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Synthesis and Characterisation of Silver Nanoparticles Using *Withania somnifera* and Antifungal Effect against *Fusarium solani*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

In the present study, an attempt was being made to use *Withania somnifera* plant leaf extract for the synthesis of silver nanoparticles and establish the antifungal activity against plant pathogen *Fusarium solani* NVS671. Among different phytoextract concentration studied, 4% was found optimum for nanoparticles synthesis in combination with 100mM $AgNO_3$. UV spectra analysis revealed peak at 420nm. The Transmission Electron Microscopy analysis provided further information about the morphological characteristics of the nanoparticles and confirmed that the particles were spherical with size in the range of 6.43-23.27 nm. Following TEM and spectrometry analysis, nanoparticles were amended into PDA media for the evaluation of their *In vitro* antifungal potential at concentrations of 25ppm, 50ppm, 100ppm and 250ppm was studied against *F. solani* NVS671. In all cases, Ag Nanoparticles exhibited higher inhibition of mycelial growth. *In vivo* analysis of silver nanoparticles against pathogenic fungus *F. solani* on sugarcane crop under greenhouse condition also sown promising results when evaluated plant growth and disease

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severity in comparison with control. It is concluded that *W. somnifera* leaf extract was found suitable for the green synthesis of AgNPs and suggested as an alternative and effective approach with antifungal potential which is eco-friendly and cost-effective for the control of pathogenic fungus like *F. solani.*

Keywords: Antifungal; Fusarium solani; pathogen; silver nanoparticles; Withania somnifera.

1. INTRODUCTION

Agricultural production is reduced worldwide every year due to disease caused by fungal pathogens in the field. Among the various fungal pathogen which affects the crop yield, *Fusarium solani* is well known for its severity and vigorous infection in many crops including sugarcane [1,2]. Chemical fungicide application is one of the widely used approaches for the protection of plants from fungal diseases but it is associated with environmental hazards including soil and water pollution. Further, the emergence and increase of fungal resistance to multiple fungicides are becoming a challenge for farmers. Therefore scientists in the agricultural field are searching for alternative measures against fungal pathogens. As an alternative to chemically manufactured fungicides, green synthesis of silver nanoparticles using phytoextract for antifungal activity against fungal plant disease has gained attention in the scientific community [3,4]. Development of a reliable and eco-friendly process for the synthesis of nanoparticles is an important step in the field of nanotechnology. The method is suitable for nano scale metal synthesis due to the absence of any requirement to maintain an aseptic environment. Silver displays multiple modes of inhibitory action against microorganisms [5]. Therefore, it may be used with relative safety for control of various plant pathogens compared to synthetic fungicides. Primary requirements for the potential use of silver in control of plant disease include the need for more information on the antifungal activity of various silver compounds to plant pathogens and development of better application strategies to increase the efficacy of disease suppression.

Withania somnifera; family Solanaceae, a traditional medicinal plant is a source of reductant and stabilizers and has been used as an antibacterial, antioxidant, aphrodisiac, liver tonic, and anti-inflammatory agent [6]. In our previous research, we have reported antifungal potential of *W. somnifera* to extract against sugarcane red rot pathogen *Colletotrichum falcatum* [7]. In the present study, an attempt is being made to establish the antifungal activity of plant extracts based AgNPs against pathogenic *Fusarium solani* isolated from sugarcane.

Fusarium solani is a filamentous fungus, a well-known plant pathogen, causing various types of diseases on a wide range of plants. Several Fusarium species cause root rot, wilt and premature death on infected plants in different crops [8]. So the aims of the present study are to investigate the antifungal effects of the extracts of the *W. somnifera* used to synthesise AgNPs and the combined effect on *F. solani.*

2. MATERIALS AND METHODS

2.1 Synthesis and Characterisation of AgNPs

Healthy leaves of *W. somnifera* were collected from medicinal plant garden, Uka Tarsadia University. The leaves were washed thoroughly with distilled water to remove the adhering dust and other contaminants. Twenty-five-gram leaves were cut into small pieces and boiled for 10min in 100ml doubled distilled water. The extract was filtered twice with Whatman filter paper and stored at -4°C for further study. Different concentrations (1%, 4% and 10%) of phytoextract were challenged with 1mM $AgNO_3$ solution to the formation of silver nanoparticles. A change in colour was observed and nanoparticles were subjected to UV spectra for a peak at 430nm. Further Silver nanoparticles size was measured by TEM analysis at Bombay IIT.

2.2 *In vitro* Antifungal Activity against *F. solani NVS671*

Fusarium solani NVS671, a plant pathogenic fungus used in this research was isolated from infected sugarcane (Data not published). To evaluate the antifungal potential of AgNPs, Potato Dextrose Agar (PDA) medium were inoculated with different concentration of nanoparticles (25ppm, 50ppm, 100ppm and 250ppm respectively). Five mm agar block with actively grown mycelia were inoculated into the centre of the plate with a sterile toothpick and

incubated at 28°C for 7 days. The growth of the pathogenic fungus was observed on PDA without AgNPs after one week.

2.3 Green House Study

The single eye sugarcane stems were sterilized with 70% ethanol and in 2% sodium hypochlorite, followed by washing five times in sterile distilled water to remove traces of chemicals. The surface sterilized stems were planted in an appropriate pot containing 2 kg of autoclaved soil under green house condition. *F. solani NVS671* spore suspension ($1X10^4$ spores/ml) was prepared in sterile distilled water. The 100µl spore suspension was inoculated at both open end of sugarcane stem and 200µl of 100ppm nanoparticles was inoculated on eye bud. In total, the following treatments with three replicates were investigated with two individual experiments: (i) control without fungus or nanoparticles inoculation (ii) pathogen (iii) nanoparticles and (iv) nanoparticles with the pathogen. The pots were arranged in a completely randomised factorial design in a greenhouse and maintained at an optimum condition for sugarcane crop. For each species and treatment, the plants of three pots were harvested 4 weeks after the emergence of seedlings, washed and morphological characteristics viz., root length, shoot length, dry and wet weight of stem and root of each plant was recorded.

3. RESULTS AND DISCUSSION

Metallic nanoparticles can synthesize using various physical and chemical methods. However, development of simple and eco-friendly biological synthesis systems would help in agriculture. The key advantages of using plants for the synthesis of silver nanoparticles are that they are easily available, safe to handle and possess a broad variability of metabolites that may help in the production process. In the present study different concentration of *W. somnifera* extract with 1mM $AgNO_3$ were used for the formation of silver nanoparticles. The synthesis of AgNPs was initially observed by the coloured change (Fig. 1). It is well known that silver nanoparticles exhibit reddish brown colour in aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles.

UV- visible spectroscopy is one of the most vital techniques to determine and evaluate the formation and stability of metal nanoparticles in aqueous solution [9]. It helps in the characterisation and analysis of metallic nanoparticles. Normally 300-800nm light wavelength is used for the characterisation of size range 2 to 100nm [10]. So, nanoparticles were subjected to UV spectra and peak was recorded at 430nm. Among the various concentration analysed, 4% was found best for nanoparticles synthesis.

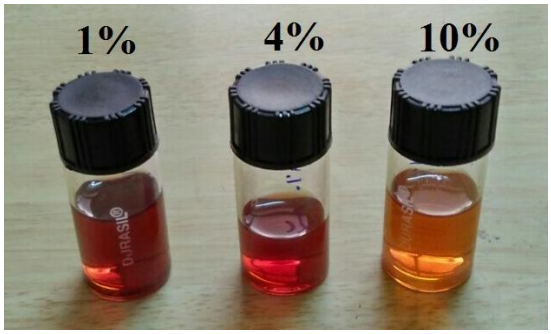

Fig. 1. Colored change observed in synthesis of AgNPs

TEM analysis provided further information about the morphological characteristics of the particles and confirmed that the particles were spherical with a size of prepared nanoparticles that is 10-21nm (Fig. 2).

The results of antifungal efficacy of AgNps against *F. solani* NVS671 were encouraging, (Fig. 3). In all cases, Ag Nanoparticles exhibited higher inhibition of mycelial growth and gave significant results as compared to control. The highest inhibition was observed with 250ppm AgNps and the lowest inhibition with 25ppm AgNps.

For *In vivo* analysis in each treatment, the plants of three pots were harvested 4 weeks after the emergence of seedlings, washed and morphological characteristics viz., root length, shoot length, dry and wet weight of stem and root of each plant were recorded. The sugarcane infested with pathogen had the lowest yield and was significantly different from all other treatments in all of the growth parameters. Sugarcane treated with pathogen had shown lowest 1.237 gm wet shoot weight due to pathogen invasion while the treatment nanoparticles had shown 1.9 gm weight which is comparable with control (Fig. 4a). These findings were in agreement with Elamawi and Al-Harbi who reported that Fusarium disease incidence on tomato was reduced by silver nanoparticles [11].

Similarly, good weight and increased plant height were reported when treated with nanoparticles (88.5 cm) as compared to pathogen treatment (69.66 cm). This indicates the efficacy of silver nanoparticles against *F. solani* NVS671 (Fig. 4b). Jo et al. found that silver nanoparticles reduced the disease severity by *Bipolaris sorokiniana* which causes seedling blight, root rot, crown rot and leaf spot blotch on various gramineous species and *Magnaporthe grisea* which causes blast on rice [12]. The potential of AgNPs for their use as drug carriers in cancer therapy, as biosensors for metabolites and pollutants, as catalyst etc. is quite high and requires intensive and integrated research activity for harnessing it [13].

In recent years, resistance to commercially available fungicides by phytopathogenic fungi has been increasing and has become a serious problem. Recently, more efforts have been given to developing safe management methods that pose less danger to humans and animals, and have focused on overcoming deficiencies of synthetic fungicides. Previous studies indicated that AgNPs influences colony formation of spores and disease progression of different plant pathogenic fungi [14,15]. The present study reveals that *W. somnifera* leaf extract was found suitable for the green synthesis of AgNPs. The reduction of silver ions by leaf extract resulted in the formation of stable particles with nano size. The microscopic images and spectroscopic characterizations using TEM, and UV-Vis were useful in proving the formation of nanoparticles and also in final confirming their size, shape, and morphology. The antifungal efficacy against fungal strain *F. solani* NVS671 confirmed that the AgNPs are capable of minimizing fungal disease and support good growth of sugarcane plants.

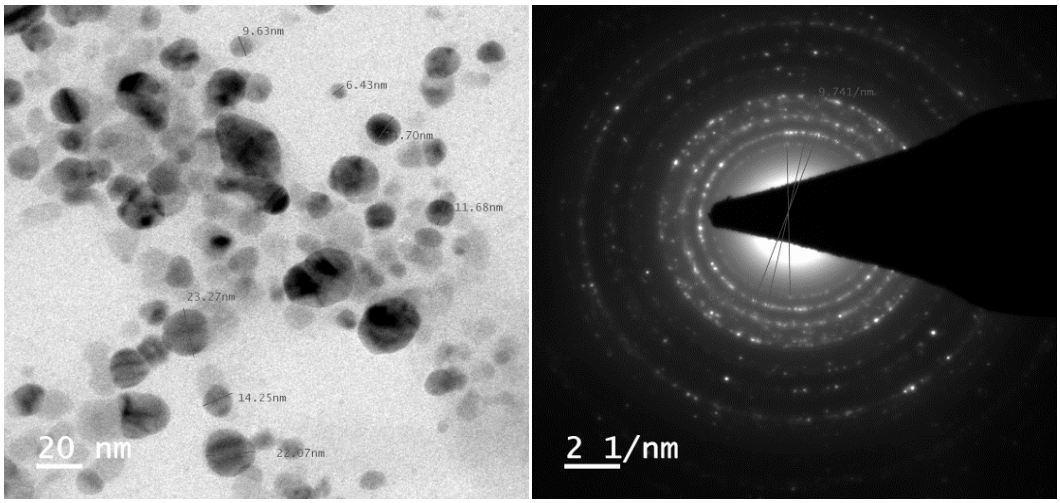

Fig. 2. TEM analysis of AgNPs particles

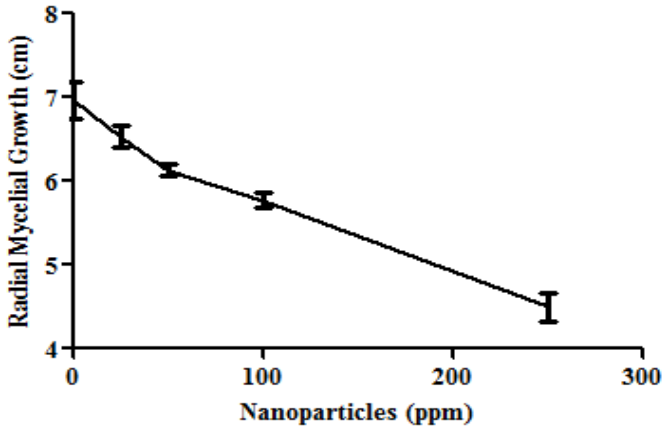

Fig. 3. Radial mycelial growth of *F. solani* NVS671 at different concentrations of AgNPs

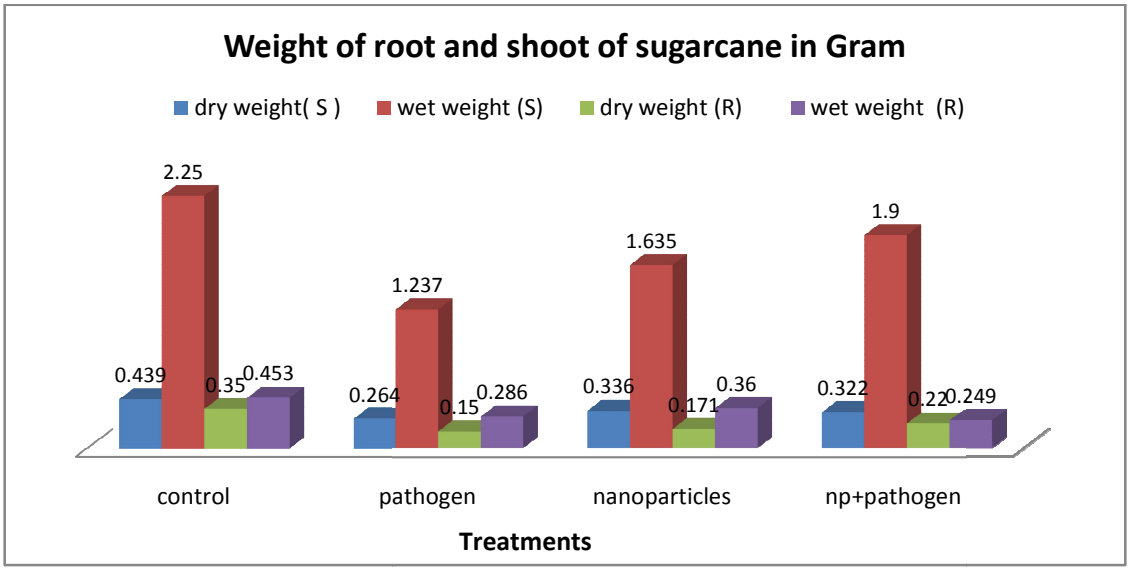

Fig. 4a. Effect of nanoparticles on weight of root and shoot of sugarcane under green house condition

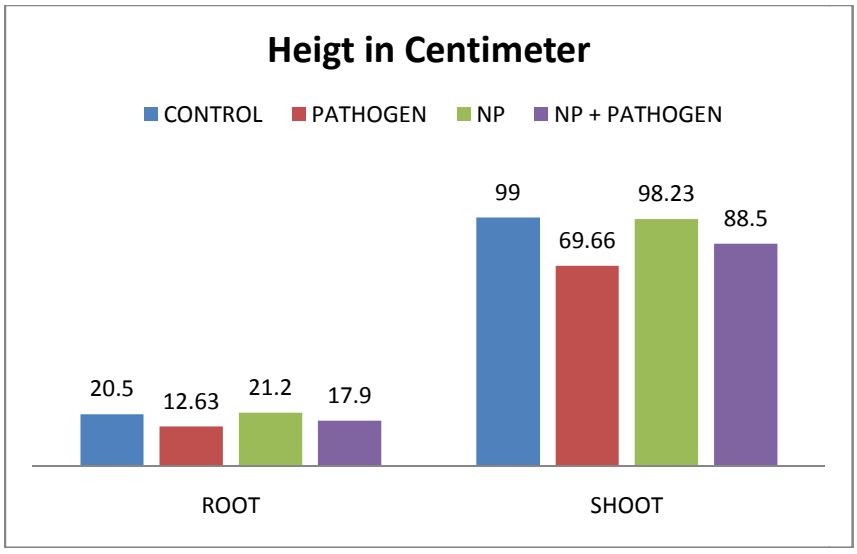

Fig. 4b. Effect of nanoparticles on plant length parameter of sugarcane root and shoot

4. CONCLUSION

The synthesis of silver nanoparticles was carried out using *W. somnifera* extract at room temperature, atmospheric pressure and was made with a universal solvent indicating a green process that presents a reliable and economic method. Further, in vitro and in vivo inhibition of plant pathogenic fungus *F. solani* indicates its possible pesticidal application in agriculture.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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