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Antimicrobial activity of silver nanoparticles biosynthesized using leaf extract of *Brassica juncea* against plant pathogens

Rita Singh, Antaryami Singh, Vikas Yadav Patade
Defence Institute of Bio-Energy Research Field Station, Defence Research and Development Organisation, Pithoragarh, Uttarakhand, India

Abstract
Silver nanoparticles have broad spectrum and highly effective antimicrobial properties. One of the potential applications in which silver nanoparticles can be utilized is in the management of plant diseases. Biological synthesis of silver nanoparticles using plants is gaining importance. In the present study, the potential of *Brassica juncea* for biosynthesis of silver nanoparticles was explored. Silver nanoparticles were synthesized using different concentrations 10 to 50% (v/v) leaf extract to obtain most effective nanoparticles with maximum inhibition of microbial growth. Antifungal effects of 50 ppm and 100 ppm silver nanoparticles synthesized with different concentrations of plant extract were evaluated against *Fusarium oxysporum* and *Colletotrichum gloeosporioides* using agar block method. Inhibitory effect of silver nanoparticles on bacteria was tested using *Bacillus* and *Enterobacter* strains. Silver nanoparticles at 100 ppm concentration biosynthesized using 20% (v/v) leaf extract of *Brassica juncea* were found to be most effective against fungi and bacteria.

Keywords: *Brassica juncea*, silver nanoparticles, antimicrobial activity, biosynthesis, leaf extract

1. Introduction

Silver nanoparticles are increasingly used in various fields including health care and food industry due to their unique physical, chemical and biological properties. Recently, silver nanoparticles are gaining interest for applications in the field of agriculture due to their antibacterial, antifungal and antiviral activity. Silver nanoparticles as antimicrobial agents can be utilized in the management of plant diseases as an alternative to chemically manufactured pesticides [1]. Silver nanoparticles due to presence multiple modes of inhibitory action against microorganisms are considered a relatively safe and effective method [2] compared to synthetic fungicides for controlling plant pathogens.

Various methods have been adopted for the synthesis of silver nanoparticles and can be classified into three broad categories: physical, chemical and biological (or green) synthesis [3]. Biosynthesis or green synthesis of metal nanoparticles is proposed as a cost-effective and environmental friendly alternative to conventional physical and chemical methods that are very expensive and hazardous. Biosynthesis of silver nanoparticles has received extensive attention due to the environmental and safety concerns with the use of organic solvents and toxic reagents [4]. Various species of bacteria, fungi, yeast, algae and plants have been reported for the biogenic synthesis of silver nanoparticles [5-8].

Brassica juncea belongs to the family Brassicaceae and is commonly known as brown mustard, Indian mustard or vegetable mustard. The leaves, seeds, and stems of this mustard variety are edible. *B. juncea* is grown for their greens and for the production of oil seed. It is also grown as a green manure and act as mulch, covering the soil to suppress weeds between crops. In the present study, the use of *Brassica juncea* for biosynthesis of silver nanoparticles was explored. The potential of silver nanoparticles biosynthesized using *Brassica juncea* was studied for controlling plant pathogens.

2. Materials and methods

2.1 Preparation of plant extract

Extract of leaves of *Brassica juncea* was prepared. Fresh leaves were collected from the fields of Seed Science & Technology Division at DIBER Field Station, Pithoragarh, Uttarakhand. Clean leaves were weighed and chopped into small pieces for crushing in mortar and pestle. 25 g crushed leaves were boiled in 250 ml of distilled water for 20 min for extraction. The extract was allowed to cool and filtered using Whatman No. 1 filter paper.

2.2. Synthesis of silver nanoparticles using plant extract

The aqueous extract of *Brassica juncea* was added at different concentrations of 10, 20, 30, 40 and 50% (v/v) to 1 mM silver nitrate solution. The biosynthesis of silver nanoparticles was carried out at room temperature for 24 h and heating at 60^{o} C for 15 minutes.

2.3. UV-Vis characterization of biosynthesized silver nanoparticles

Silver nanoparticles biosynthesized using extract of *Brassica juncea* were analyzed by ultraviolet-visible (UV-Vis) spectroscopy. The optical absorbance of silver nanoparticles was measured using an Eppendorf Bio spectrometer. The absorption spectra of the samples were recorded in the wavelength range of 300 to 800 nm to evaluate the reduction of silver and formation of silver nanoparticles by the extract of *Brassica juncea* leaves.

2.4. Testing of silver nanoparticles for antimicrobial activity

Silver nanoparticles were tested for antifungal activity using plant pathogenic fungi- *Fusarium oxysporum* and *Colletotrichum gloeosporioides.* Different concentrations (50 and 100 ppm) of biosynthesized silver nanoparticles were tested using agar block method. Testing of silver nanoparticles for antibacterial activity was carried out using well diffusion method. Bacterial cultures – *Bacillus* and *Enterobacter* were used and zone of inhibition in presence of silver nanoparticles was measured.

3. Results

Synthesis of silver nanoparticles using extract of plant *Brassica juncea* (Figure 1a) was studied. Effect of concentrations of plant extract on biosynthesis of nanoparticles was evaluated. Silver nitrate (1mM $AgNO_3$) was treated with different concentrations 10, 20, 30, 40 and 50% (v/v) of leaf extract of *Brassica juncea* (Figure 1c).

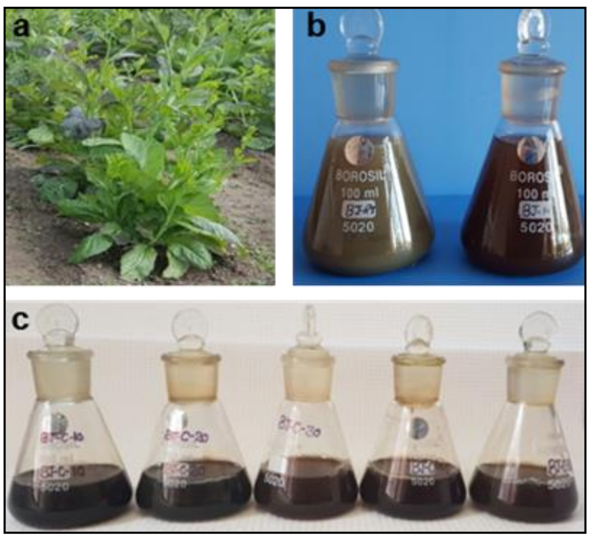

Fig 1: Biosynthesis of silver nanoparticles (a) using the leaf extract *of Brassica juncea* (b) at different temperatures (c) with different concentrations of plant extract

Changes in colour to dark brown were recorded at different concentrations indicating formation of silver nanoparticles. The synthesis of nanoparticles by *Brassica juncea* was compared at room temperature and heating at 60^{o} C for 15 minutes (Figure 1b). The comparison suggested that the low rate of biosynthesis at room temperature was enhanced at high temperature. UV-visible absorption spectra were recorded to confirm the formation of silver nanoparticles by the leaf extract of *Brassica juncea* (Figure 2). The UV-Vis spectrum exhibited an absorption band at around 440 nm which is characteristic of silver nanoparticles.

The microbicidal effect of biosynthesized silver nanoparticles on pathogenic fungi-*Fusarium oxysporum* and *Colletotrichum gloeosporioides* affecting vegetable crops was evaluated. Radial growth of *Fusarium oxysporum* in media without silver nanoparticles and in presence of 50 ppm (Figure 3a) and 100 ppm (Figure 3b) silver nanoparticles was measured. Variation in antifungal activity of silver nanoparticles synthesized with different concentrations (10-50%) *Brassica juncea* leaf extract was observed. The activity was found to decrease in presence of the highest concentration of 50% plant extract.

The inhibition of *Fusarium oxysporum* in presence of 50 ppm silver nanoparticles synthesized with 10-40% plant extract was 47.23±8.34 to 84.38±3.13% (Figure 4). No growth was observed in presence of 100 ppm biosynthesized silver nanoparticles. Silver nanoparticles with 50% plant extract showed less inhibition of *Fusarium oxysporum* both at 50 ppm and 100 ppm concentration.

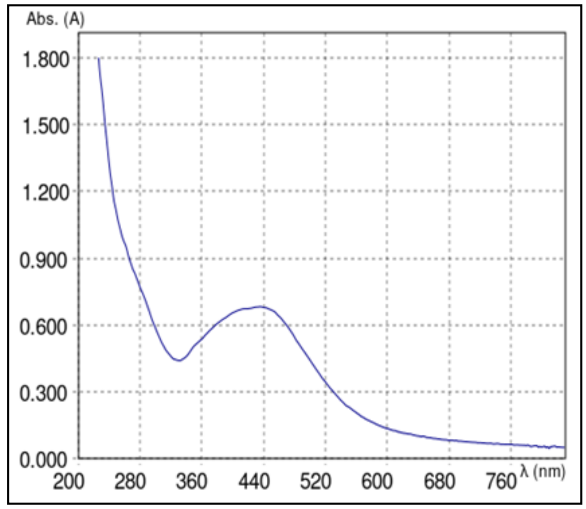

Fig 2: UV-Vis absorption spectra of biosynthesized silver nanoparticles

Growth of *Colletotrichum gloeosporioides* in presence of silver nanoparticles biosynthesized with 10, 20, 30, 40 and 50% leaf extract of *Brassica juncea* is presented in Figure 5. Significant reduction in radial growth of fungi was observed at 100 ppm (Figure 5b) as compared to radial growth at 50 ppm (Figure 5a). Percent inhibition of *Colletotrichum gloeosporioides* in presence of 50 ppm and 100 ppm silver nanoparticles is presented in Figure 6. In presence of 50 ppm biosynthesized silver nanoparticles, inhibition was observed to be 47.62±2.76% at 50% plant extract concentration to 73.64±0.91% at 20% concentration of plant extract.

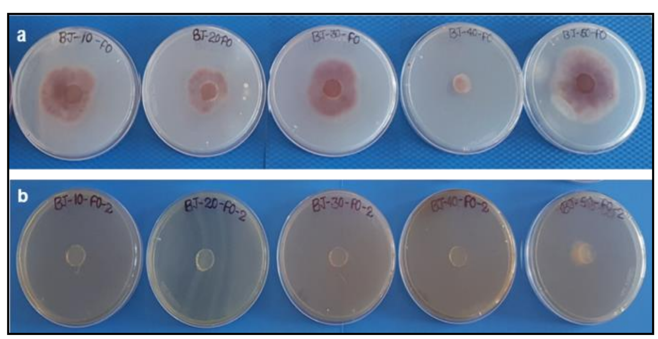

Fig 3: Growth of *Fusarium oxysporum* in presence of (a) 50 ppm and (b) 100 ppm silver nanoparticles synthesized with different concentrations (10-50%) of plant extract

Percent inhibition at higher concentration of 100 ppm silver nanoparticles was also lowest at 50% plant concentration. Complete inhibition of fungi was observed with silver nanoparticles synthesized with 20% and 30% plant extract. Comparison of radial growth at 50 ppm and 100 ppm concentrations demonstrate the highest efficacy of silver nanoparticles synthesized with 20% plant extract.

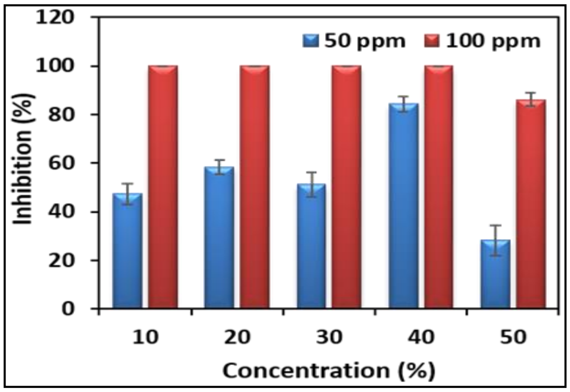

Fig 4: Inhibition of *Fusarium oxysporum* by silver nanoparticles synthesized in presence of different concentrations of plant extracts

Silver nanoparticles synthesized with different concentrations of 10 to 50% plant extract were tested for antibacterial activity. Zone of inhibition at 50 ppm and 100 ppm concentration for two bacterial cultures *Bacillus* and *Enterobacter* are presented in Table 1. Zone of inhibition was higher at 100 ppm silver nanoparticles for all the concentrations of plant extract used for biosynthesis.

Table 1: Zone of inhibition in presence of silver nanoparticles synthesized with different concentrations of plant extract

Treatment	*Enterobacter*		*Bacillus*	
	50 ppm	100 ppm	50 ppm	100 ppm
AgNP-PE10	2.67 ± 0.47	5.33 ± 1.25	4.33 ± 0.47	6.33 ± 0.47
AgNP-PE20	9.67 ± 1.40	14.33 ± 1.68	6.67 ± 0.47	9.00 ± 0.82
AgNP-PE30	4.67 ± 0.47	6.67 ± 1.70	5.67 ± 0.94	8.00 ± 1.41
AgNP-PE40	4.00 ±0.82	5.67 ± 1.25	5.00 ± 0.82	7.00 ± 0.82
AgNP-PE50	4.00 ± 1.63	5.00 ± 0.82	3.67 ± 0.47	4.67 ± 1.15

Values are mean of zone of inhibition in mm of 3 replicates ± SE.
AgNP, Silver nanoparticles; PE, Plant extract at different concentrations of 10 to 50%.

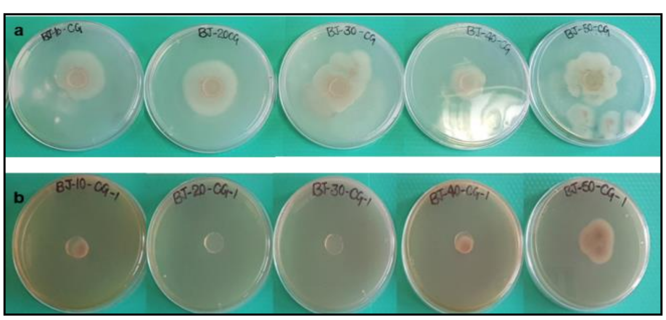

Fig 5: Growth of *Colletotrichum gloeosporioides* in presence of (a) 50 ppm and (b) 100 ppm silver nanoparticles synthesized with different concentrations (10-50%) of plant extract

Most significant inhibition was observed for silver nanoparticles synthesized with 20% and 30% plant extract as compared to other concentrations.

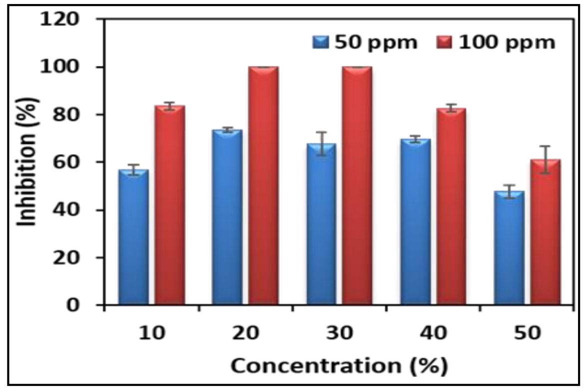

Fig 6: Inhibition of *Colletotrichum gloeosporioides* by silver nanoparticles synthesized in presence of different concentrations of plant extracts

4. Discussion

Silver nanoparticles have vast potential in agriculture for the management of pathogenic microorganisms affecting crops, thereby increasing agricultural production. Biological synthesis is an economical method for production of silver nanoparticles which is extensively useful for agricultural applications. In the present study, silver nanoparticles were synthesized using leaf extract of *Brassica juncea*. *Brassica juncea* is reported to contain bioactive phytochemicals including glycosides, sterols, flavonoids, and phenolic compounds [9, 10]. The phytochemicals in the leaf extract of *Brassica juncea* reduce silver ions to silver nanoparticles.

Silver nanoparticles have shown strong antifungal activity against a range of phytopathogens [11-13]. The antifungal mechanism of silver nanoparticles may be due to the production of free radicals from the nanoparticles that could damage the membrane lipids and disrupt the membrane functions. It is also proposed that the membrane could be deteriorated by the formation of pits on the surface of the cell wall membrane [14, 15]. The toxicity of silver nanoparticles on fungal hyphae and conidial development has also been suggested [16, 17].

Antibacterial effect of silver nanoparticle has been demonstrated to be due to the sustained release of free silver ions from the nanoparticles. The formation of free radicals and subsequent free radical-induced membrane damage is a potential mechanism. Silver nanoparticles can penetrate the bacterial cell wall, thereby causing structural change of the cell membrane and increasing cell permeability, leading to cell death. It has also been found that silver ions released by silver nanoparticles interact with the thiol groups of vital enzymes and phosphorus-containing bases, thus preventing cell division and DNA replication [18]. Silver nanoparticles have antibacterial effect against a broad range of Gram-negative bacteria, Gram-positive bacteria and antibiotic-resistant bacterial strains [19]. The results of the present study are in corroboration with other studies available on the effect of silver nanoparticles on plant pathogens.

5. Conclusion

The study has demonstrated the potential of leaf extract of commonly grown vegetable plant *Brassica juncea* for the synthesis of silver nanoparticles. The highest antimicrobial properties were observed for silver nanoparticles biosynthesized in presence of 20% aqueous leaf extract. The results of antimicrobial evaluation have demonstrated the efficacy of biosynthesized silver nanoparticles for control of bacterial and fungal plant pathogens

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