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Assessment of the Inhibitory Effect of Nanoparticles and Salicylic Acid on *Alternaria solani*

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ABSTRACT

Background and Objective: *Alternaria solani* is responsible for causing early blight, leaf spot, collar rot, fruit rot and stem canker diseases in solanaceous crops and is very difficult to control. Several management strategies are employed but chemical fungicides are most effective. However, due to their established negative consequences, much greener and eco-friendly options are needed. Nanotechnology could offer new approaches for solving this problem and the present study was carried out to explore the fungicidal activity of twelve nanoparticles against the mycelial inhibition of *Alternaria solani*. **Materials and Methods:** The comparative inhibitory effect of twelve Nanoparticles (NPs) viz., Cobalt ferrite ($CoFe_2O_4$), Elemental copper (Cu^-), Ferric oxide (Fe_2O_3), Nickel ferrite ($NiFe_2O_4$), Nickel peroxide (NiO_2), Polyamelene (PANI), Polyanelene+ferrite (P600), Tin oxide (SnO_2), Titanium oxide (TiO_2), Zinc ferrite ($ZnFe_2O_4$), Zinc oxide (ZnO), Zinc peroxide (ZnO_2) and Salicylic acid (SA) were evaluated *in vitro* against *Alternaria solani*. All the data were processed by using Analysis of Variance (ANOVA) and R-software at ($p \leq 0.05$). **Results:** *In vitro*, screening of NPs and SA at 5, 10, 15, 20 and 25 µg/mL revealed substantial variability to inhibit the mycelial growth of *A. solani*. In general, the NPs which caused significant inhibition in fungal growth, their effectiveness increased with increase in the concentrations. Among the NPs, zinc peroxide (20 µg/mL), salicylic acid (20 µg/mL), zinc oxide (20 µg/mL), elemental copper (20 µg/mL), nickel peroxide (25 µg/mL) and ferric oxide (25 µg/mL) caused the 100% inhibition in the mycelia growth of *A. solani*. **Conclusion:** This is the first study on the fungicidal effects of nano-zinc peroxide on *A. solani*. However, the mechanism involved in the fungicidal activity and toxicological studies are required to be confirmed.

KEYWORDS

Plant-pathogenic fungi, early blight, nanoformulation, mycelial inhibition, induce resistance

INTRODUCTION

Alternaria solani is regarded as an important menace and causes huge damage to solanaceous crops[1]. The fungus is responsible for causing diseases like early blight, leaf spot, collar rot, fruit rot, stem canker, etc. and is very difficult to control[2]. Several management strategies are employed against *A. solani* in various

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crops[3] and chemical fungicides are one of the most effective methods to manage this fungus. However, due to their established negative consequences, much greener and more effective fungicides are needed[4].

Nanotechnology could offer new approaches for solving this problem as the performance of conventional pesticide/fungicide formulae is often improved by nanotechnology construction[5,6]. A multitude of chemical, biological and physical techniques are still being developed which lead to the production of noble nanoparticles, some of which are used against fungal pathogens[7,8]. Recent research has demonstrated that the foliar application of nano-CuO, MnO and ZnO significantly declined fungal pathogens without physiological toxicity in a greenhouse[9]. In other studies, antifungal activity of copper-based[10,11], Ag-based[12] against *Alternaria alternata* and Zn-based nanoparticles against *Alternaria mali* was shown by Ahmad *et al*.[8].

Phenolic compounds including salicylic acid (SA) are involved in plant defence against plant pathogens[13]. Several studies revealed the ability of SA to induce systemic defense in various crops against fungal pathogens[4]. Further, exogenous application of SA induced a strong systemic defense response in rice crops against *Rhizoctonia solani*[14,15], tobacco against *Pythium aphanidermatum*[16] and cowpea for root-knot pathogen[17]. Jayakumar *et al*.[18] reported that SA application at 50, 100 and 200 ppm improved plant height, root length, shoot and root weight and significantly decreased root-knot disease in tomato plants.

With the above background, the present *in vitro* study was carried out to explore the fungicidal activity of twelve nano particles viz., cobalt ferrite, elemental copper, ferric oxide, nickel ferrite, nickel peroxide, polyamelene, polyanelene+ferrite, tin oxide, titanium oxide, zinc ferrite, zinc oxide and zinc peroxide and salicylic acid against the mycelial inhibition of *Alternaria solani*.

MATERIALS AND METHODS

Study area: The present study was carried out during September-December, 2023 at the Department of Plant Protection, Faculty of Agricultural Science, Aligarh Muslim University, Aligarh, India.

Nanoparticles and their doses: Twelve nanoparticles viz., Cobalt ferrite ($CoFe_2O_4$), Elemental copper (Cu^-), Ferric oxide (Fe_2O_3), Nickel ferrite ($NiFe_2O_4$), Nickel peroxide (NiO_2), Polyaniline (PANI), Polyanelene+ferrite (P600), Tin oxide (SnO_2), Titanium oxide (TiO_2), Zinc ferrite ($ZnFe_2O_4$), Zinc oxide (ZnO) and Zinc peroxide (ZnO_2) were obtained from Department of Chemistry, Aligarh Muslim University, India. Pure and Lab grade salicylic (Qualigen, India) was procured from a chemical dealer in Aligarh, India. To investigate their nematicidal action in terms of mortality and hatching inhibition of *M. javanica* J2s *in vitro*, five concentrations viz., 5, 10, 15, 20 and 25 μg/mL of each chemical were used.

Inoculum of *Alternaria solani*: The isolation, purification and morphological identification of a pathogenic isolate of *Alternaria solani* AMUAS-1 was previously done and used in the present study[1]. Further molecular identification using 18s rDNA was carried out at Macrogen Co., Ltd. (Seol, South Korea) and phylogenetic tree was constructed using the neighbor-joining method in MEGA 11.0[19], with bootstrap values based on 1000 replications. The partial gene sequence was submitted to the NCBI gene bank with accession number KY062572 (Fig. 1).

Determination of minimum inhibitory concentration of nanoparticles and salicylic acid against mycelial growth of *Alternaria solani in vitro*: The sensitivity of *Alternaria solani* to twelve nanoparticles (NPs), as mentioned in the previous section and salicylic acid (SA) was tested by poisoned food technique at five different concentrations (5, 10, 15, 20 and 25 μg/mL) as described by Dhingra and Sinclair[20]. Sterilized potato dextrose agar medium was poured in Petri plates having desired NPs and SA concentrations separately. Thereafter, the medium was allowed to solidify. A mycelial disc (9 mm dia.) of

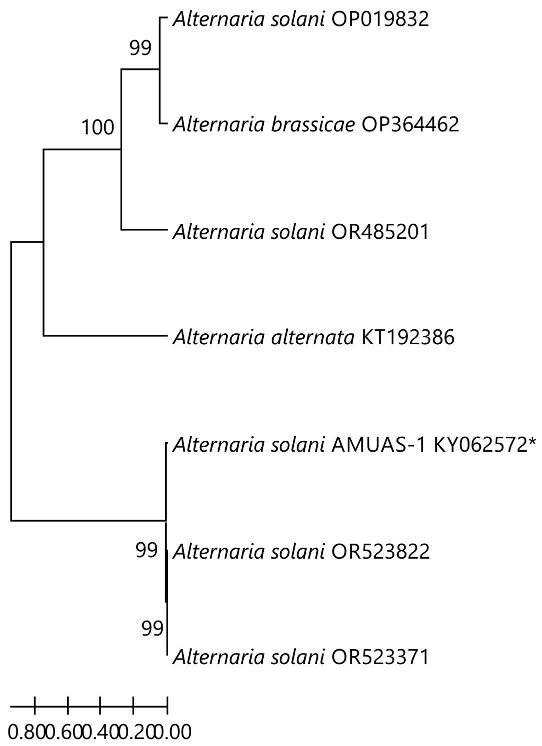

Fig. 1: Dendrogram showing the homology similarity search for *Alternaria solani* AMUAS-1

the pure culture of *A. solani* AMUAS-1 was placed in the middle of the Petri plates and appropriate control was maintained without adding NPs and SA. Every treatment was maintained in five copies, one of which was the untreated control. The plates were incubated at room temperature (25±2°C) and the diameter of colonies was recorded on 7th day and expressed in millimetres (mm). The formula utilized to compute the percent inhibition (PI) was:

$$PI = \frac{C - T}{C} \times 100$$

Where:

C = Growth of test pathogen (mm) in control
T = Growth of test pathogen (mm) in the amended medium

Statistical analysis: The *in vitro* study was repeated for more accuracy. Analysis of Variance (ANOVA) was used to process all of the data using R-software for Windows 11. The data were pooled (10 replicates per treatment) because the differences between the two repeated experiments were non-significant at $p \leq 0.05$. The data were analysed using a two-factor ANOVA, with chemicals (nanoparticle and salicylic acid) as factor one and chemical doses (concentrations) as the second factor and F-values were also examined to identify important treatments at $p \leq 0.05$.

RESULTS

***In-vitro* determination of minimum inhibitory concentration of different nanoparticles and salicylic acid against *Alternaria solani*:** *In vitro* screening of NPs and SA at 5, 10, 15, 20 and 25 µg/mL exhibited substantial variability to inhibit the mycelial growth of *A. solani*. In general, the NPs which caused significant inhibition, their effectiveness increased with higher concentrations (Table 1). Among the NPs, zinc peroxide, salicylic acid and elemental copper at 20 µg/mL caused the 100% inhibition in the mycelia growth of *A. solani* (Table 1). While zinc oxide (25 µg/mL), nickel peroxide (25 µg/mL) and ferric oxide

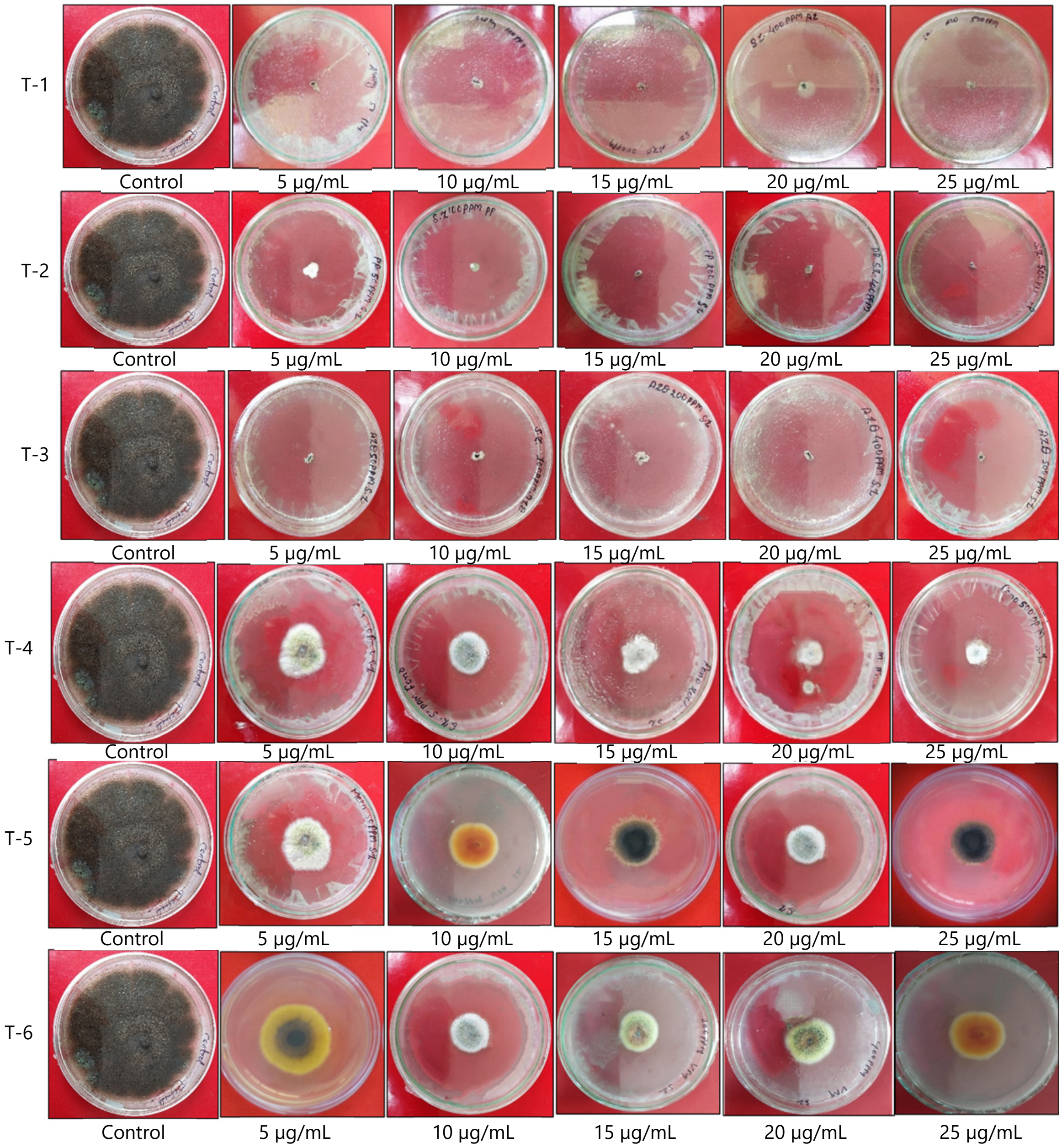

Fig. 2: Effect of nanoparticles and salicylic acid at different concentrations on the mycelial growth of *Alternaria solani*

T1 = Zinc peroxidase, T2 = Salicylic acid, T3 = Elemental copper, T4 = Zinc oxide, T5 = Nickel peroxide and T6 = Ferric oxide

(25 µg/mL) exhibited over 90% inhibition in the mycelia growth of test fungus (Fig. 2). The ferrite (cobalt ferrite, nickel ferrite, zinc ferrite and polyanelene+ferrite) were recorded least effective NPs (Table 1). Overall the inhibitory effectiveness of NPs and SA against *A. solani* at 25 µg/mL were in order of Zinc peroxide>Salicylic acid>Elemental copper>Zinc oxide>Nickel peroxide>Ferric oxide>Tin oxide>Titanium oxide>Polyamelene>Polyanelene+ferrite>Zinc ferrite>Nickel ferrite>Cobalt ferrite (Table 1).

DISCUSSION

The present study evaluated the effectiveness of twelve nanoparticles (NPs) and salicylic acid (SA) against *Alternaria solani* at five concentration levels *in vitro* conditions. Screening of NPs has shown significant heterogeneity to cause mycelial growth inhibition of *A. solani*. Among the twelve NPs evaluated, zinc peroxide, elemental copper, zinc oxide, nickel oxide, ferric oxide and tin oxide were demonstrated to be

Table 1: Effect of the different concentrations of nanoparticles and salicylic acid on the percentage inhibition of mycelial growth of *Alternaria solani in vitro*

	Mycelial inhibition (%)					
Treatments	5 µg/mL	10 µg/mL	15 µg/mL	20 µg/mL	25 µg/mL	LSD ($p \leq 0.05$)
Control	1.1	1.3	1.2	1.0	1.5	0.11
Cobalt ferrite	5.0	12	15	18	20	1.75
Elemental copper	80	85	93	100	100	8.90
Ferric oxide	56	64	78	85	93	8.70
Nickel ferrite	8.0	13	19	24	28	2.03
Nickel peroxide	36	47	63	80	94	7.23
Polyamelene	13	15	20	25	39	3.72
Polyanelene+ferrite	20	24	30	33	37	2.77
Salicylic acid	68	80	92	100	100	9.03
Tin oxide	42	61	70	85	90	8.67
Titanium oxide	51	59	63	65	70	6.60
Zinc ferrite	15	19	22	24	29	2.40
Zinc oxide	76	84	93	95	100	9.83
Zinc peroxide	65	78	97	100	100	10.0
LSD $p \leq 0.05$	2.34	3.46	5.76	6.12	6.27	-
F-values ($p \leq 0.05$)						
NPs (df = 12)	11.3	13.4	15.2	15.6	15.3	-
Dose (df = 4)	9.6	8.9	11.7	9.2	5.9	-
NPs×dose (df = 12)	5.4	4.8	5.1	4.6	NS	-

Data are means of ten replicates, F-values are significant otherwise not significant (NS) at $p \leq 0.05$ and LSD: Least significant difference

most effective against the test pathogen and cause 90-100% inhibition of mycelia growth of *A. solani*. Antifungal activity of copper-based by Ouda[10], Kanhed *et al.*[11] and Krishnaraj *et al.*[12] also examined the effect of nanoparticles on *Alternaria* spp., as observed in this study.

In the current study, zinc peroxide was relatively more effective than salicylic acid and elemental copper. Zinc peroxide is a surgical antiseptic chemical and its antimicrobial activity is well documented historically[21]. However, the anti-fungal properties of zinc peroxide are seldom reported and a majority of the studies are focused on zinc oxide[22-24]. In this study also zinc oxide and titanium oxide were found inhibitory to *A. solani* growth. Khan *et al.*[25] also reported the induction of oxidative stress, growth inhibition and reduction in biofilm formation of *Streptococcus mitis* by titanium dioxide and zinc oxide nanoparticles.

Malandrakis *et al.*[26] used copper nanoparticles against foliar and soil-borne plant pathogens. In current study, elemental copper was the second most effective NP against *A. solani* and caused 100% mycelial inhibition at 20 µg/mL concentration. Similarly, the *in vitro* fungicidal activity of copper nanoparticles against *Alternaria* spp. was also evaluated by Ouda[10] and Kanhed *et al.*[11], corroborating current study.

Several studies have reported the application of nanoparticles in the management of various plant-pathogenic fungi. Seku *et al.*[27] reported that *Candida albicans*, *Candida parapsilosis*, *Aspergillus niger* and *Aspergillus oryzae* are subject to antifungal activity by Cu-NP. Rubina *et al.*[28] have found that Cu-chitosan nanocomposites worsen *Rhizoctonia solani* and *S. rolfsii* fungal mycelium. Gold nanoparticles are altered and ruptured by the fungal cell membrane of *Puccinia graminis tritici*, *Aspergillus flavus*, *A. niger* and *Candida albicans*[29]. Elmer and White[9] have demonstrated that *Fusarium* wilt and *Verticillium* diseases have declined without physiological toxicity in the foliar application of nano-CuO, MnO and ZnO in a greenhouse.

Application of salicylic acid (SA) was also found very effective in suppressing the mycelial growth of *A. solani* in the present study. The SA has been identified as one of the essential elements in the signal transduction pathway that results in plant resistance to a number of diseases, including Alternaria blight[7]. Several studies revealed that the exogenous application of SA was inhibitory to the fungal pathogens as observed in the present study[14-16].

In the present study, zinc peroxide, elemental copper, zinc oxide and nickel oxide were found most effective against *Alternaria solani*. However, the mechanism involved in the fungicidal activity and toxicological studies are yet to be confirmed. Further, pot and field trials are required to confirm their performance before commercial use. In addition to this, high processing costs, problems with scalability, industrial production, public perception, environment, health and safety concerns related to the application of nanoparticles are need to addressed properly.

CONCLUSION

Among the NPs screened, zinc peroxide was found most effective in inhibiting the mycelia growth of *Alternaria solani*. To the best of our knowledge, this is the first report of fungicidal properties of zinc peroxide against early blight fungus, *A. solani*. Hence, zinc peroxide may be explored as another substitute for chemical fungicide in the management of Alternaria blight, early blight, leaf spot, collar rot, fruit rot and stem canker caused by *Alternaria* spp. However, the mechanism involved in the fungicidal activity and toxicological studies are required to be confirmed before its commercial use in field conditions.

SIGNIFICANCE STATEMENT

Alternaria solani is regarded as an important fungal pathogen of solanaceous crops and causes severe yield loss to the crop. To explore much greener management options, twelve nanoparticles (NPs) and salicylic acid were evaluated against the mycelial inhibition of *Alternaria solani in vitro* conditions. Among the NPs screened, zinc peroxide was found most effective and inhibited 100% mycelial growth of *Alternaria solani*. According to previous studies, this is the first report of fungicidal properties of zinc peroxide against early blight fungus, *A. solani*. Hence, zinc peroxide may be explored as another substitute for chemical fungicide in the management of Alternaria blight, early blight, leaf spot, collar rot, fruit rot and stem canker caused by *Alternaria* spp.

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