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Anshu Kumar
[a] Department of Plant Pathology, Bihar Agricultural University, Sabour, Bihar, India
[b] Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Abhijeet Ghatak
Department of Plant Pathology, Bihar Agricultural University, Sabour, Bihar, India

Huma Nazneen
Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Venu Emmadi
Department of Plant Pathology, Indian Agricultural Research Institute, New Delhi, India

Badavath Kishore
Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Wahid Ul Hasan
Department of Genetics and Plant Breeding, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Corresponding Author:
Abhijeet Ghatak
Department of Plant Pathology, Bihar Agricultural University, Sabour, Bihar, India

Testing inhibitory efficacy of copper and silver nanoparticles against *Alternaria brassicicola* and *Bipolaris sorokiniana*

Anshu Kumar, Abhijeet Ghatak, Huma Nazneen, Venu Emmadi, Badavath Kishore and Wahid Ul Hasan

Abstract
The antifungal activity of copper nanoparticle (CuNP) and silver nanoparticle (AgNP) was investigated against the two foliar pathogen *Alternaria brassicicola* and *Bipolaris sorokiniana*. The efficacy of CuNP and AgNP were determined in reducing the mycelia growth of the pathogen at 100 ppm and 1000 ppm. Mycelial growth is moderately inhibited by CuNP. At 100 ppm concentrations, AgNP demonstrated radial growth inhibition in *A. brassicicola* by and *B. sorokiniana* 49.6% and 68.2%, respectively. Radial growth inhibition further increased to 61.7% in *A. brassicicola* and 86.4% in *B. sorokiniana* at 1000 ppm application of AgNP. CuNP has been shown to suppress the fungi; however, it was far less efficient than AgNP and the fungicide carbendazim. The information revealed in this work may be useful in synthesizing nanoparticles commercially for management of diseases caused by *A. brassicicola* and *B. sorokiniana*.

Keywords: AgNP, *Alternaria brassicicola*, *Bipolaris sorokiniana*, CuNP, nanoparticle

Introduction
Alternaria brassicicola and *Bipolaris sorokiniana* are the wide-spreading and sporulating pathogens causing aerial infection. The large losses in winter (*Rabi*) crops grown in India's Gangetic plains and northern areas are thus attributed to both foliar pathogens. They produce conidia by numerous cycles during the entire stage of their growth. The foliar pathogen, *A. brassicicola*, is a necrotrophic fungus capable of infecting a wide range of economically important Brassicaceae crops. *B. sorokiniana* isolates differ in their virulence and aggressiveness (Duveiller and Altamirano, 2000) [7]. The disease epidemic caused by *B. sorokiniana* is exacerbated by the presence of collateral hosts (Bashyal *et al*., 2010) [4].
The harmful phytotoxic chemicals used in managing these diseases create an adverse impact on the environment. As a result, there is an urgent need to bring in some safe and effective components to manage these diseases. Furthermore, biological management methods are not always efficient against these infections (Vallad and Goodman, 2004) [24]. Many commercial fungicides are on verge of getting banned due to their harmful effect on the environment; furthermore, because to the recent rise in microbial resistance, their utility is dwindling (Lamsal *et al*., 2011; Ouda, 2014) [13, 19]. So, phytopathological research is looking for an alternate control tool in the form of nanoparticles. The introduction of nanoparticles (NPs) may provide a new way in the management of plant diseases (Mishra *et al*., 2014; Kim *et al*., 2012) [17, 10]. Although the benefits of NPs have not been extensively studied, several studies suggest that they have the potential to suppress disease (Banik and Sharma 2011; Patel *et al*., 2014; Mishra *et al*., 2015) [3, 21, 18].
Copper is an important component of plant defence mechanisms, hence it has been the subject of recent research on the nanoscale (Borgatta *et al*., 2018; Elmer *et al*., 2018) [5, 8]. Copper oxide nanoparticle's biocidal effect is principally due to the copper (I)-peptide complex produced by cuprous oxide (Cu_2O) and the formation of free radicals by cupric oxide (CuO) (Meghna *et al*., 2015) [16]. Until now, only a little amount of study had been done to show that silver may be used to control plant diseases (Park *et al*., 2006) [20]. Nano-silver (AgNP) has recently been used in plant pathological research. *In vitro* investigation on fungal mycelial growth in the presence of AgNP may suggest that it can be used to limit pathogen proliferation. Thus, this investigation focused on assessing the two NPs, CuNP and AgNP, under *in-vitro* conditions against the two foliar pathogens, *A. brassicicola* and *B. sorokiniana*.

Materials and Methods

At the Bihar Agricultural University's Sabour Agricultural Farm, isolates of *A. brassicicola* and *B. sorokiniana* were collected from infected mustard and barley crops, respectively. The specimens were washed with flowing tap water and sliced into little pieces and rinsed thrice for 20-30 s each with a 1% sodium hypochlorite solution, followed by a final wash with distilled water. Different fungi, including isolates from different hosts, require a specific substrate to grow and thrive (Balodi *et al.*, 2015) [1], we preferred potato dextrose agar (PDA) as a common medium for cultivation of the tested fungi. Therefore, the specimens were transferred onto the Petriplates containing PDA and incubated at 25±2 °C for 2-3 days after for growth. The fungal mycelium picked from the periphery of the colony was transferred to PDA slants, kept in the incubator for 5-6 days. The culture slants were stocked in a refrigerator at 4 °C and employed during the experiment whenever needed. The PDA slants can be stored for up to 4-5 months.

In this work, copper nanoparticles (CuNP) and silver nanoparticles (AgNP) were tested to observe their efficacy against the mentioned foliar pathogens. The nanoparticles (NPs) were purchased from Sisco Research Laboratory Pvt. Ltd., India. Under the in-vitro condition, a poisoned food assay was conducted with both CuNP and AgNP at a concentration of 100 ppm and 1000 ppm. Both nanoparticles were put into a Petriplate at their precise concentrations (100 ppm and 1000 ppm). Then, using a flame-sterilized cork borer, 5 mm of actively developing mycelium was placed in the center of the PDA plate. The treated mycelia with 100 ppm and 1000 ppm of both NPs were compared to a control in a Petriplate with no inhibitory chemical. As suggested by Kumar *et al*. (2018) [12], the radial growth was assessed at 48 h intervals.

Results and Discussion

Silver has been shown to disrupt a range of biological activities in microbes, including cell membrane construction and function (McDonnell *et al*., 1999) [15]; thus, as a result of its heightened chemical reactivity, AgNP finds it much easier to assault. AgNP is known to easily penetrate fungal microbiological cells, meaning that lower AgNP concentrations are required for antibacterial action (Samuel and Guggenbichler, 2004) [23]. In comparison to metallic silver, nanosized silver has the potential for fast chemical reactions. Silver at the nanoscale has a higher surface energy and surface area than its bulk counterpart, making it easier to penetrate microbial cells. Banik and Pérez-de-Luque (2017) [2] found that a mixture of CuNPs and simple copper like copper oxychloride, inhibited the growth of oomycetes plant diseases by 76%. Ponmurugana *et al*. (2016) [22] evaluated the antifungal activity of biosynthesized CuNP against *Poria hypolateritia*, a fungus that causes red root-rot in tea, and found that a 2.5 ppm soil treatment provided promising disease control outcomes.

In this experiment, AgNP and CuNP were tested for their potentiality to control pathogen *A. brassicicola* and *B. sorokiniana* under the *in-vitro* conditions (Fig 1). This investigation was conducted following a poisoned food assay. In the current study, nanosized silver had a substantial impact on the inhibitory features of foliar pathogens, *A. brassicicola* and *B. sorokiniana*. The radial growth of the fungi was measured according to Kumar *et al.* (2018) [12]. When compared to CuNP, AgNP found to be more effective at controlling the pathogen's radial development at 100 ppm. *A. brassicicola* mycelial development was also significantly reduced when AgNP was applied at a concentration of 100 ppm (Fig 1). Furthermore, the observation of *A. brassicicola* and *B. sorokiniana* mycelial growth reduction at 100 ppm AgNP is in agreement with Kim *et al*., (2012) [10], who reported that AgNP successfully suppressed pathogen mycelial development at 25 ppm. In *A. brassicicola* and *B. sorokiniana*, Kriti *et al*. (2020) [11] found that 100 ppm AgNP significantly suppressed conidial germination and inhibited mycelial growth. Mahdizadeh *et al*. (2014) [14] observed that AgNP at concentrations of 6, 10, 12, 14, and 16 ppm completely inhibited the growth of *Pythium aphanidermatum* and *Sclerotinia sclerotiorum*. Silver ions and NPs, according to Jo *et al*., (2009) [9], have a significant inhibitory effect on colony formation from *B. sorokiniana* and *M. grisea* conidia. The fact that mycelial growth was decreased on NP-treated plates implies that NPs can potentially reduce fungal growth.

AgNP effectively controlled the fungal growth of *A. brassicicola* and *B. sorokiniana* (Fig 2). AgNP showed 49.6% and 68.2% growth inhibition in *A. brassicicola* and *B. sorokiniana*, respectively at 100 ppm concentration. This finding is in consistent with Mishra *et al*., (2014) [17], who found that biosynthesized AgNP is effective in inhibiting *B. sorokiniana* mycelial growth. The reduction in mycelial development attended up to 61.7% in *A. brassicicola* and 86.4% in *B. sorokiniana* at 1000 ppm of application. CuNP moderately inhibited the mycelial growth of both pathogens (Fig 2). In *A. brassicicola*, CuNP showed 14.7% mycelial growth inhibition at 100 ppm whereas 23.7% reduction in growth was observed at 1000 ppm. The radial growth inhibition was found to be 3% and 7.6% at 100 ppm and 1000 ppm, respectively in *B. sorokiniana.* CuNP showed to limit the mycelial growth in *A. brassicicola* and *B. sorokiniana*, although it was shown to be significantly less efficient than the fungicide, carbendazim. This fungicide hampering the biosynthesis of DNA, rendered 27.8% and 18.2% inhibition in radial growth in *A. brassicicola* and *B. sorokiniana*, respectively at 100 ppm concentration whereas, at 1000 ppm, it restricted the radial growth by 43.6% and 25.8% in the respective pathogen.

Conclusion

Results presented in this study confirm that AgNPs have significant inhibitory effects on *A. brassicicola* and *B. sorokiniana.* In this investigation, AgNP at 100 ppm was observed to suppress *B. sorokiniana* and *A. brassicicola* mycelial growth of the fungi. This point to the possibility of using AgNP to limit pathogenic growth and can be tested for different concentration for effective disease management. The major focus of using nanomaterials is to combat diseases as these possess antifungal and antimicrobial properties. AgNP should be evaluated against a wide range of pathogens. Gene expression analysis should be targeted to manage the pathogen at the molecular level. Furthermore, our forthcoming research should be focused on developing silver compounds, formulation and integrating them with carriers to enhance their stability, safety and efficiency in the field. Nanocomposites such as silver chitosan composites are gaining popularity for exploration in the field of plant disease management. Future research should be laid down to explore the best time and concentration of nanoparticle application in order to make greater and better use of nanoparticles in fungal control. It is very important that our future experiments should be focused on objectives to determine the risk on the environment and human health during the application of silver-based nano-compounds in the field. The generated information in this work will be helpful in conducting applied experiments and commercialization of nanoparticles as a crop protection fungicide.

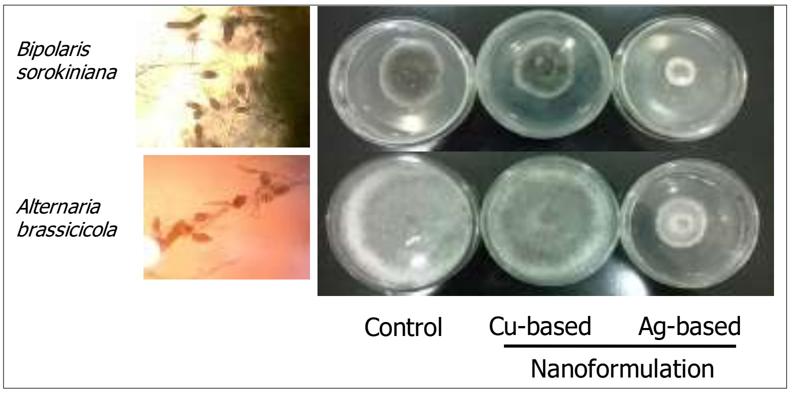

Fig 1: Efficacy of CuNP and AgNP on radial growth of *Bipolaris sorokiniana* and *Alternaria brassicicola*.

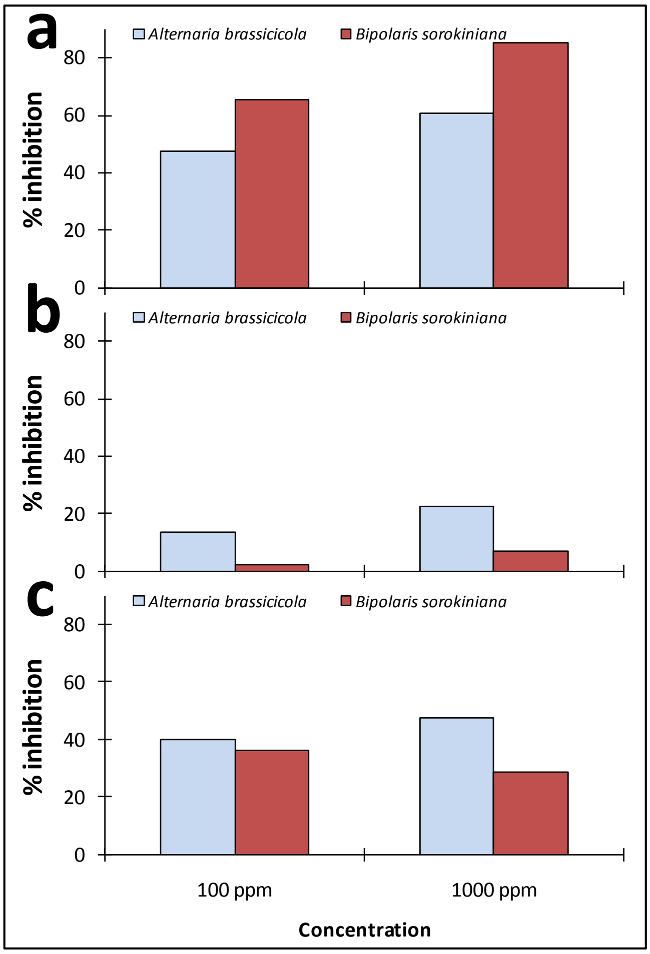

Fig 2: Radial growth inhibition (%) of *Alternaria brassicicola* and *Bipolaris sorokiniana* for (a) AgNP, (b) CuNP and (c) Carbendazim under *in-vitro* conditions.

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