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Silver Nanoparticles: The Powerful Antidote for Disease in Pomegranate

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ABSTRACT

In this study, simple approach was applied for the synthesis, characterization and application of silver nanoparticles from the leaves of *Bryophyllum p*., garlic and jamun which is cost-effective, eco-friendly and easy to synthesis. The extract acts both as reducing as well as capping agent. The color change of the solution yellowish brown to reddish brown confirms the formation of silver nanoparticles. The synthesized silver nanoparticles were characterized by UV-visible spectroscopy which showed a peak between 420 and 430 nm corresponding to the absorbance of the AgNPs that confirmed the reduction of Ag^{+} to Ag^{0} nanoparticles. The anti-bacterial activity of AgNPs was investigated against *Xanthomonas axonopodis* pv. *punicae* and its identity was confirmed by morphological, biochemical and pathogenicity test. The results indicated that garlic AgNPs shoed maximum inhibition at a concentration of 15 mM. Hence, they can be used in the disease management in pomegranate.

1. Introduction

Recently, nanotechnology is rapidly growing field with great application for generating nanoparticles by using medicinal plants in agriculture due to their diverse effect [1]. Silver nanoparticles of size smaller than 100 nm contain about 10,000–15,000 silver atoms [2]. It has various role in field of high sensitivity bimolecular detection, catalysis [3] and medicine; it is been acknowledged to have strong bactericidal effects along with the anti-fungal activities [4-6]. Silver nanoparticles have showed to be the most effective against bacteria and some eukaryotic micro-organisms. The synthesis of nanoparticles by biological method showed better results than the chemical methods, it is easily scaled up and cost effective [7]. Biosynthetic methods can use microorganism or the plant extract for production of nanoparticles [8]. Pomegranate is one of the known edible fruit [9], growing in tropical and subtropical regions, belongs to the Punicaceae family. In recent years the area under pomegranate has been increased due to low maintenance costs, drought resistance and high yields. It is rich in vitamin C, antioxidants [10, 11] and containing polyphenols which inhibit estrogen synthesis. In India, pomegranate is commercially cultivated in Maharashtra, Karnataka, Andhra Pradesh, Gujarat, Uttar Pradesh, and Tamil Nadu [12]. There are several production constraints of pomegranate crop which severe yield losses among them bacterial blight diseases caused by *Xanthomonas axonopodis* pv. *Punicae;* a major threat [13, 14]. Initial symptoms showed small irregular water soaked, dark colored spots on leaves which later on enlarge and led to defoliation, result in heavy economic losses and complete destruction [15]. In the present study the causative agent was isolated from the infected fruits and to control of this disease, silver nanoparticles were synthesized from leaf extracts of *Bryophyllum p.* garlic and jamun at variable concentrations. The results were confirmed by UV-visible spectroscopy and antimicrobial activity of silver nanoparticles was evaluated against bacterial blight disease to control and manage disease which will be beneficial to farmers.

2. Experimental Methods

2.1 Sample Collection

The infected fruit samples were collected from the farmer's fields of major pomegranate growing areas of Bori, Indapur, Maharashtra (India), showing typical symptoms like yellowish brown spots, water-soaked lesions, cankers lesions and brown patches (Fig. 1). Higher disease infected fruits were selected according to disease severity scale [16].

Fig. 1 *Xanthomonas sp.*III Sample collection of infected fruit from the areas of Bori, Indapur, Maharashtra (India)

2.2 Isolation and Characterization of Pathogen

Infected fruit samples were collected; surface sterilized with 0.1% $HgCl_2$ solution for 1 minute and washed three times with sterile distilled water. Then it was squeezed gently with a sterile scalpel to get a suspension. The suspension was serially diluted and plated in sterilized petriplates containing YDC agar medium and incubated at 30 °C for 72 hours. After the incubation, isolates were showing typical characters of *Xanthomonas axonopodis* with light yellow mucoid shining colonies (Fig. 2). The isolated colonies of *X. axonopodis* were named as isolate III based on disease severity scale. Further isolated colonies were screened based on biochemical and morphological characterization according to 9th edition of Bergey's manual of Determinative Bacteriology [17].

2.3 Pathogenicity Test

Pathogenicity tests were performed of isolates III. For this the healthy pomegranate fruit was selected, surface sterilized with 10% sodium

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hypochlorite for 1 minute. Fruit were priced by a sterile needle and sprayed with suspension of *Xanthomonas axonopodis* isolates as per their same fruit variety. The inoculated fruit packed with sterile polythene bags. Disease symptoms were observed after 10-15 days. The pathogens were reisolated from the diseased fruits and compared with their original cultures for their verification [18-20].

2.4 Synthesis of Silver Nanoparticles by using Plant Extracts

5 g of fresh *Bryophyllum p*. garlic and jamun leaves were collected from the bio-village of VPASC College, Baramati. The leaves were crushed in deionized water; the extract was filtered by Whatman filter paper number one. The 5 mL of extract filtrate was collected and added in various concentrations of silver nitrate solution (5 mM, 10 mM and 15 mM). This reaction was kept under dark condition for 15 days; tubes were monitored after every 24 hours. After 15 days, the color change of the solution containing silver nanoparticles was analyzed by UV spectroscopy. Reaction showing peaks at around 420 nm – 430 nm. This solution was centrifuged at 10,000 rpm for 10 min and the precipitate of silver nanoparticles was washed in methanol three times. The resulting pellet was dried in oven at 65 °C for 24 hours and then collected for further use.

2.5 In-vitro Efficiency of Biosynthesized Silver Nanoparticles against Xanthomonas axonopodis Isolates

The antibacterial test was carried out by agar well diffusion method on nutrient agar medium [21]. Different concentration of silver nanoparticles was prepared in Erlenmeyer flasks. For antimicrobial activity freshly prepared 100 µL of the isolated pathogen was spread on the sterile nutrient agar plates. The milli-Q water and silver nanoparticles were used as a positive control; *Bryophyllum p*. garlic and jamun used as a test sample. Plates were incubated at 37 °C for 24 hours, and then the zone of inhibition was measured.

3. Results and Discussion

3.1 Colony Characteristics

After the incubation, *Xanthomonas sp*. III isolates was showing typical characters with light yellow mucoid shining colonies (Fig. 2).

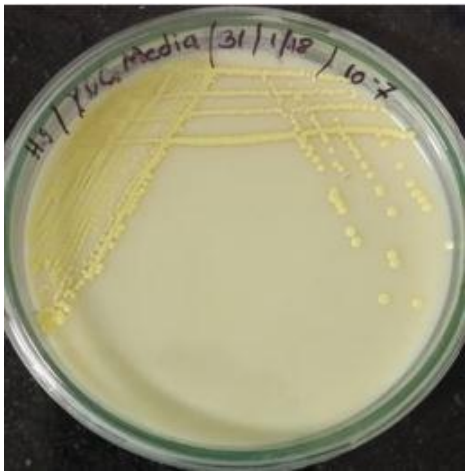

Fig. 2 The results of isolates *Xanthomonas sp*. III on YDC agar plates

Table 1 Colony characters of isolate *Xanthomonas sp*. III

Colony characters	*Xanthomonas sp. III*
Size	3mm
Shape	Circular
Colour	Yellow
Margin	Regular
Elevation	Elevated
Consistency	mucoid
Opacity	Opaque
Gram nature	Gram Negative rods
Motility	Motile

3.2 Biochemical Characteristics of Isolates Xanthomonas sp. III

The biochemical characters of isolate *Xanthomonas sp*. III, are given in Table 1. Identification of isolates was done by comparing morphological and biochemical tests according to Bergy's manual (Fig. 3).

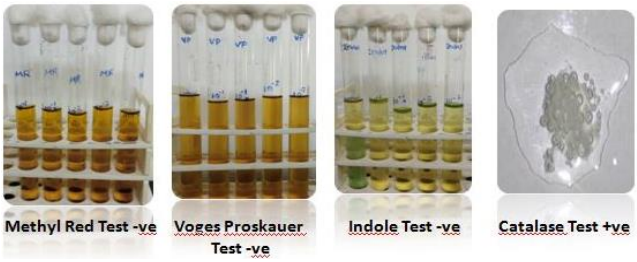

Fig. 3 Biochemical tests for identification of isolates

3.3 Pathogenicity Test

Pathogenicity tests were performed on the field of VSBT farm. For this the healthy pomegranate fruit were selected, surface sterilized with 10% sodium hypochlorite. Pomegranate were priced by a sterile needle and sprayed with suspension of *Xanthomonas axonopodis* isolates as per their same fruit variety. The inoculated fruits packed with sterile polythene bags. After 15-25 days observation shows the development of disease symptoms. The pathogens were reisolated from the diseased fruits and compared with their original cultures for their verification (Fig. 4).

Fig. 4 Pathogenicity test of *Xanthomonas spp*.III a) Inaculation of *X.axonopodis pv.Punicae* on pomegranate plant b) Symptoms of bacterial blight observed on fruits after 15-25 days of incubation

3.4 Synthesis of Silver Nanoparticles

As the leaf extracts were added to different concentrations of aqueous silver nitrate solution, the color of the solution changed from colourless to brown indicating AgNP formation (Fig. 5). The completion of reaction between leaf extract and $AgNO_3$ was observed. Absorption spectra of AgNPs formed in the reaction media has absorption maxima in the range of 420 to 460 nm (Fig. 6).

Fig. 5 Silver Nanoparticles of garlic, *Bryophyllum p.* and jamun

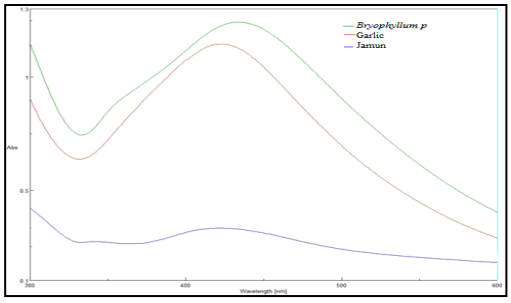

Fig. 6 Spectral analysis of silver nanoparticles at 420 to 430 nm

3.5 Anti-Microbial Activity of Extract

The antimicrobial activity shows most rapid bioreduction in 5 mL extract of *Bryophyllum* p. garlic and jamun at 15 mM concentration of silver nitrate solution. Among three medicinal plant extract, garlic extract is a good source for the synthesis of silver nanoparticles to overcome the problems of bacterial blight of pomegranate. The inhibition zones obtained indicates maximum antibacterial activity of the prepared test sample (Figs. 7, 8). The silver nanoparticles produced by tulsi, neem, tridax

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and drumstick showed the inhibition of *Xanthomonas* sp. [22] where the different cyanobacterial stains also produced extracellular silver nanoparticles and these nanoparticles have an antibacterial activity [23].

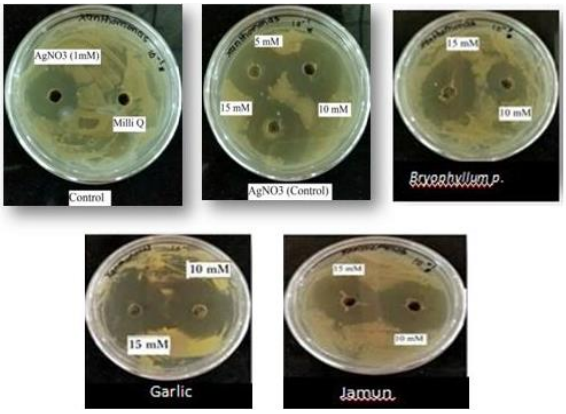

Fig. 7 Antibacterial activity of control, $AgNO_3$ (control), *Bryophyllum p*. garlic and jamun at (10mM conc.) against *Xanthomonas axonopodis pv. punicae*

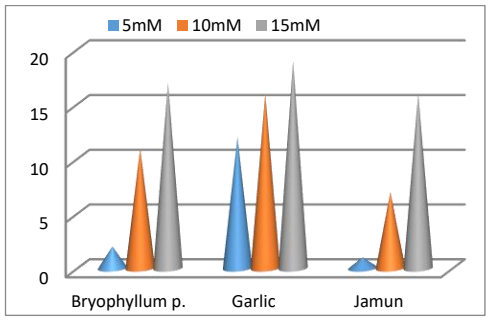

Fig. 8 Antimicrobial activity of AgNP's, against *Xanthomonas sp*. III at different concentration

4. Conclusion

Present study results showed that, bacterial blight of pomegranate was found to be highly destructive and wide spread disease. *Xanthomonas sp*. III was obtained from the highly infected plant and its identity was confirmed by morphological, biochemical and Pathogenicity tests. We found that garlic extract is a good source for the synthesis of silver nanoparticles to overcome the problems of bacterial blight of pomegranate. Therefore, silver nanoparticles would be used as powerful weapons against *Xanthomonas axonopodis pv. punicae*.

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