Efficacy of Zinc oxide nanoparticles against anthracnose and southern blight disease of green gram

Abstract

Nanotechnology has rapidly advanced in the 21st century, with significant developments in the synthesis and application of nanoparticles. ZnNps were synthesized using extracts from mehandi (*Lawsonia inermis*), neem (*Azadirachta indica*), marigold (*Tagetus erecta*) and ginger (*Zingiber officinalis*).

This study investigates the antifungal activity of green synthesized zinc nanoparticles (ZnNPs) against *Colletotrichum lindemuthianum*, the causative agent of anthracnose in mung beans and *Sclerotium rolfsii* causing southern blight in mung bean using the poisoned food technique. Various concentrations (100ppm, 250ppm and 500ppm) of zinc nanoparticles derived from neem, mehandi, marigold and ginger were evaluated alongside corresponding plant extracts and Zinc nitrate hexahydrate at 250ppm. Results indicated significant fungistatic activity, with Mehandi ZnNps at 500ppm exhibiting the highest efficacy, demonstrating minimal mycelial growth (28.00mm) and a growth inhibition rate of 68.88% followed by Marigold and Neem ZnNPs against *Colletotrichum*. As for *Sclerotium*, Neem ZnNps at 500 ppm exhibiting the highest efficacy, demonstrating minimal mycelial growth (15.00 mm) and a growth inhibition rate of 83.33% followed by ginger and Mehandi. All tested ZnNP concentrations outperformed their respective plant extracts and Zinc nitrate hexahydrate, confirming the potential of green synthesized ZnNPs as effective agents.

(**Keywords:** Anthracnose, *Colletotrichum lindemuthianum*, Zinc nanoparticles, *In vitro*, Southern blight, *Sclerotium rolfsii*)

Introduction

Green gram or Mung bean (*Vigna radiata*) belongs to the family Leguminaceae. Numerous fungal diseases frequently hinder the ability of the affected plants to germinate, kill them or

significantly lower their capacity for production. Various nanoparticles can be used against seedborne pathogens and soil borne pathogens on green gram. Zinc nanoparticles were also used against *Colletotrichum sp*, which causes anthracnose diseases in fabaceae family (Mosquera *et al.* 2020) [4]. Zinc nanoparticles were effectively used against *Sclerotium rolfsii*, which causes southern blight on mung bean crop Kamel (2024). [3]. This study was conducted to analyze the efficacy of zinc oxide nanoparticles against *Colletotrichum lindemuthianum* causing anthracnose on green gram and *Sclerotium rolfsii* causing southern blight on green gram.

Material and method

The efficacy of zinc nanoparticles synthesized from neem (*Azadirachta indica)*, mehandi (*Lawsonia inermis*), marigold (*Tagetus erectus*) and ginger (*Zingiber officinalis*) were assessed against *Colletotrichum lindemuthianum* and *Sclerotium rolfsii* using Poisoned food technique (Nene and Thapliyal, 1993) [5] by utilizing Potato dextrose agar medium as the base culture. Zinc nanoparticles synthesized from neem, mehandi and marigold plant extracts were evaluated against *Collettotrichum lindemuthianum* at 100, 250 and 500 ppm. Neem plant extracts at 250 ppm, mehandi plant extracts at 250ppm, marigold plant extracts at 250ppm and Zinc nitrate hexahydrate at 250 ppm were also evaluated against *Colletotrichum lindemuthianum* in order to find out the potential of antifungal activity of zinc nanoparticles against the fungus.

Zinc nanoparticles synthesized from neem, mehandi and ginger plant extracts were evaluated against *Sclerotium rolfsii* at 100, 250 and 500 ppm. Neem plant extracts at 250 ppm, mehandi plant extracts at 250ppm, ginger plant extracts at 250ppm and Zinc nitrate hexahydrate at 250 ppm were also evaluated against *Sclerotium rolfsii* in order to find out the potential of antifungal activity of zinc nanoparticles against the fungus.

The PDA medium containing appropriate quantity of zinc nanoparticles or plant extracts or zinc nitrate hexahydrate at 250ppm were poured aseptically into Petri dishes (90 mm in diameter), where it solidifies at room temperature. All of the plates were inoculated aseptically with a 5 mm culture disc made from an actively growing pure culture of a pathogen. The disc was positioned in the middle of the petri plate on PDA, and the plates then be incubated at a temperature of 27 ± 2°C. Three replication of treatment were made. Petri plates with basic PDA containing the pathogen's culture disc were continued to be used as the untreated control. Thus replications with 14 treatments and by using design CRD for *Colletotrichum* and *Sclerotium*. Treatment details are as follows,

list 1 : **Treatment details for *Colletotrichum***

Tr. No	Treatments	Tr. No	Treatments
T_1	Neem ZnNps @100ppm	**T_8**	Marigold ZnNps @ 250ppm
T_2	Neem ZnNps @250ppm	**T_9**	Marigold ZnNps @ 500ppm
T_3	Neem ZnNps @ 500ppm	**T_{10}**	Neem extracts @ 250ppm
T_4	Mehandi ZnNps @ 100ppm	**T_{11}**	Mehandi extracts @ 250ppm
T_5	Mehandi ZnNps @ 250ppm	**T_{12}**	Marigold extracts @ 250ppm
T_6	Mehandi ZnNps @ 500ppm	**T_{13}**	Zinc Nitrate Hexahydrate @250ppm
T_7	Marigold ZnNps @ 100ppm	**T_{14}**	**Control (Untreated)**

list 2 : **Treatment details for *Sclerotium***

Tr. No	Treatments	Tr. No	Treatments
T_1	Neem ZnNps @100ppm	**T_8**	Ginger ZnNps @ 250ppm
T_2	Neem ZnNps @250ppm	**T_9**	Ginger ZnNps @ 500ppm
T_3	Neem ZnNps @ 500ppm	**T_{10}**	Neem extracts @ 250ppm
T_4	Mehandi ZnNps @ 100ppm	**T_{11}**	Mehandi extracts @ 250ppm
T_5	Mehandi ZnNps @ 250ppm	**T_{12}**	Ginger extracts @ 250ppm
T_6	Mehandi ZnNps @ 500ppm	**T_{13}**	Zinc Nitrate Hexahydrate @250ppm
T_7	Ginger ZnNps @ 100ppm	**T_{14}**	**Control (Untreated)**

Observations on radial colony diameter of the test isolates were recorded at an interval of 24 hrs and continued till untreated control plates were fully covered with growth of the test pathogen. Per cent growth inhibition of the test pathogens over untreated control was calculated by following formula (Vincent, 1927) [8].

$$\text{Per cent inhibition (I)} = \frac{(C-T) \times 100}{(C)}$$

Where, I = Per cent inhibition

C = Growth of test fungus on untreated control plates

T = Growth of test fungus pathogen on treated plates

Result and Discussion

In case of *Colletotrichum*, the results indicated that solutions of zinc nanoparticles derived from Neem, Mehandi and Marigold at concentrations of 100ppm, 250ppm and 500ppm exhibited significant fungistatic effects. Notably, Mehandi zinc nanoparticles at 500ppm showed the greatest efficacy, resulting in minimal mycelial growth (28.00mm), followed closely by Marigold and Neem at the same concentration (29.00mm and 30.00mm, respectively). In terms of growth inhibition percentages, Mehandi zinc nanoparticles achieved the highest inhibition rate at 68.88%, with Marigold and Neem following at 67.77% and 66.66%, respectively. All tested concentrations of the zinc nanoparticles proved to be more effective than the corresponding plant extracts and zinc nitrate hexahydrate. Results shows that all the treatments at all concentration were significant over the control in inhibiting the growth of test pathogen. (Table1, Plate1, Fig 1).

These findings align with previous research indicating that zinc nanoparticles possess notable antifungal properties. For instance, Mosquera *et al*. (2020) [4] reported that ZnO nanoparticles significantly inhibited *Colletotrichum* growth, while Thienprasert *et al* (2021) [7] found a substantial inhibition rate using nanoparticles derived from banana peel extract. Additionally, Saranya *et al*. (2023) [6] highlighted that zinc nanoparticles from mustard seeds also demonstrated strong inhibitory effects on mycelial growth. Overall, the results underscore the potential of green synthesized zinc nanoparticles as effective antifungal agents against *Colletotrichum lindemuthianum*, suggesting their applicability in agricultural practices for managing fungal diseases.

In case of *Sclerotium rolfsii* the results indicated that solutions of zinc nanoparticles derived from Neem, Mehandi and Ginger at concentrations of 100ppm, 250ppm and 500ppm exhibited significant fungistatic effects. The results showed that all treatments significantly inhibited fungal growth compared to the untreated control. Among the treatments, Neem ZnNps at 500ppm exhibited the highest effectiveness, resulting in the lowest mycelial growth (15.00mm), followed by Ginger ZnNps (19.00mm) and Mehandi ZnNps (27.00mm) at the same concentration. Lower concentrations also demonstrated antifungal activity, though less effectively. In terms of growth

inhibition percentages, Neem zinc nanoparticles achieved the highest inhibition rate at 83.33%, with Ginger zinc nanoparticles and Mehandi following at 78.88% and 70.00%, respectively. All tested concentrations of the zinc nanoparticles proved to be more effective than the corresponding plant extracts and zinc nitrate hexahydrate. (Table 2, Platec2, Fig 2). These findings align with the previous research indicating that zinc nanoparticles possess notable antifungal properties. For instance, Ali *et al* [1]. (2020) found that 60 mg ZnO-NPs from *Azadirachta indica* inhibited mycelial growth of *Sclerotium rolfsii* by 41%, while chemically synthesized ZnO-NPs had no effect. Zaki *et al* [9]. (2021) tested various concentrations (20, 40, and 100 µg/mL) of ZnO-NPs against *Rhizoctonia solani, Fusarium sp.* and *Macrophomina phaseolina*, observing significant growth inhibition across all concentrations. Jomeyazdian *et al* [2] (2024) reported that ZnO-NPs synthesized from *Trichoderma harzianum* inhibited the growth of *Fusarium oxysporum* in a dose-dependent manner, with higher concentrations leading to greater inhibition.

Table 1. *In vitro* evaluation of green synthesized Zinc nanoparticles against *Colletotrichum lindemuthianum* by Poisoned Food Technique

Tr. No.	Treatment at different concentration	Colony Diameter* (mm)	% Inhibition
T_1	Neem ZnNps @100ppm	40.00	55.55 **(48.18)**
T_2	Neem ZnNps @250ppm	35.00	61.11 **(51.41)**
T_3	Neem ZnNps @ 500ppm	30.00	66.66 **(54.73)**
T_4	Mehandi ZnNps @ 100ppm	42.00	53.33 **(46.90)**
T_5	Mehandi ZnNps @ 250ppm	38.00	57.77 **(49.47)**
T_6	Mehandi ZnNps @ 500ppm	28.00	68.88 **(56.09)**
T_7	Marigold ZnNps @ 100ppm	41.00	54.44 **(47.54)**
T_8	Marigold ZnNps @ 250ppm	34.00	62.22 **(52.07)**
T_9	Marigold ZnNps @ 500ppm	29.00	67.77 **(55.40)**
T_{10}	Neem extracts @ 250ppm	55.00	38.88 **(38.52)**
T_{11}	Mehandi extracts @ 250ppm	57.00	36.66 **(37.26)**

T_{12}	Marigold extracts @ 250ppm	56.00	37.77 **(37.92)**
T_{13}	Zinc Nitrate Hexahydrate @250ppm	47.00	47.77 **(43.72)**
T_{14}	Control (Untreated)	90.00	0.00 **(0.00)**
	S.E. ±	**0.55**	**0.58**
	C.D. (P=0.01)	**2.17**	**2.28**

Mean of three replications. **Figures in parenthesis are arc sine transformed values

Table 2. *In vitro* evaluation of green synthesized Zinc nanoparticles against *Sclerotium rolfsii* by Poisoned Food Technique

Tr. No.	Treatment at different concentrations	Colony Diameter* (mm)	% Inhibition
T_1	Neem ZnNps @100ppm	36.00	60.00 **(50.76)**
T_2	Neem ZnNps @250ppm	30.00	66.66 **(54.73)**
T_3	Neem ZnNps @ 500ppm	15.00	83.33 **(65.90)**
T_4	Mehandi ZnNps @ 100ppm	50.00	44.44 **(41.80)**
T_5	Mehandi ZnNps @ 250ppm	40.00	55.55 **(48.18)**
T_6	Mehandi ZnNps @ 500ppm	27.00	70.00 **(56.78)**
T_7	Ginger ZnNps @ 100ppm	52.00	42.22 **(40.52)**
T_8	Ginger ZnNps @ 250ppm	39.00	56.60 **(48.79)**
T_9	Ginger ZnNps @ 500ppm	19.00	78.88 **(62.64)**
T_{10}	Neem extracts @ 250ppm	70.00	22.22 **(28.12)**
T_{11}	Mehandi extracts @ 250ppm	60.00	33.33 **(35.26)**
T_{12}	Ginger extracts @ 250ppm	58.00	35.55 **(36.60)**
T_{13}	Zinc Nitrate Hexahydrate @250ppm	55.00	38.88 **(38.52)**
T_{14}	Control (Untreated)	90.00	0.00 **(0.00)**

	S.E. ±	**0.55**	**0.56**
	C.D. (P=0.01)	**2.17**	**2.20**

Mean of three replications. **Figures in parenthesis are arc sine transformed values

Plate 1: *In vitro* evaluation of biosynthesized Zinc nanoparticles against *Colletotrichum lindemuthianum* by poisoned food technique

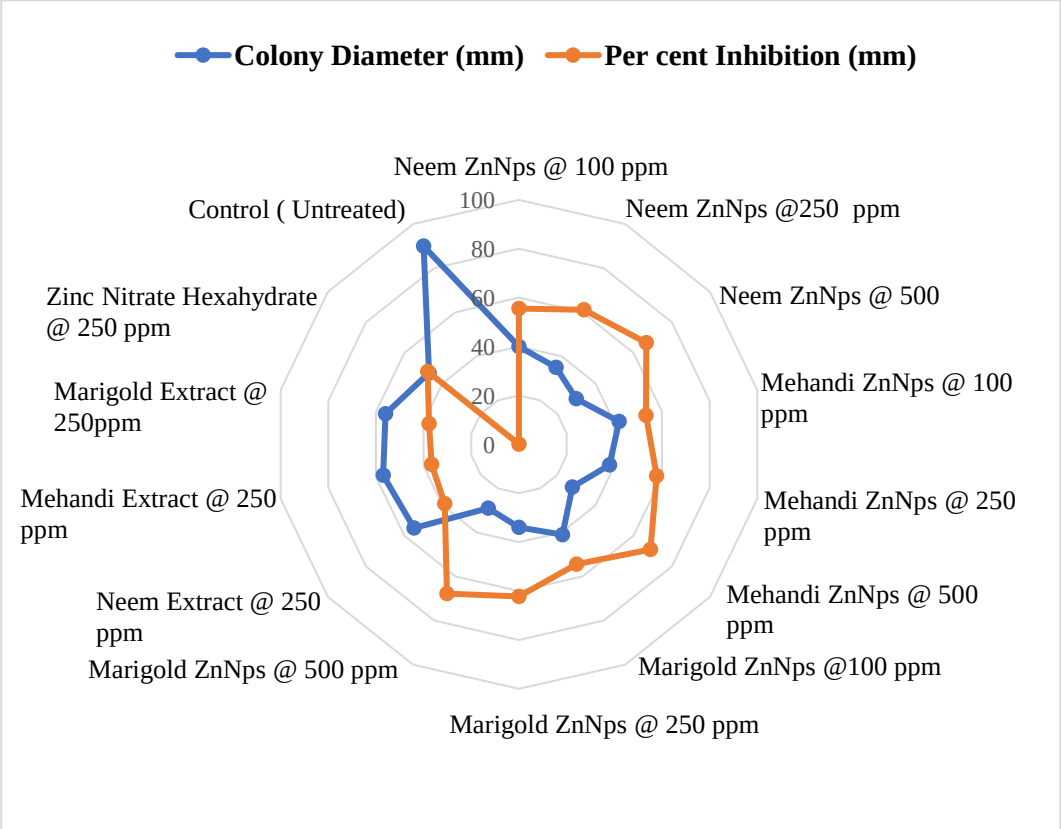

Fig 1: *In vitro* evaluation of biosynthesized Zinc nanoparticles against *Colletotrichum lindemuthianum* by poisoned food technique

Plate 2: In vitro evaluation of biosynthesized Zinc nanoparticles against *Sclerotium rolfsii* by poisoned food technique

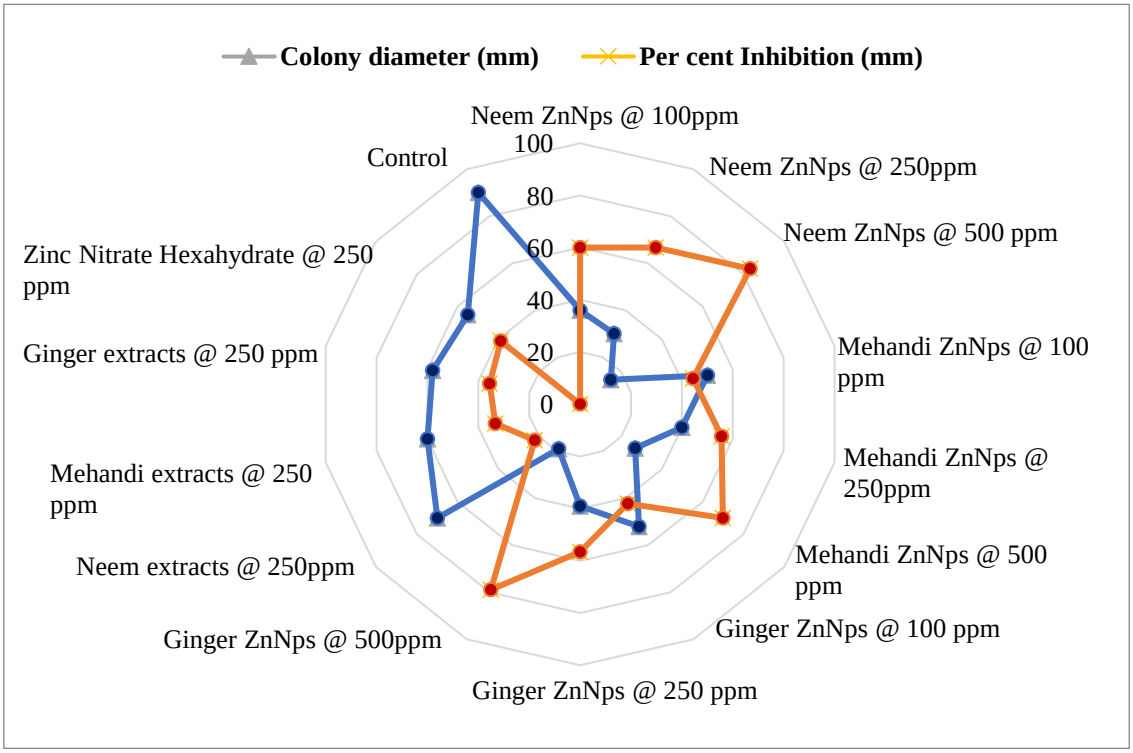

Fig 2: In vitro evaluation of biosynthesized Zinc nanoparticles against *Sclerotium rolfsii* by poisoned food technique

Conclusion

The study demonstrated that zinc nanoparticles (ZnNps) derived from Mehandi, Marigold and Neem significantly inhibit the radial mycelial growth of *Colletotrichum lindemuthianum*, the pathogen responsible for anthracnose in mung bean and in case of *Sclerotium rolfsii* the nanoparticles synthesized from neem, mehandi and ginger were effective. Among the treatments, Mehandi ZnNps at 500 ppm exhibited the highest inhibition rate at 68.88%, followed closely by Marigold and Neem ZnNps at the same concentration in case of *Colletotrichum* while, for *Sclerotium* Neem ZnNp at 500ppm followed by ginger and mehandi. The effectiveness of these nanoparticles surpassed that of traditional plant extracts and zinc nitrate hexahydrate, indicating their potential as a novel approach for managing fungal diseases in crops. These findings underscore the importance of utilizing zinc nanoparticles as a promising alternative for enhancing plant disease resistance and improving agricultural productivity. Further research may explore the mechanisms behind this efficacy and the potential for practical applications in field.

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