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ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES (AgNPs) AGAINST *Erwinia carotovora* subsp. *atroseptica* & *Alternaria alternata*

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In the current research antimicrobial activity of silver nanoparticles (AgNPs) i.e. Ag, AgP, AgIB, AgAE and AgBE and antibiotics (Nystatin and Streptomycin) were evaluated against plant pathogens *Erwinia carotovora* subsp. *atroseptica* and *Alternaria alternata*. The experiment was carried out in completely randomized design (CRD) with three replications. The antibiotic Nystatin was used as a standard antibiotic reference against antifungal activity while Streptomycin against antibacterial activity. For the antifungal and antibacterial activity different concentrations (150ppm, 200ppm and 250ppm) and zone of inhibition (mm) for all AgNPs and antibiotics were prepared and inhibition zone was measured in millimeters (mm). The results revealed that AgAE and AgIB showed the largest inhibition zone with the tested *Erwinia carotovora* subsp. *atroseptica* where the activity was 14.33mm and 13.13mm, respectively followed by AgBE (10.40mm), AgP (10.33) and Ag (7mm) while the reference antibiotic Streptomycin produced the lowest inhibition zone (5.66mm). In case of *Alternaria alternata* maximum inhibition zones were achieved from AgAE and AgIB where the antifungal activity was 27mm and 24mm followed by AgBE (22.33mm), AgP (21.66mm) and Ag (18.66) while the reference antibiotic Nystatin produced minimum inhibition zone (4mm). Further, it was noticed that increasing the concentration of AgNPs significantly ($p<0.05$) increased the inhibition zones of the test plant pathogen and higher concentration (250ppm) possesses strong antimicrobial activity. It is concluded that AgNPs had maximum inhibitory effect against *Erwinia carotovora* subsp. *atroseptica and Alternaria alternata* when compared with the antibiotics.
Keywords: Silver nanoparticles, antibiotics, antimicrobial activity, inhibition zones.

INTRODUCTION

The application of silver nanoparticles (AgNPs) in the management of plant diseases has become more common with the advances of the technology, making its production more economical. Since AgNPs display multiple mode of action against plant pathogens, therefore they can be used for controlling various plant diseases in relatively safe way than the synthetic chemicals (Mendes *et al.* 2014). Until now, few reports are available for their applicability against plant diseases. In the present work we evaluated the antifungal and antibacterial activity of AgNPs against two important plant pathogens, *Erwinia carotovora* subsp.*atroseptica* and *Alternaria alternata*

MATERIALS AND METHODS

***Preparation of stock suspension of plant pathogen*:** The purified and identified cultures of *Erwinia carotovora* subsp. *atroseptica* and *Alternaria alternata* were obtained from the culture collection lab, Department of Plant Pathology, University of Agriculture, Peshawar. To prepare stock suspension, one ml from old culture was aseptically distributed onto potato dextrose agar (PDA) and incubated at 37°C for 24 hours. The bacterial growth was washed off with sterilized distilled water to produce suspension and then it was stored in refrigerator at 4°C. For the plant pathogenic fungi, a disc of 0.5 cm in diameter was taken from the old culture and poured into 250 ml sterilized distill water in a flask and incubated at 30°C for the fungal suspension.

***Antifungal and antibacterial assay through well diffusion method*:** Nystatin and Streptomycin were purchased from the local pharmaceutical market of Peshawar, Pakistan while five AgNPs viz. Ag, AgP, AgIB, AgAE and AgBE were obtained from the HEJ research institute, Karachi. The well diffusion method of Kavanagh, 1972 was followed in this study. Concentrations of each antibiotic and AgNPs were prepared as 150ppm, 200ppm, and 250ppm. Then 2 ml from the bacterial and fungal stock suspension were thoroughly mixed with 20ml molten sterilized potato dextrose agar (PDA) and poured into sterilized petri-dishes. The PDA media was left to set and in each of the 3 wells of 10mm in diameter in petri dish. Streptomycin was used as standard antibiotic reference for antibacterial activity whereas Nystatin for antifungal activity. The wells of petri-dishes were filled with 50µl sample of each of prepared concentration of antibiotic and AgNPs using micropipette and incubated in the upright position at 30°C for 24 hours in case of bacteria and for 4 days in case of fungi. Three replicates were used against each tested organism.

***Statistical analysis*:** The experiment was arranged in completely randomized design (CRD). After the incubation periods the diameters of the inhibition zones were measured in mm. Data obtained were subjected to analysis of variance technique (ANOVA) and the least significant difference (LSD) at 5% level of significance was calculated using statistical software Statistix 8.1.

RESULTS AND DISCUSSION

***Antibacterial activity*:** The results regarding the antimicrobial activity of different AgNps and antibiotic (Streptomycin) against the *Erwinia carotovora* subsp. *atroseptica* are presented in Table 1 and Figure 1. Maximum zone of inhibition was obtained for AgAE (13mm) followed by AgIB (12mm). A slightly lower inhibition zone (9mm) was obtained for both AgP and AgBE whereas the minimum inhibition zone was found for Ag (6mm) and Streptomycin (4mm) at 150ppm concentration. Moreover, at this concentration the silver nanoparticles AgAE and AgIB were found to be statistically identical with zone of inhibitions 13mm and 12mm. Similarly, AgP and AgBE as well as Ag and Streptomycin were found to be statistically similar at $p<0.05$ level of probability. As compared to 150 ppm concentration, 200 ppm had shown highest inhibition zone with 14, 13, 10.1 and 10 mm for AgAE, AgIB, AgBE and AgP, respectively. Though maximum inhibition zone was obtained for AgAE but it was similar to AgIB at $p<0.05$ level of probability. The lowest inhibition zone at 200ppm was obtained for Ag and Streptomycin with 7mm and 6mm values, respectively. When concentration was increased upto 250 ppm, AgAE gave maximum inhibition zone (16mm) followed by AgIB, AgBE, Agp with 15 mm, 12.11 mm and 12 mm while lowest inhibition zone was found for Ag and Streptomycin with 8mm and 7mm, respectively.

Table 1. Antibacterial activity of Silver nanoparticles (AgNPs) against *Erwinia carotovora* subsp. *atroseptica*.

Treatments	Inhibition zones (mm)			Mean
	150ppm	200ppm	250ppm	
Ag	6c	7c	8c	7
AgP	9b	10.b	12.b	10.33
AgIB	12a	13a	15a	13.33
AgAE	13a	14a	16a	14.33
AgBE	9b	10.1b	12.11b	10.40
Streptomycin	4c	6c	7c	5.66
LSD (0.05)	2.17	1.77	1.92	1.99

Means within a column followed by different letters are significantly different at 5% level of significance (P<0.05); Values are mean of three replicates.

It is, therefore, concluded that the antibiotic Streptomycin showed the lowest inhibition zone (5.33m) whereas among the silver nano particles, the AgAE was found = superior in performance against the test bacterium with maximum inhibition zone of 14.33 mm. An increase in the concentrations significantly ($p<0.05$) increase the inhibition zones of the *Erwinia carotovora* subsp. *atroseptica.*

***Antifungal activity*:** The inhibition zones (mm) of different AgNPs and Nystatin determined for *Alternaria alternata* are presented in Table 2 and Fig. 2. At the concentration of 150ppm, maximum inhibition zone was found for AgAE followed by AgIB with 25mm and 23mm, respectively whereas same inhibition zone was obtained for AgP and AgBE with 20 mm. AgP and AgBE at 150 ppm concentration were not significantly different at $p<0.05$ whereas at the same concentration lowest inhibition zone was observed in Ag (17mm) followed by the antibiotic Nystatin (3mm).

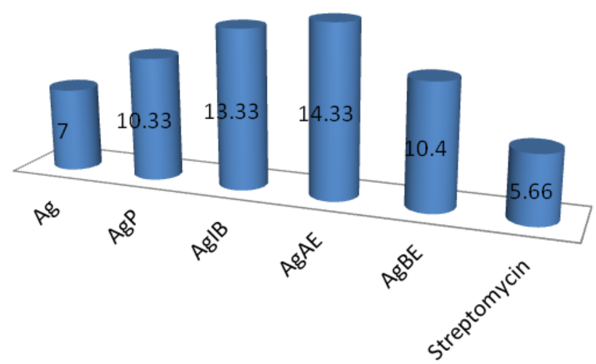

Figure 1. Mean zone of inhibition for *Erwinia carotovora* subsp. *atroseptica*.

AgNPs had more inhibitory effect on the growth of *Alternaria alternata* at 200ppm. At this concentration highest inhibition zone was recorded from AgAE (27mm) followed by AgIB (24mm), AgBE (23mm) and AgP (22mm) whereas Ag(19mm) and Nystatin (4mm) showed lowest inhibition zone against the investigated fungi. AgP, AgIB and AgBE were not statistically different at $p<0.05$. In all the cases of silver nanoparticles and antibiotic concentrations, the concentration 250 ppm produced a greater inhibition zone. At this concentration highest inhibition zone (29mm) was recorded from AgAE at the concentration of 250ppm followed by AgIB (25mm) while the silver nanoparticle AgBE and AgP showed slightly lower inhibition zone (24mm) and (23mm) against the tested fungi. Minimum inhibition zone at this concentration was obtained for Ag (20mm) followed by Nystatin (5mm). Further increase in the concentrations of AgNPs significantly increased the inhibition zones. Overall, results showed that AgNPs showed highest inhibitory action against *Alternaria alternata* as compared to antibiotic Nystatin.

Table 2. Antifungal activity of Silver nanoparticles (AgNPs) against *Alternaria alternata*.

Treatments	Inhibition zones (mm)			Mean
	150ppm	200ppm	250ppm	
Ag	17d	19c	20c	18.66
AgP	20c	22b	23b	21.66
AgIB	23b	24b	25b	24.00
AgAE	25a	27a	29a	27.00
AgBE	20c	23b	24b	22.33
Nystatin	3e	4d	5d	4.00
LSD(0.05)	1.77	2.17	2.81	2.25

Mean within a column followed by different letters are significantly different at 5% level of significance (P<0.05); Values are mean of three replicates.

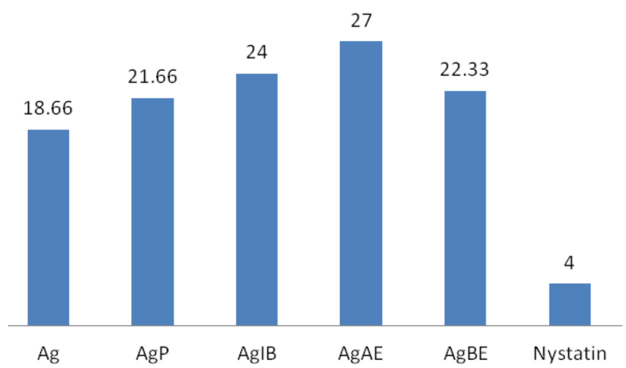

Figure 2. Mean zone of inhibition (mm) for *Alternaria alternata*.

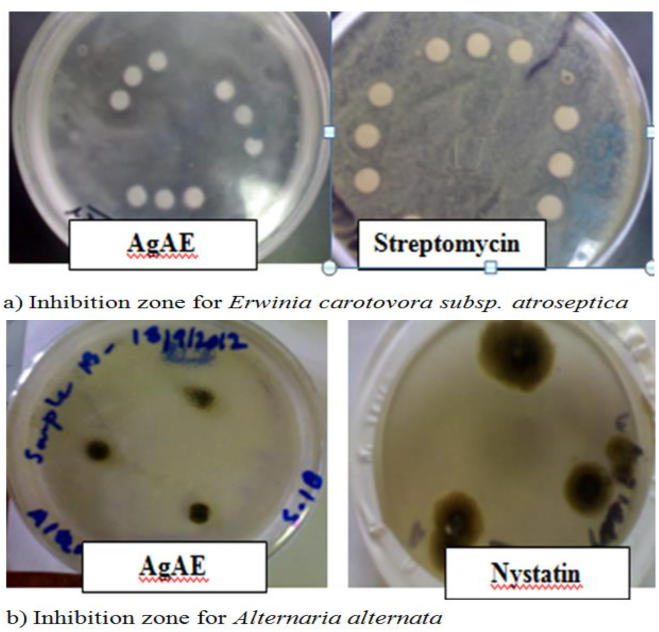

Figure 3. Antimicrobial activity of Silver nanoparticles (AgNPs) and commercial antibiotics.

DISCUSSION

Plant diseases reduce agricultural production every year worldwide. Millions of dollars have been invested to manage these plant diseases. Various components of integrated disease management (IDM) have been applied to manage these diseases. Among these components use of chemicals such as pesticides are the most prevalent. These pesticides have created environmental hazards therefore to manage these diseases alternative measures such as use of silver nanoparticles has become more common. The synthesis of these silver nanoparticles is also economical and environmentally friendly. Our findings that AgNPs had more inhibitory effect against *Erwinia carotovora* subsp.*atroseptica* as compared to antibiotic Streptomycin are also consistent with the findings of Sarkar *et al*. (2007) who reported that AgNPs showed greater antibacterial activity as compared to antibiotic penicillin. The same results were also obtained by Allahverdiyev *et al. (*2011) who concluded that AgNPs had high antibacterial efficiency as compared to antibiotic amoxicillin. Further Lok *et al.* (2006) proved that AgNPs after attaching with the cell wall of bacteria denature the proteins resulting in the death of bacteria. Zawrah *et al.* 2011 stated that AgNPs also rupture plasma membrane of bacteria and thereby depletion of cellular energy. Another mechanism proposed by Kumar *et al.,* 2004 is that AgNPs block the cellular respiration as a result of reaction between the Ag groups of AgNPs and sulphohydral (SH) group of the bacterium cell wall. Morones *et al.* (2005) proved that AgNPs have capability to break the defense system of bacterial cell. Jo *et al.* (2009) investigated that silver nanoparticles have a high antifungal activity and can be used to manage plant diseases. He tested various form of silver nanoparticles against *Bipolaris sorokiniana* and *Magnaporthe grisea*. His results indicated that sivler nanoparticles had a significant effect on the growth of these two fungal plant pathogens. Kim *et al.* (2012) studied fungal properties of nano-size silver colloidal solutions against various fungal plant pathogens. Eighteen different plant pathogenic fungi were treated with these solutions at concentrations of 10, 25, 50 and 100 ppm. Their results revealed that most significant inhibition of plant pathogenic fungi was recorded at 100 ppm concentrations. These findings that AgNPs had more inhibitory effect against *Alternaria alternata* are in agreement with the results of Al-Askar *et al.* (2013) who investigated the antifungal activity of AgNPs against *Fusarium oxysporum, Alternaria alternata* and *Aspergillus flavus.* Their results showed that AgNPs have potent antifungal activity as compared to Nystatin and Griseofulvin. Inhibition zones obtained for AgNPs ranged between 10-26mm when compared with the 5-8mm for antibiotics. These findings are also consistent with the results of Huang *et al.* (2007) who reported maximum inhibitory effect of AgNPs against sclerotia forming fungi as compared with the antibiotics. Mendes *et al.* (2014) evaluated antifungal activity silver colodial nanoparticles against *Phomopsis* sp. in soybean seeds. Hundred percent inhibition of growth of *Phomopsis* sp. was observed on treated with a 270 and 550 µg ml^{-1} concentrations of nanoparticles. Mahdizadeh *et al.* (2015) studied in vitro antifungal activity of silver nanoparticles against five phytopathogenic fungi and a bicontrol agent (*Trichoderma harzianum*). The most sensitive fungi to silver nanoparticles were found to be *Sclerotinia sclerotiorum* and *Macrophomina phaseolina*. Further he suggested that as compared to chemical fungicides the silver nanoparticles can be used against plant pathogen as a safer alternative.

Conclusion**:** The plant pathologists are facing the challenges of continuous increase in resistant of plant pathogen against the conventional pesticides. The present research was focused on silver nanoparticles (AgNPs) and their efficacy against commonly found plant pathogens i.e. *Erwinia carotovora* subsp.*atroseptica* & *Alternaria alternata*. The silver nanoparticles (AgNPs) proved excellent antimicrobial activity against these plant pathogens with agar well diffusion method.

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