FT-IR instrument, specifically the Digital Excalibur 3000 series from Japan.

X-Ray Diffraction Analysis (XRD) involved subjecting the ZnO nanoparticles to centrifugation for 15 minutes at 10,000 rpm, followed by dissolution in sterilized water. The precipitates obtained were then oven-dried at 50°C and examined using an XRD spectrometer (Pan Analytical, X-pert seasoned, Netherland). This analysis allowed for the determination of the crystalline nature, range, and particle size of the synthesized zinc nanoparticles. Particle size calculations were performed using Scherrer's equation, which is expressed as follows: D =Kλ/ (β1/2 cos θ), Where D represents the average crystal size, radians correspond to the width of the line (full width at half maximum of the peak in radians), λ stands for the X-ray wavelength, β is the Bragg angle, and K is a fixed geometric factor with a value of 0.94, as outlined by Monshi et al. in 2012.

Inhibitory Effect of ZnONPs:

To assess the inhibitory effect of ZnO NPs on the mycelial growth of F. oxysporum in vitro, an agar dilution protocol was employed. Sterilized Petri dishes were filled with twenty milliliters of Potato Dextrose Agar (PDA), and specific amounts of ZnO NPs from the stock solution were added to achieve the desired concentrations. The final concentrations in the growth medium ranged from 0.01 to 15 µg of ZnO NPs per mL. Negative control plates contained PDA without ZnO NPs, while positive control plates were treated with a fungicide. A fungal disc, measuring 4 mm in diameter, was aseptically excised from the periphery of a seven-day-old culture of F. oxysporum and inoculated at the center of each PDA solid media plate. The plates were then sealed and incubated at 28°C until the mycelial growth in the control plates reached the outer edge. Each treatment was conducted in triplicate, and photographs were taken. Mycelial radial growth was measured after seven days.

The percentage of inhibition of radial growth caused by different concentrations of ZnONPs, in comparison to the control, was calculated using the following equation:

Inhibition (%) = $R1-R2/ R1 \times 100$

Where:

- $R1$ represents the mean radial growth in the control group.
- $R2$ represents the mean radial growth in the treatment group [30].

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798314

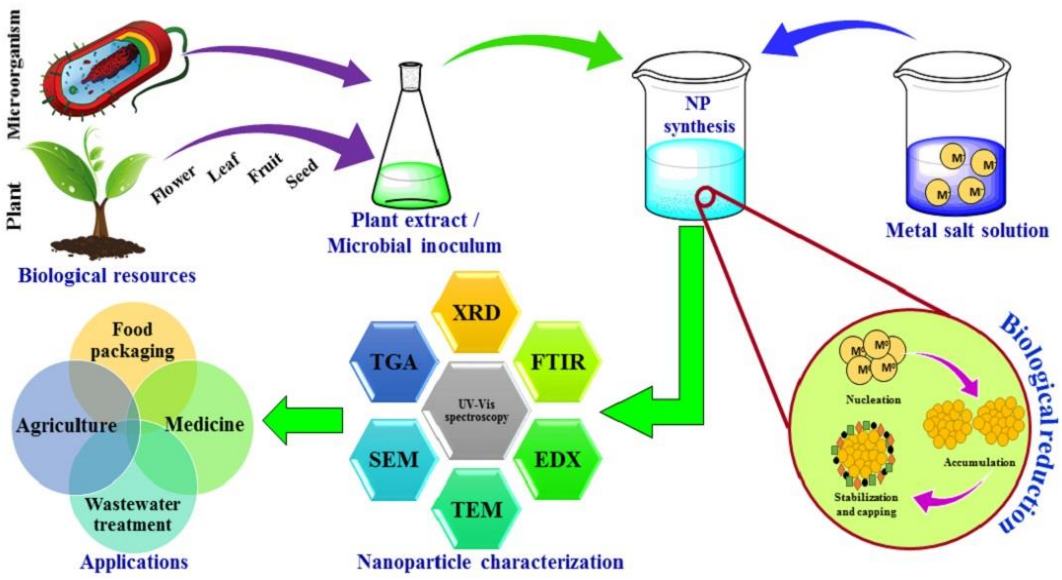

Figure 2: Fungal Growth Inhibition Analysis Incited by Zinc Oxide Nanoparticles

Antifungal Activity Assays:

In vitro assays are conducted to evaluate the antifungal activity of the synthesized ZnO nanoparticles against various soil-borne fungal pathogens. This involves exposing fungal cultures to different concentrations of ZnO NPs and assessing their inhibitory effects on fungal growth. In recent times, various eco-conscious and effective alternatives have emerged for managing phytopathogenic fungi. These alternatives include plant extracts, biological control, essential oils, and engineered nanomaterials (singh, 2021). Zinc oxide nanoparticles (ZnO-NPs) find extensive use across various domains due to their exceptional attributes, which encompass affordability, straightforward production, chemical durability, and non-harmful properties (Hazarika 2022, Naikoo, 2021). Within the realm of agriculture, numerous investigations have explored the utilization of ZnO-NPs as innovative antifungal agents, yielding encouraging outcomes (Rojas 2021, Sardar 2022).

This study introduced a novel approach for biosynthesizing nanoparticles (NPs) using zinc salts and a bryophytic extract, emphasizing its environmentally friendly nature. The bryophytic extract's minerals and biomolecules facilitated NP formation through biochemical reactions with metallic precursors. In vitro tests evaluated the antifungal properties of the biosynthesized NPs against Fusarium Oxysporum f.sp. Capsici, revealing that higher NP concentrations resulted in stronger inhibitory effects, with a minimum inhibitory concentration (MIC) of 10 ppm and an effective concentration (EC50) of 4 ppm. Compared to prior research, these NPs exhibited superior antibacterial efficacy against P. aeruginosa, possibly attributed to their unique non-spherical shape and polydispersity. The study also demonstrated significant inhibition of Fusarium Oxysporum f.sp. Capsici growth using dual culture and zone of inhibition methods, with the most

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798314

effective inhibition observed at 900 ppm and 1000 ppm concentrations of ZnO NPs (86.38% and 87.89% growth inhibition, respectively).

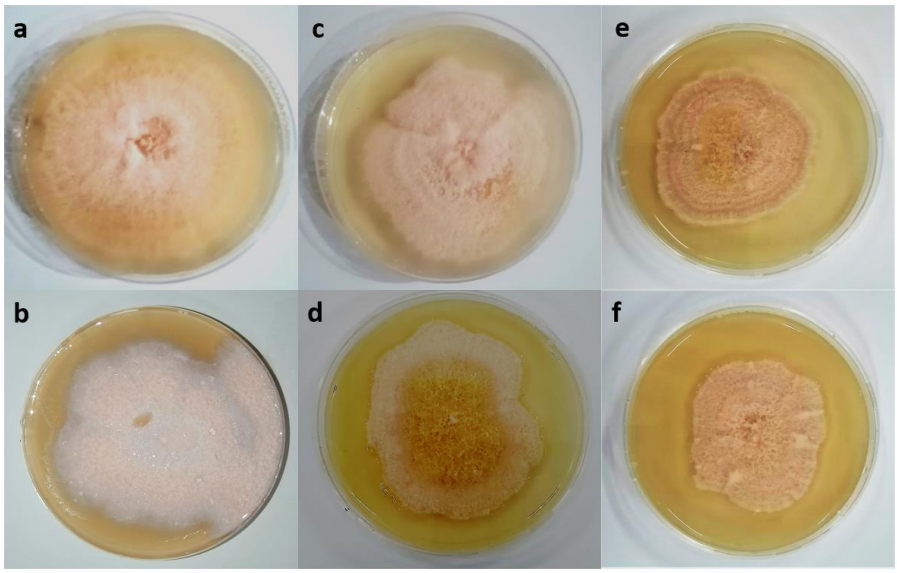

Figure 3: Effect of various concentations of green synthesized zinc oxide nanoparticles on the colonial growth of fusarium oxysporum fsp capsici (a) control, (b) 100ppm, (c)200ppm, (d)300ppm (e)400ppm (f) 500ppm

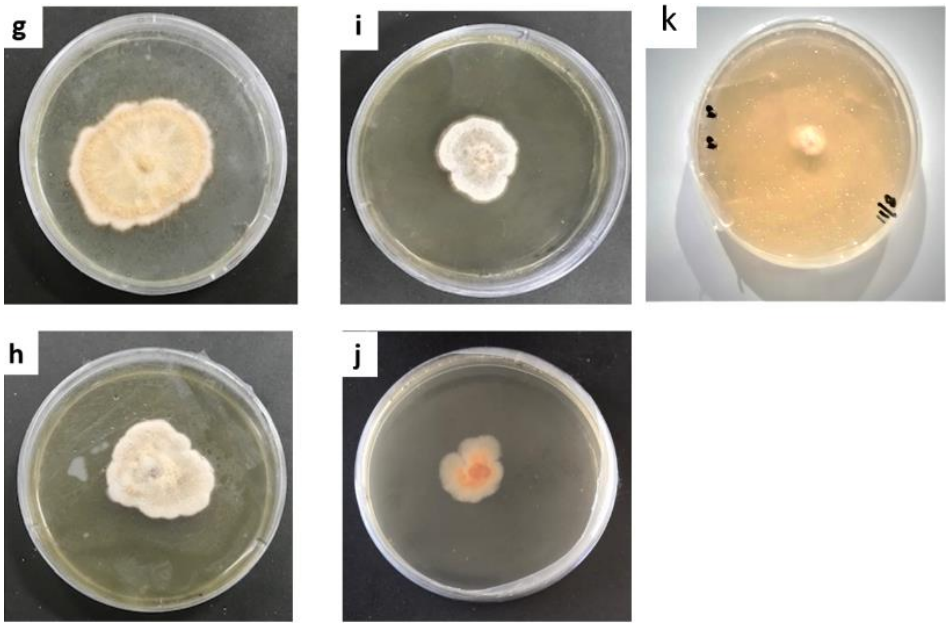

Figure 4: Effect of Various Concentations of Green Synthesized Zinc Oxide Nanoparticles on the Colonial Growth of Fusarium Oxysporum fsp Capsici (g) 600ppm, (h) 700ppm, (i) 800ppm (j) 900ppm (k) 1000ppm.

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798314

Among all the applied concentrations of NPs of ZnO, 1000ppm (T11) and 900ppm (T10) exhibited more than 80% growth inhibition against *Fusarium oxysporum* f.sp *capsici* while minimum inhibition was observed with the 100ppm (T2) concentration. The graph results revealed that fungal growth inhibition was increased with the increase of NPs concentration (Fig.)

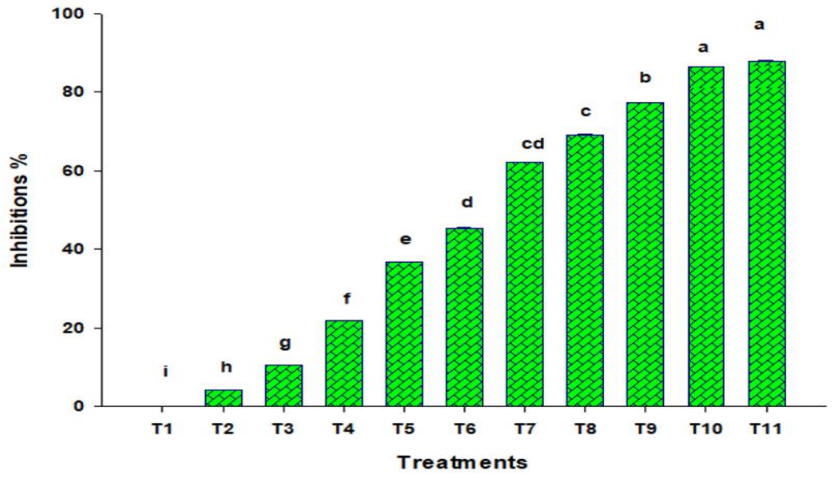

Figure 5: Growth inhibition percentage of ZnO NPs against *Fusarium oxysporum*

Mean values sharing the same letters on each bar for each parameter are statistically indistinguishable based on the LSD test (P≤ 0.05). Treatment groups (T1 to T11) involved the application of different concentrations of ZnO NPs in conjunction with the pathogen inoculum, ranging from 100ppm to 1000ppm, to evaluate their impact on F. oxysporum f.sp. Capsici.

Assessment of Green-Synthesized ZnO Nanoparticles on Chilli Growth Parameters: An In-Vivo Study.

This study investigated the impact of green-synthesized zinc oxide nanoparticles on the growth parameters of the host plant. The experiments were divided into two sets: Set A involved applying the nanoparticles solution to the host plant before introducing the fungal pathogen, while Set B introduced the pathogen first, followed by the nanoparticles solution. This division allowed for assessing the nanoparticles' efficacy in controlling the disease during both pre-emergence and post-emergence conditions. In field conditions, zinc oxide nanoparticles demonstrated effectiveness against Fusarium oxysporum f.sp. capsici in both scenarios. Set A yielded more promising results, with the highest root length (22.23 cm), root fresh weight (4.83 g), root dry weight (1.1 g), shoot fresh weight (45.24 g), and dry shoot weight (6.18 g) observed in plants treated with nanoparticles. Even the negative control treatment showed notable differences in root length, fresh weight, and dry weight compared to the positive control treatment. Additionally, the most

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798314

effective treatments exhibited shoot lengths of 45.7 cm and 70.4 cm, representing significant increases in shoot growth compared to the positive control.

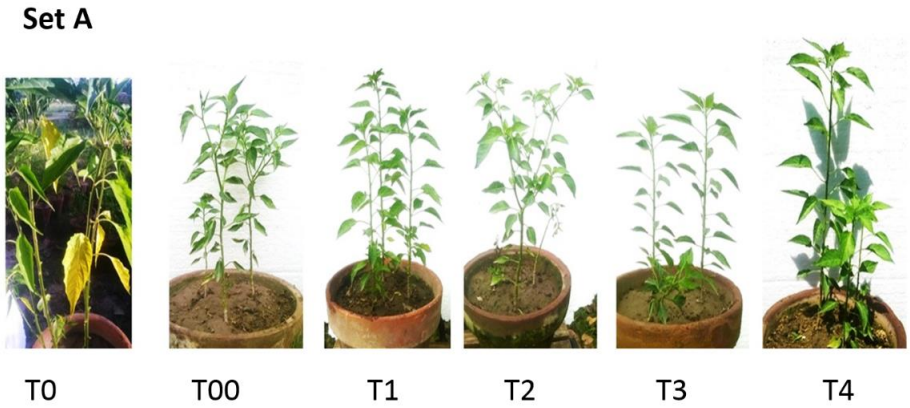

Figure 6: Effect of zinc oxide nanoparticles on the growth of chilli plant (T0 positive control), (T00 Negative control), (T1 5ppm of ZnO NPs/50 ml), (T2 10ppm of ZnO NPs/50 ml), (T3 20ppm of ZnO NPs/50 ml), (T4 30ppm of ZnO NPs/50 ml)

In Set B, where the host plants were already exposed to the disease, the nanoparticles still exhibited robust growth in the host plants. The highest root length (17.3 cm), root fresh weight (3.12 g), root dry weight (0.84 g), shoot fresh weight (41.24 g), and dry shoot weight (5.51 g) were observed in these plants. Even in the case of the negative control, there were noticeable differences in root length, fresh weight, and dry weight compared to the positive control treatment. Furthermore, the most effective treatments displayed shoot lengths of 65.4 cm and 65.6 cm, signifying a significant increase in shoot growth compared to the positive control.

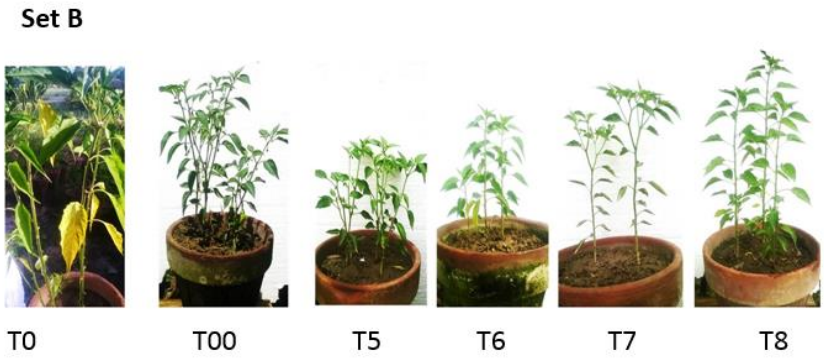

Figure 7: Effect of zinc oxide nanoparticles on the growth of chilli plant (T0 positive control), (T00 Negative control), (T1 5ppm of ZnO NPs/50 ml), (T2 10ppm of ZnO NPs/50 ml), (T3 20ppm of ZnO NPs/50 ml), (T4 30ppm of ZnO NPs/50 ml)

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798314

The significant increase in these vegetative parameters of root reflects that application of nanoparticles not only had reduced the disease attack but also shown a healthy effect on plants growth.

Effect of ZnO NPs on growth parameters of Chili before inoculum of *Fusarium oxysporum*

Effect of ZnO NPs on the Shoot Length

The impact of ZnO NPs on chili shoot length is depicted in Figure 3.14. The graph illustrates a significant influence of ZnO NPs on the shoot length of chili plants, with increasing concentrations of NPs of ZnO leading to enhanced shoot growth. The positive control (T0), involving only pathogen application, exhibited notably shorter shoot lengths compared to other treatments. Notably, the 10ppm concentration of ZnO NPs (T2) resulted in the highest shoot length (78.8 cm), surpassing the negative control (T00) where neither pathogen nor NPs treatment was applied. Additionally, the 5ppm concentration of NPs (T1) also had a positive effect on shoot length (78.5 cm) compared to the negative control. However, shoot lengths of 75 cm were observed for the 20ppm concentration (T3) and 70 cm for the 30ppm concentration (T4).

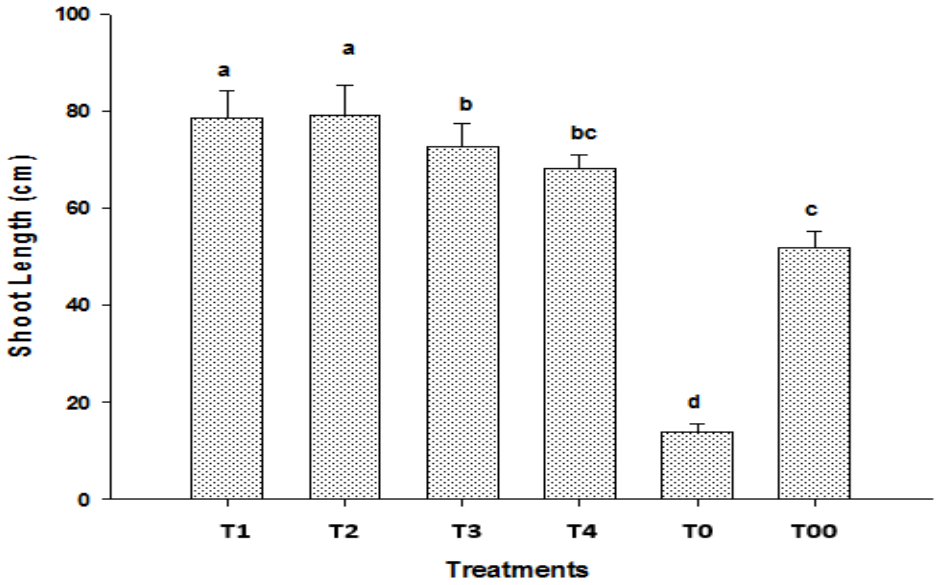

Figure 8: Effect of ZnO NPs on the Shoot Length

Effect of ZnO NPs on the Root Length

The negative control (T00), along with the 10ppm (T2) and 30ppm (T4) NPs concentrations, had nearly identical effects on the root length of chili plants, all measuring at 10 cm. In contrast, the positive control (T0) displayed the shortest root length at 6.6 cm. Both T1 and T3 exhibited similar root length values of 8.05 cm, which were less than those of T2, T4, and T00 (Fig. 3.15).

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798314

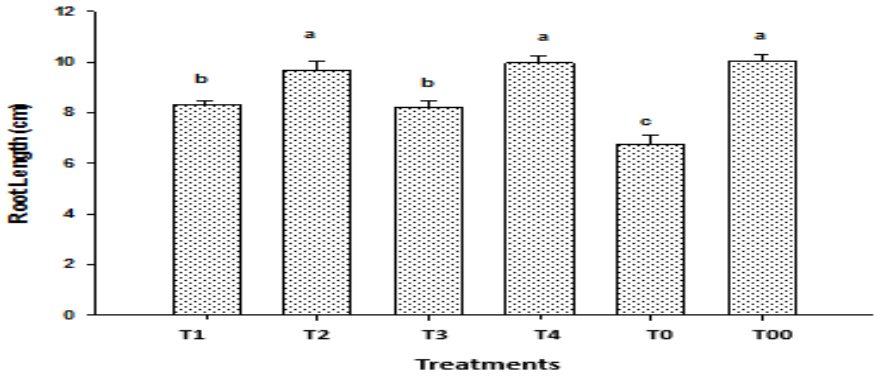

Figure 9: Effect of ZnO NPs on the Root Length

Effect of ZnO NPs on Fresh Shoot weight

The negative control (T00), as well as the 10ppm (T2) and 30ppm (T4) NPs concentrations, demonstrated virtually identical effects on the root length of chili plants, all measuring at 10 cm. Conversely, the positive control (T0) exhibited the shortest root length at 6.6 cm. Both T1 and T3 showed similar root length values of 8.05 cm, which were less than those observed for T2, T4, and T00 (Fig. 3.15).

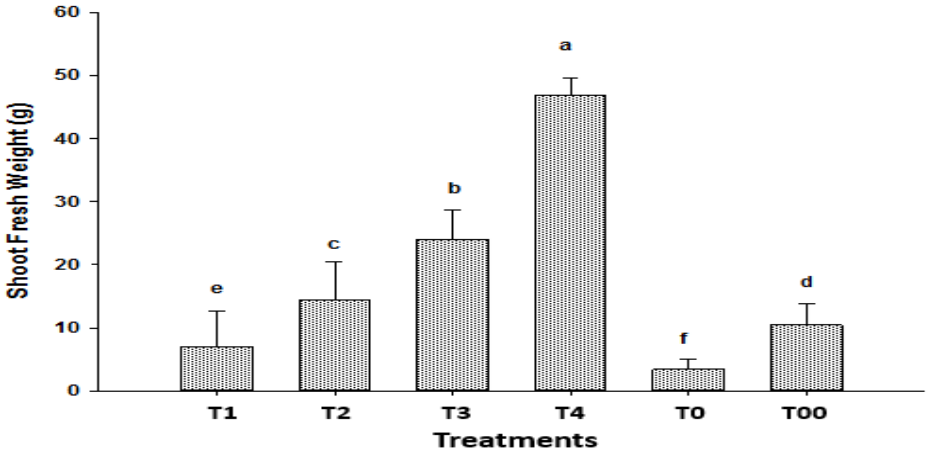

Figure 10: Effect of ZnO NPs on Fresh Shoot Weight

Effect of ZnO NPs on Dry Shoot Weight

In the context of root length in chili plants, the negative control (T00), along with the 10ppm (T2) and 30ppm (T4) NPs concentrations, exhibited nearly identical effects, with all measuring at 10 cm. In contrast, the positive control (T0) displayed the shortest root length at 6.6 cm. Both T1 and T3 showed similar root lengths of 8.05 cm, which were less than those observed for T2, T4, and T00 (Fig. 3.15).

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798314

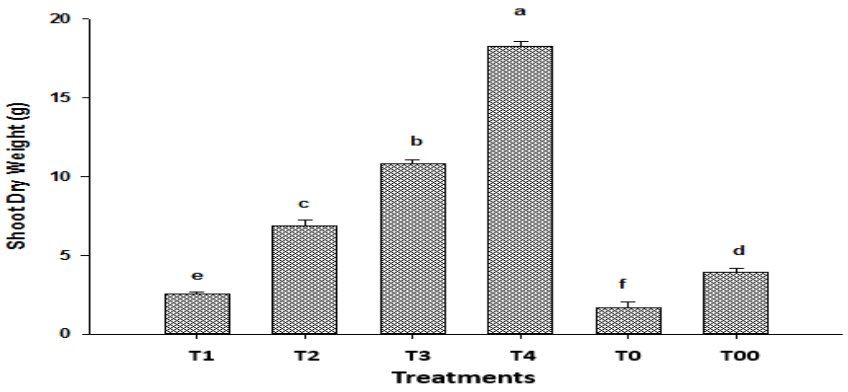

Figure 11: Effect of ZnO NPs on Dry Shoot Weight

Effect of ZnO NPs on Fresh Root Weight

The negative control (T00) and the positive control (T0) had fresh root weights of 1.5g and 0.58g, respectively. The figure indicates that the plant treated with 5ppm of ZnO NPs exhibited the highest fresh root weight of 28g. However, as the treatment concentration increased, the root weight decreased, with T2, T3, and T4 showing fresh root weights of 27.8g, 21.5g, and 18g, respectively (Fig. 3.18).

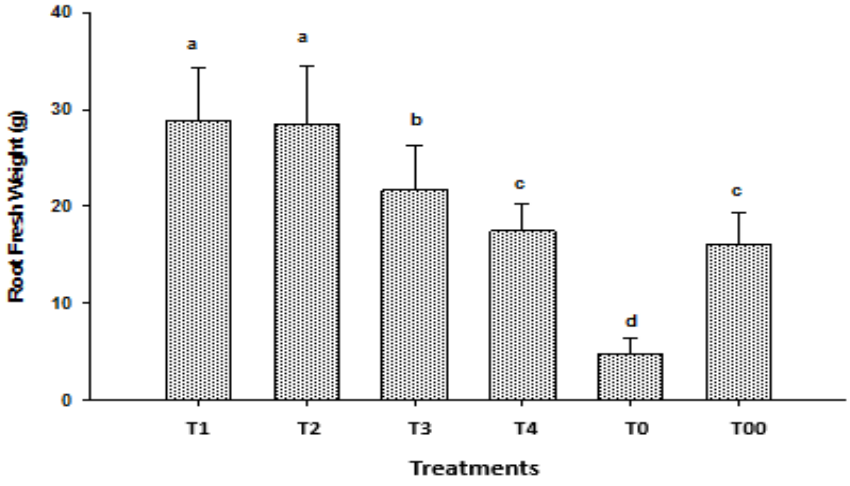

Figure 12: Effect of ZnO NPs on Fresh Root Weight

Effect of ZnO NPs on Dry Root Weight

T1 and T2 exhibited similar dry root weights of 23g, which represented the highest gain, while T0 showed the lowest dry root weight at 2.5g. In contrast, T3 had a dry root weight of 15g compared to T00 and T0. Treatment T4, with 30ppm ZnO NPs, showed the lowest dry root weight of 13.5g among all the NPs concentrations (Fig. 3.19).

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798314

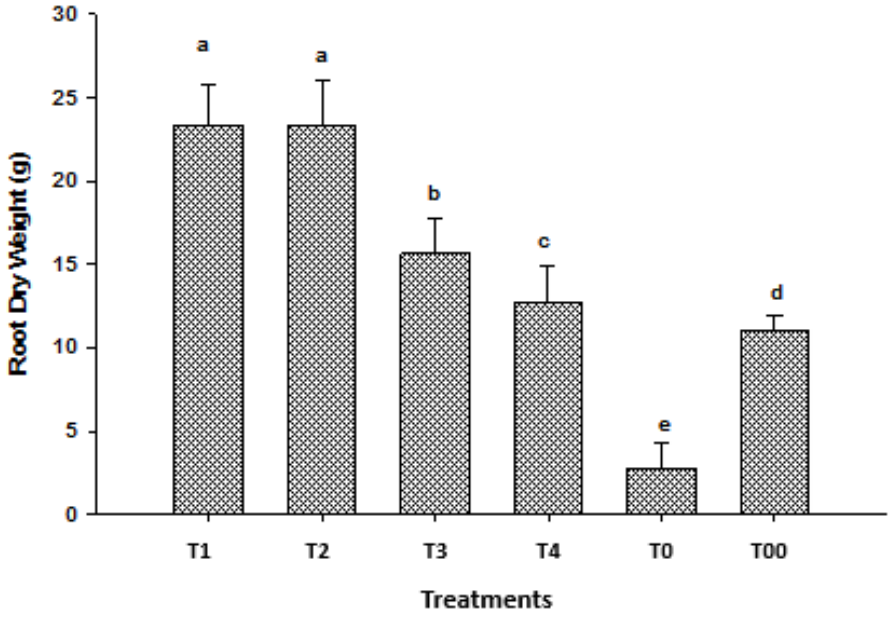

Figure 13: Effect of ZnO NPs on Dry Root Weight

Effect of ZnO NPs on growth parameters of Chili after inoculum of *Fusarium oxysporum*

Effect of ZnO NPs on the Shoot Length

The use of ZnO NPs also demonstrated a positive impact on the growth parameters of chili plants, even when applied after the development of pathogen symptoms. Shoot length increased with the higher concentrations of ZnO NPs. In the case of the positive control (T0), which only involved pathogen application, the shoot length was notably shorter compared to other treatments.

Notably, the concentrations of 5 ppm (T5) and 10 ppm (T6) of ZnO NPs resulted in the highest shoot lengths at 79 cm, outperforming the negative control (T00), where neither pathogen nor NPs treatment was applied. Additionally, the 20 ppm concentration (T7) of NPs also had a positive effect on shoot length, reaching 73 cm compared to the negative control. However, the 30 ppm concentration (T8) exhibited the shortest shoot length at 68.3 cm, which was lower than both the negative control (T00) and positive control (T0) (Figure 13).

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798314

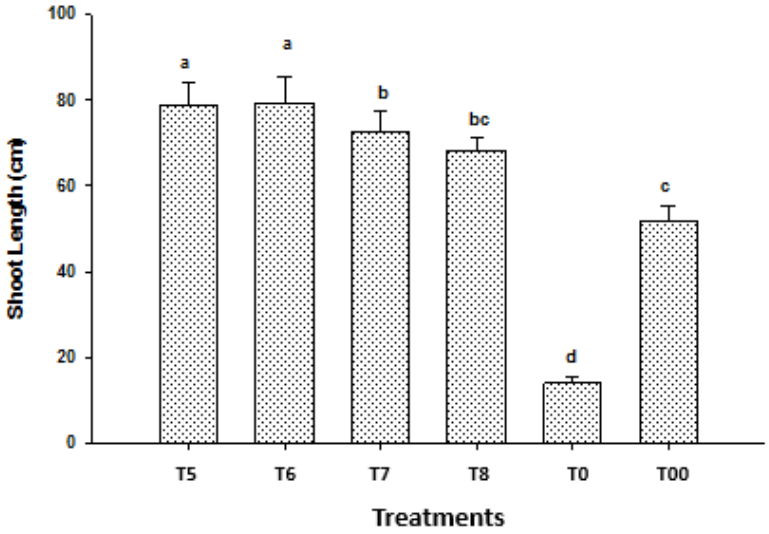

Figure 14: Effect of ZnO NPs on the Shoot Length

Effect of ZnO NPs on the Root Length

The NPs concentration of 30ppm (T8) and 20ppm (T7) had nearly identical effects on the root length of chili plants, measuring at 40cm and 39cm, respectively, with T8 showing the longest root length among all treatments. In contrast, the positive control (T0) exhibited the shortest root length at 7cm. T5 and T6 showed increasing root length values, reaching 21cm and 28cm, respectively (Fig. 14).

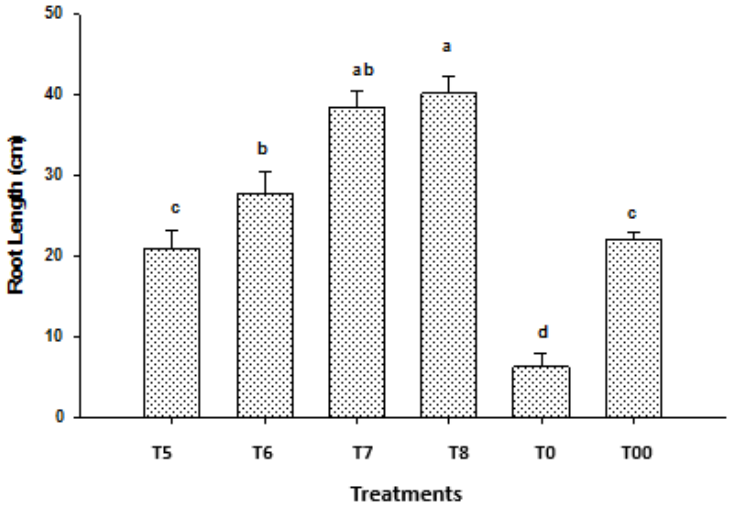

Figure 15: Effect of ZnO NPs on the Root Length

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798314

Effect of ZnO NPs on Fresh Shoot Weight

In terms of the fresh shoot mass of chili plants measured in grams, the NPs concentration of 30ppm (T8) exhibited the highest fresh weight, reaching 48.7g for the shoots, while the lowest fresh shoot weight (4g) was observed in the positive control (T0). The graph further indicated that plants treated with 20ppm ZnO NPs (T7) displayed a fresh shoot weight of 23.05g. Additionally, the NPs concentrations of 5ppm and 10ppm resulted in fresh shoot weights of 7g and 14g, respectively, in comparison to both the negative control (T00) and positive control (T0) (Fig. 15).

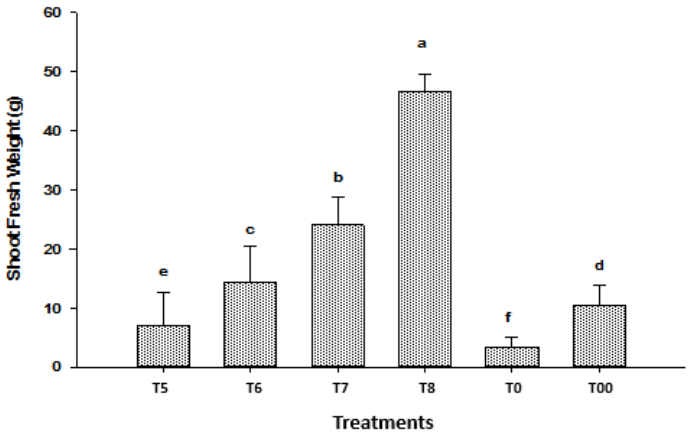

Figure 16: Effect of ZnO NPs on Fresh Shoot Weight

Effect of ZnO NPs on Dry Shoot Weight

In the case of dry shoot mass for chili plants, the NPs treatment T8 displayed the highest dry shoot weight of 18.77g, while T0 exhibited the lowest dry shoot weight at 2.23g. The figure also indicated that plants treated with 5ppm (T5) and 10ppm (T6) of ZnO NPs exhibited dry shoot weights of 3g and 7.4g, respectively. Additionally, treatment T7 with 20ppm of ZnO NPs resulted in a dry shoot weight of 11.18g (Fig. 16).

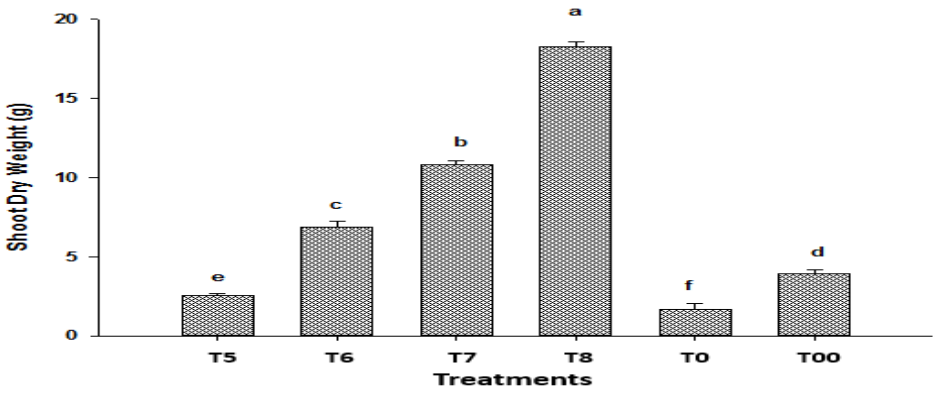

Figure 17: Effect of ZnO NPs on Dry Shoot Weight

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798314

Effect of ZnO NPs on Fresh Root Weight

In terms of root fresh weight, the negative control T00 and positive control (T0) had root weights of 16g and 5g, respectively. The figure illustrates that the plant treated with 20ppm (T7) of ZnO NPs exhibited the highest root weight at 27g. Among the ZnO NPs treatments, T5 (5ppm) displayed the lowest root fresh weight at 17g. Additionally, T8 and T6 showed root fresh weights of 25g and 26.5g, respectively, with the highest NPs concentration (T8) displaying a lower root weight than T7 (Fig. 17).

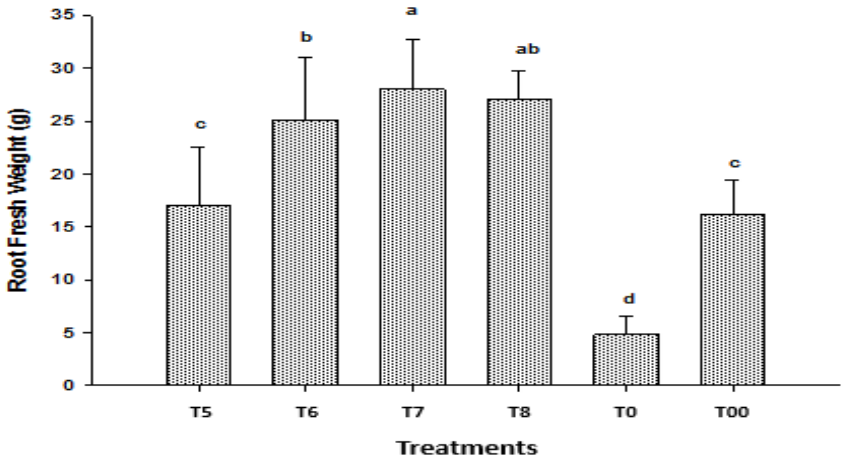

Figure 18: Effect of ZnO NPs on Fresh Root Weight

Effect of ZnO NPs on Dry Root Weight

T7 displayed the highest dry root weight at 24g, while T0 exhibited the lowest dry root weight at 3g. T6 and T8 showed nearly identical dry root weights, both measuring 20.5g, which were higher than the values observed for T00 and T0. Treatment T5 with 5ppm ZnO NPs showed the minimum dry root weight at 14.5g among the different NPs concentrations, but it was still higher than that of T0 and T00 (Fig. 18).

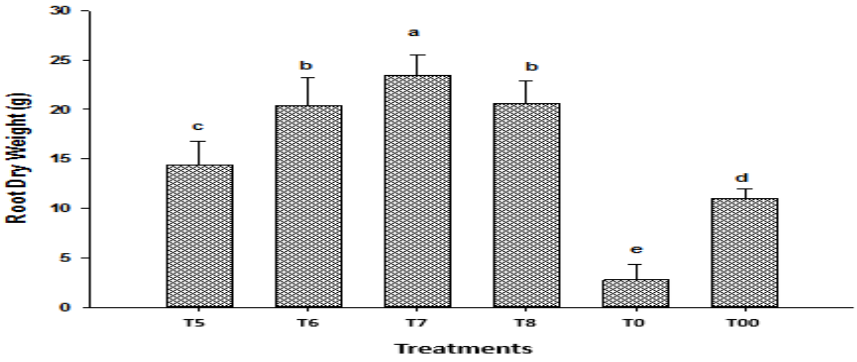

Figure 19: Effect of ZnO NPs on Dry Root Weight

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798314

DISCUSSION

The use of nanotechnology in agriculture has gained significant attention due to its potential to address challenges related to food security and sustainable production. Zinc oxide nanoparticles (ZnO NPs), in particular, have emerged as a promising tool with various applications, including antimicrobial and plant growth-promoting properties. In this study, green-synthesized ZnO NPs derived from bryophytes were evaluated for their antifungal efficacy against Fusarium oxysporum f. sp. capsici, a soil-borne pathogen affecting chili plants. These characteristics highlight the potential utilization of nanoparticles as antifungal agents for controlling the proliferation and contamination of various pathogens (Raskar & Laware, 2014). ZnO nanoparticles, due to their remarkable chemical and thermal stability, cost-effectiveness, and environmental friendliness, are the most commonly employed nanomaterials for safeguarding plants (Rajiv et al., 2013; Sabri et al. 2013, as cited in Kriti et al., 2020). By investigating the release of ZnO from nanoparticles, both microparticles and nanoparticles were assessed to determine their role in antimicrobial activity. This study revealed the unique impact of ZnO at the nanoscale on the level of antifungal effectiveness. The bioactivity of nanoparticles was found to be superior, underscoring that their nanosize enhances the potency of ZnO by elevating its toxicity compared to larger microparticles and hindering the mycelial growth of the Fusarium graminearum fungus (Dimkpa et al., 2013

Green Synthesis of ZnO NPs: The green synthesis of ZnO NPs using bryophytes as a starting material represents an environmentally friendly and cost-effective approach. The use of plant-mediated biofabrication offers several advantages, including the availability of large quantities of plant material, the presence of secondary metabolites, reduced processing time, and minimal cross-contamination. This green synthesis method aligns with sustainable practices and avoids the use of toxic chemicals, high temperatures, and pressure.

Antifungal Activity of ZnO NPs: In vitro assays demonstrated the antifungal activity of green-synthesized ZnO NPs against Fusarium oxysporum f. sp. capsici. The inhibitory effect of ZnO NPs was concentration-dependent, with higher concentrations leading to stronger growth inhibition. Notably, ZnO NPs exhibited a minimum inhibitory concentration (MIC) of 10 ppm and an effective concentration (EC50) of 4 ppm. These findings highlight the potential of ZnO NPs as a natural alternative for managing fungal pathogens, offering an eco-conscious and effective solution. In Esparza's study conducted in 2016, it was noted that at a concentration of 1,000 mg/L, the antifungal activity of ZnO nanoparticles (with a size of 23.44 nm) resulted in a remarkable 91.13 percent inhibition of growth in F. oxysporum. This suggests that ZnO nanoparticles exhibit substantial effectiveness in restraining the growth of F. oxysporum and could potentially serve as an alternative approach for managing this phytopathogen (Esparza, 2016).

Influence on Chili Growth Parameters: The study further investigated the impact of green-synthesized ZnO NPs on chili plants' growth parameters under both pre-emergence and post-emergence conditions, simulating pathogen exposure scenarios.

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798314

Pre-Emergence Conditions:

- **Shoot Length:** ZnO NPs application led to enhanced shoot growth, with the 10 ppm concentration resulting in the highest shoot length (78.8 cm). This concentration outperformed both the negative control (T00) and positive control (T0), highlighting the positive effect of NPs on shoot development.
- **Root Length:** ZnO NPs promoted root length, with the 10 ppm and 30 ppm concentrations exhibiting similar effects. The positive control had the shortest root length, emphasizing the role of NPs in root development.
- **Fresh and Dry Shoot Weight:** NPs treatments positively influenced shoot weight, with higher concentrations leading to increased fresh and dry shoot weights. The positive control showed the lowest values, underscoring the importance of NPs in enhancing shoot biomass.
- **Fresh and Dry Root Weight:** ZnO NPs significantly increased root fresh and dry weights, with the 5 ppm concentration resulting in the highest fresh root weight. These results indicate that NPs application not only reduced disease incidence but also promoted healthy root growth.

Post-Emergence Conditions:

- **Shoot Length:** Even when applied after pathogen exposure, ZnO NPs continued to positively impact shoot length. The 5 ppm and 10 ppm concentrations exhibited the highest shoot lengths, surpassing controls and emphasizing their role in mitigating pathogen-induced stress.
- **Root Length:** NPs treatments maintained their positive effect on root length, with the 30 ppm concentration displaying the longest roots. The positive control exhibited the shortest root length.
- **Fresh and Dry Shoot Weight:** NPs application resulted in increased fresh and dry shoot weights, demonstrating their ability to enhance shoot biomass even under pathogen pressure.
- **Fresh and Dry Root Weight:** ZnO NPs treatments significantly increased root fresh and dry weights, with the 20 ppm concentration showing the highest values.

CONCLUSION

This study highlights the potential of green-synthesized ZnO NPs as an effective antifungal agent against Fusarium oxysporum f. sp. capsici, a soil-borne pathogen affecting chili plants. The environmentally friendly synthesis method using bryophytes provides an eco-conscious alternative for producing ZnO NPs. In vitro assays demonstrated concentration-dependent inhibitory effects of ZnO NPs on fungal growth.

Moreover, the study showed that ZnO NPs positively influenced various growth parameters of chili plants, including shoot and root length, fresh and dry shoot weight, and fresh and dry root weight. Importantly, NPs application exhibited beneficial effects

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798314

even when applied after pathogen exposure, highlighting their potential for disease management and plant growth promotion.

Overall, this research offers valuable insights into the use of green-synthesized ZnO NPs as a sustainable and effective approach for mitigating soil-borne fungal pathogens and enhancing crop growth, contributing to sustainable agriculture practices and food security. Further studies can explore the practical application of these NPs in field conditions and their long-term effects on soil health and plant ecosystems.

References

1) Adisa, I.O.; Reddy Pullagurala, V.L.R.; Rawat, S.; Hernandez-Viezcas ,J.A, Dimkpa, C.O.; Elmer, W.H.; White, J.C.; Peralta-Videa, J.; Gardea-Torresdey, J.L. Role of cerium compounds in fusarium wilt suppression and growth enhancement in tomato (*Solanum lycopersicum*). *J Agric Food Chem*. **2018,** *66*, 5959–70.

2) Ahmed, S.; Chaudhry, S. A.; & Ikram, S. A review on biogenic synthesis of ZnO nanoparticles using plant extracts and microbes: a prospect towards green chemistry. *Journal of Photochemistry and Photobiology B: Biology*, **2017,** *166*, 272-284.

3) Basnet, P.; Chanu, T. I.; Samanta, D.; & Chatterjee, S. A review on bio-synthesized zinc oxide nanoparticles using plant extracts as reductants and stabilizing agents. *Journal of Photochemistry and Photobiology B: Biology*, **2018**, *183*, 201-221.

4) Dimkpa C.O., McLean J.E., Britt D.W., and A.J. Anderson. 2013. Antifungal activity of ZnO nanoparticles and their interactive effect with a biocontrol bacterium on growth antagonism of the plant pathogen *Fusarium graminearum*. BioMetals, 26: 913–924.

5) Esparza A.I.J.E. 2016. Potencial antimicrobial de nanopartículas metálicas en microorganismos fitopatógenos y su potencial como promotores de crecimiento en plantas. Universidad Autónoma Agraria Antonio Narro. Thesis.

6) Elgorban, A. M.; Aref, S. M.; Seham, S. M.; Elhindi, K. M.,; Bahkali, A. H., Sayed.; S. R., & Manal, M. A. Extracellular synthesis of silver nanoparticles using Aspergillus versicolor and evaluation of their activity on plant pathogenic fungi. *Mycosphere*, **2016**, *7*, 844-852.

7) Fu, L.; Wang, Z.; Dhankher, O.P.; Xing, B. Nanotechnology as a new sustainable approach for controlling crop diseases and increasing agricultural production*. J Exp Bot*. **2020**; *71*, 507–19.

8) Hazarika, A.; Yadav, M.; Yadav, D.K.; Yadav, H.S. An overview of the role of nanoparticles in sustainable agriculture. *Biocatal. Agric. Biotechnol.* **2022**, *43*, 102399.

9) Kadiyala, U.; Kotov, N. A.; & VanEpps, J. S. Antibacterial metal oxide nanoparticles: challenges in interpreting the literature. *Current pharmaceutical design*, **2018**, *24*, 896-903.

10) Kah, M.; Tufenkji, N.; White, J.C. Nano-enabled strategies to enhance crop nutrition and protection. *Nat Nanotechnol*. **2019**, ***14,532***–40.

11) Babajani, A.; Iranbakhsh, A.; Oraghi Ardebili, Z.; Eslami, B. Diferential growth, nutrition, physiology, and gene expression in *Melissa Officinalis* mediated by zinc oxide and elemental selenium nanoparticles. *Environ Sci Pollut Res Int.* **2019,** *26*, 24430–44

12) Kriti A., Ghatak A., and N. Mandal. 2020. Antimycotic efficacy of zinc nanoparticle on dark-spore forming phytopathogenic fungi. J. Pharmacog Phytochem. 9: 750–754.

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798314

13) Moradbeygi, H.; Jamei, R.; Heidari, R.; Darvishzadeh, R. Investigating the enzymatic and non-enzymatic antioxidant defense by applying iron oxide nanoparticles in *Dracocephalum moldavica* L. plant under salinity stress. *Sci Hortic***. 2020,** 272.

14) Naikoo, G.A.; Mustaqeem, M.; Hassan, I.U.; Awan, T.; Arshad, F.; Salim, H.; Qurashi, A. Bioinspired and green synthesis of nanoparticles from plant extracts with antiviral and antimicrobial properties: A critical review. *J. Saudi Chem. Soc.* **2021**, *25*, 101304.

15) Raskar S.V., and Laware S.L. 2014. Effect of zinc oxide nanoparticles on cytology and seed germination in onion. The Int J. Current Microbiol and Appl Sci. 3: 467–473.

16) Rajiv P., Rajeshwari S., Venckatesh R. 2013. Bio-Fabrication of zinc oxide nanoparticles using leaf extract of *Parthenium hysterophorus* L. and its size-dependent antifungal activity against plant fungal pathogens. *Spectrochimica Acta* Part A*:* Molecu Biomolecu Spectrosco. 112, 384–387.

17) Rojas-Chávez, H.; Miralrio, A.; Hernández-Rodríguez, Y.M.; Cruz-Martínez, H.; Pérez-Pérez, R.; Cigarroa-Mayorga, O.E. Needle-and cross-linked ZnO microstructures and their photocatalytic activity using experimental and DFT approach. *Mater. Lett.* **2021**, *291*, 129474.

18) Sardar, M.; Ahmed, W.; Al Ayoubi, S.; Nisa, S.; Bibi, Y.; Sabir, M.; Khan, M.M.; Ahmed, W.; Qayyum, A. Fungicidal synergistic effect of biogenically synthesized zinc oxide and copper oxide nanoparticles against *Alternaria citri* causing citrus black rot disease. *Saudi J. Biol. Sci.* **2022**, *29*, 88–95.

19) Servin, A.; Elmer, W.; Mukherjee, A.; Torre-Roche, R.; Hamdi, H.; White, J.C.; Bindraban, P.; Dimkpa, C. A review of the use of engineered nanomaterials to suppress plant disease and enhance crop yield. *J Nanopart Res*. **2015***, 17*, 1–21.

20) Shang, Y.; Hasan, M.; Ahammed, G.J.; Li, M.; Yin, H.; Zhou, J. Applications of nanotechnology in plant growth and crop protection: a review. *Molecules*. **2019**, *24*: 2558.

21) Sharma, S.; Singh, B.; Bindra, P.; Panneerselvam, P.; Dwivedi, N.; Senapati, A.; Adholey, A.; Shanmugam, V.; Triple-smart eco-friendly chili anthracnose control agro-nanocarrier. *ACS Appl Mater Interfaces*. **2021**, *13*:9143–55.

22) Sidhu A, Bala A, Singh H, Ahuja R, Kumar A. Development of MgOSepoilite nanocomposites against phytopathogenic fungi of rice (*Oryzae sativa*): a green approach. ACS Omega. 2020; 5:13557–65.

23) Singh, R.P.; Handa, R.; Manchanda, G. Nanoparticles in sustainable agriculture: An emerging opportunity. *J. Control Release* **2021**, *329*, 1234–1248.

24) Singhal, M..; Chhabra V.; Kang P, and Shah D.O. Materials Research Bulletin. 1997, 32: 239-247. Dhermendre K,; Tiwari and Behari J. Prasenjiten. Application of nanoparticles in waste water treatment. *World Applied Science Journal***, 2008**, *3*, 417-433.

25) Vasantharaj, S.; Sathiyavimal, S.; Senthilkumar, P.; LewisOscar, F.L.; Pugazhendhi, A. Biosynthesis of iron oxide nanoparticles using leaf extract of *Ruellia tuberosa*: antimicrobial properties and their applications in photocatalytic degradation. *J Photochem Photobiol B*. **2019**, *192*, 74–82.

26) Vijayakumar, S.; Mahadevan, S.; Arulmozhi, P.; Sriram, S.; & Praseetha, P. K. Green synthesis of zinc oxide nanoparticles using Atalantia monophylla leaf extracts: Characterization and antimicrobial analysis. *Materials Science in Semiconductor Processing*, **2018**, *82*, 39-45.

27) Wang, C;, Zhang, L.; Su, W.; Ying, Z;, He, J;, Zhang, L.; & Wang, T. Zinc oxide nanoparticles as a substitute for zinc oxide or colistin sulfate: Effects on growth, serum enzymes, zinc deposition, intestinal morphology and epithelial barrier in weaned piglets. *PloS one*, **2017**, *12*.