



## RESEARCH PAPER

## OPEN ACCESS

## Antimicrobial activity of silver nanoparticles (AgNPs) against *Erwinia carotovora* pv. *carotovora* and *Alternaria solani*

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### Abstract

Resistant plant pathogens have emerged against the conventional antibiotics. Alternative to these conventional antibiotics application of eco-friendly nanoparticles is an important strategy to manage plant disease. In the present 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* pv. *carotovora* and *Alternaria solani*. The experiment was carried out in completely randomized Block Design (CRD) with three replications. The antibiotic Nystatin was used as a standard antibiotic reference in case of antifungal activity while streptomycin in case of antibacterial activity. For the antifungal and antibacterial activity different concentration were prepared as 150ppm, 200ppm and 250ppm and zone of inhibition(mm) for all silver nanoparticles (AgNPs) and antibiotics were prepared and inhibition zone was measured in millimeters(mm). Results revealed that the silver nanoparticles(AgNPs) i.e. AgAE and AgIB showed largest inhibition zone with the tested *Erwininacarotovorapv. carotovora* 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 lowest inhibition zone(5.66mm). In case of *Alternaria solani* maximum inhibition zones were achieved from silver nanoparticles(AgNPs), 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 silver nanoparticles(AgNPs) significantly ( $P < 0.05$ ) increased the inhibition zones of the test plant pathogen and higher concentration(250ppm) posses strong antimicrobial activity. We can conclude that silver nanoparticles(AgNPs), had maximum inhibitory effect against *Erwininacarotovorapv. carotovora* and *Alternaria solani* when compared with the antibiotics

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## Introduction

Plants are exposed to various disease caused by plant pathogens that present in their environment. These pathogens are bacteria, fungi, nematodes and viruses which are causing significant yield loss worldwide (Shabana *et al.*, 2008). Among these pathogens, bacteria and fungi are playing major role for yield losses in crops in Pakistan (Javaid and Anjum, 2006). Especially in case of bacteria, *Erwinia species* are known to cause soft rot diseases in economically important potato and tomato crops (Rahidet *al.*, 2012) whereas in fungal group *Alternariaspp* are causing several diseases of plants most common disease in Pakistan is early blight of tomato and potato (Gondalet *al.*, 2012). Plant pathogens are managed by adopting the integrated disease management strategy and application of antibiotics is one of major component of that strategy (Chanda and Rakholiya, 2011). Though the amount of antibiotics used against plant pathogens is skimpy, still the antibiotic resistant plant pathogens have emerged which further make more difficult their management (MacManus and Stockwell, 2000). Further antibiotics on the plant surfaces loses activity against plant pathogens swiftly (Stockwell and Duffy, 2012). Therefore in contrast to conventional antibiotics, application of nanoparticles is most important strategy to manage plant diseases (Sastry, 2003). These nanoparticles may be environmentally if have been extracted from plants in contrast with chemical synthetic methods (Basanagowda and Hooli, 2012). The bio-based strategy for synthesis of nanoparticles are gaining popularity due to their eco-friendly characteristics (Iravaniet *al.* 2013) Nanoparticles such as platinum, silver (Ag) and gold (Au) are inventions of nanotechnology and widely used against plant pathogens (Al-Aksaret *al.* 2013). For plant pathogens many mutations are required for plant pathogens to develop resistant against nanoparticles as compared with the conventional antibiotics (Chethana, 2013). Among these nano-particles silver particles (AgNPs) have long been used as a preservative and as antimicrobial agents (Liam *et al.*, 2010). Silver nanoparticles (AgNPs) have adverse effects on cells such as the production of reactive oxygen species

which are toxic to both bacterial and fungal species (Park *et al.*, 2009). Silver nanoparticles (AgNPs) improve their antimicrobial activity as compared with the bulk silver (Sondi and Salopek, 2004). By keeping in view, the importance of Silver nanoparticles (AgNPs) the present research was carried out to investigate the antimicrobial activity of silver nanoparticles against *Erwinia carotovora pv. carotovora* and *Alternaria solani*.

## Materials and methods

### *Preparation of stock suspension of plant pathogen*

The purified and identified cultures of *Erwinia carotovora pv. carotovora* and *Alternaria solani* were obtained from the culture collection Lab of department of plant pathology, University of Agriculture Peshawar. For preparation of stock suspension for tested bacterial isolate *E. carotovora pv. carotovora*, 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 sterilize distill 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*

Antibiotics namely nystatin and streptomycin were purchased from the local pharmaceutical market of Peshawar Pakistan while five silver nanoparticles (AgNPs) viz. Ag, Agp, AgIB, AgAE and AgBE were obtained from the H.E.J research institute Karachi. The well diffusion method of Kavanagh, 1972 was followed in this study. Concentrations of each antibiotic and silver nanoparticles were prepared as 150ppm, 200ppm, and 250ppm by using the formula ( $V_1C_1 = V_2C_2$ ). Then 2 ml from the bacterial and fungal stock suspension were thoroughly mixed with 20ml molten sterilize potato dextrose agar (PDA) and then poured into sterilized petri-dishes. The PDA media was left to set and in each of the petri-dish 3 wells of

10mm in diameter was made by using sterilize cork borer. The antibiotics streptomycin were used as standard antibiotic reference for antibacterial activity whereas nystatin for antifungal activity. The wells of petri-dishes were filled with 50ul 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 mean diameter of the inhibition zones were measured in mm. Data obtained was 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 Silver nanoparticles (AgNPs) and antibiotic (streptomycin) against the *Erwinia carotovora* pv. *carotovora* are presented in Table 1 and Fig. 1. Maximum zone of inhibition was obtained to AgAE (13mm), AgIB (12mm) whereas slightly lowest inhibition zone (9mm) was obtained to both AgP and AgBE whereas the minimum inhibition zone was found to 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 compare to 150 ppm concentration, 200 ppm had showed highest inhibition zone with 14mm, 13 mm 10.1 and 10 mm for AgAE, AgIB, AgBE and Agp respectively. Though maximum inhibition zone was obtained to AgAE but this nanoparticles is statistically same with AgIB at  $P < 0.05$  level of probability.

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

Treatments	Inhibition zones (mm)			Means
	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.

The Lowest inhibition zone at 200ppm was obtained to Ag and streptomycin with 7mm and 6mm values respectively. When concentration increased upto 250 ppm, again the silver nanoparticle AgAE gave maximum inhibition zone (16mm) followed by AgIB, AgBE, Agp with 15 mm, 12.11mm and 12 mm while lowest inhibition zone was found to Ag and streptomycin with 8mm and 7mm respectively.

Finally it can be concluded that the antibiotic streptomycin showed the lowest inhibition zone (5.33m) whereas among the silver nano particles, the AgAE was found to superior in performance against the test bacterium with maximum inhibition zone (14.33 mm). It was also noticed that increasing the concentrations significantly ( $P < 0.05$ ) increase the inhibition zones of the *Erwinia carotovora* pv.

*carotovora*. These findings are in agreement with the results of Al-Askaret *al.* (2013) who reported that silver nanoparticles (AgNPs) have high antibacterial activity against *E.amylovora*, *P. wasabia* and *P. carotovorumatrosepticum* with inhibition zones 14mm, 18mm and 19mm respectively with compared to generic antibiotics. Our findings that silver nanoparticles (AgNPs) had more inhibitory effect against *E. carotovora* as compared to antibiotic streptomycin are also in consistent with the findings of Sarkar *et al.* (2007) whom reported that AgNPs showed greater antibacterial activity as compared to antibiotic penicillin. The same results were also obtained by Allahverdiyev *et al.* (2011) whom demonstrated that

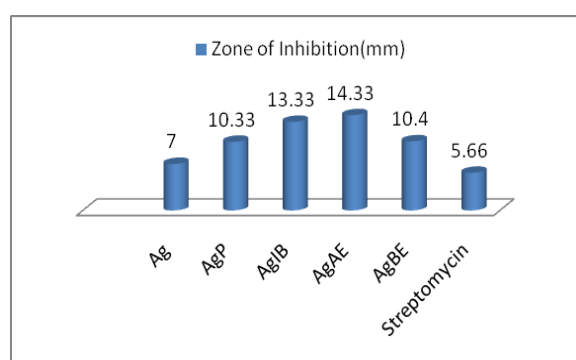
silver nanoparticles (AgNPs) had high antibacterial efficiency as compared to antibiotic amoxicillin. Further Loket *al.*, 2006 proved that AgNPs attached to cell wall of bacterium and denatures the proteins consequently death of the bacterium occurs. Zawrah *et al.* 2011 stated that AgNPs also rupture plasma membrane of bacterium and thereby depletion of cellular energy. Another mechanism proposed by Kumar *et al.* 2004 that AgNPs block the cellular respiration as a result of reaction between the Ag groups of AgNPs and SH group of the bacterium cell wall. Morones *et al.* (2005) proved that AgNPs have capability to break the defense system of bacterial cell.

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

Treatments	Inhibition zones (mm)			Means
	150ppm	200ppm	250ppm	
Ag	17d	19c	20c	18.66
Agp	20c	22b	23b	21.66
AgIB	23b	24b	25b	24
AgAE	25a	27a	29a	27
AgBE	20c	23b	24b	22.33
Nystatin	3e	4d	5d	4
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.



**Fig. 1.** Antibacterial activity.

#### Antifungal activity

The inhibition zone (mm) of different silver nanoparticles (AgNPs) and antibiotic (Nystatin) were determined for *Alternaria solani* are presented in Table 2 and Fig. 2. At the concentration of 150ppm,

maximum inhibition zone was found to AgAE followed by AgIB with 25mm and 23mm respectively whereas same inhibition zone was obtained to Agp and AgBE with 20 mm. The silver nanoparticles (AgNPs) Agp and AgBE at 150 ppm concentration were not statistically different at  $p < 0.05$  level of probability whereas at the same concentration lowest inhibition zone was observed in Ag (17mm) followed by the antibiotic nystatin (3mm). The silver nanoparticles (AgNPs) had more inhibitory effect on the growth of *Alternaria solani* at 200ppm. At this concentration highest inhibition zone was recorded from AgAE (27mm) and AgIB (24mm) followed by AgBE (23mm) and Agp (22mm) whereas Ag (19mm) and antibiotic Nystatin (4mm) showed lowest inhibition zone against the tested fungi. Silver

nanoparticles(AgNPs) Agp, AgIB and AgBE were not statistically different at  $p < 0.05$  level of probability. In all the cases of silver nanoparticles and antibiotic (Nystatin) concentrations, the concentration 250 ppm produced a greater inhibition zone against the tested fungi. 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 to Ag(20mm) followed by Nystatin(5mm). Further increasing the concentrations of AgNPs significantly increase the inhibition zones of the test fungi. Overall results showed that silver nanoparticles(AgNPs) showed highest inhibitory action against *Alternaria solani* as compared to antibiotic nystatin. These findings that silver nanoparticles(AgNPs) had more inhibitory effect against *Alternaria solani* are in agreement with the results of Al-Askaret *et al.* (2013) who investigated the antifungal activity of AgNPs against *Fusariumoxysporum*, *Alternaria alternate* and *Aspergillousflavus*. His results showed that AgNPs have potent antifungal activity as compared with the antibiotics nystatin and griseofulvin. Inhibition zones obtained to AgNPs ranged between 10-26mm when compared with the 5-8mm for antibiotics. These findings are also in consistent with the results of Huang *et al.* (2007) who reported maximum inhibitory effect of AgNPs against sclerotium forming fungi as compared with the antibiotics.

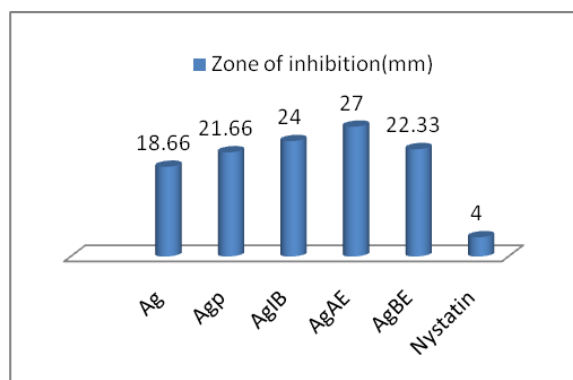


Fig. 2. Antifungal activity.

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