

Use of Silver Nano Particle for Controlling the Powdery Mildew Diseases at Celastrales Plants

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Abstract: The antifungal effectiveness against powdery mildew caused by fungus *Erysiphe* sp. on the Celastrales plant (*Euonymus* sp, Celastraceae) by using silver nanoparticles (AgNps) was investigated. Commercial brand (nanonasp pars COM, Iran) of Silver nanoparticles component was prepared. Before use, size particles and stability investigated by spectrophotometer and Scanning Tunneling Microscope (STM). The silver nanoparticles concentration of 4000 ppm was diluted in to 10, 50, 100, 200 ppm and was sprayed on plants surface which were infected by powdery mildew in advance. Under *in vitro* conditions, cut branch were put in the vials containing mentioned dosages of silver nanoparticles, then antifungal effects were observed by an optical microscope. Under *in vivo* trails, Plants were investigated for one month after treatment. No remarkable disease reduction was observed, in contrast to the finding of some researchers. Our hypothesis for this contrariety is related to he sensitivity of silver nanoparticles to conditions and materials at field and variety of AgNps at different brands. It is necessary to use standard protocol for these anti microbial and toxicity tests of AgNps and new formulation of AgNps for different crops and conditions.

Key word: Silver nanoparticles · Powdery mildew · *Erysiphe* sp

INTRODUCTION

The present study addressed the efficacy of Silver nanoparticles (AgNps) for controlling plant pathogenic microorganisms. Today's AgNps is one of the best and powerful particles for anti microbial usage especially for bacteria which have been resistant to common antibiotics [1]. Silver (Ag) is known as a powerful disinfecting agent for killing unicellular microorganisms by inactivating enzymes having metabolic functions in the microorganisms by oligodynamic action [2]. Silver nanoparticles which have high Specific surface area and high fraction of surface atoms, will have high antimicrobial activity compared to bulk silver metal. The interaction of metal nanoparticles with microorganisms (from fungi to viruses, e.g. HIV) is an expanding field of research [3]. It is believed that the mechanism of the antibacterial effect of silver ions (Ag⁺) involve their absorption and accumulation by bacterial cells and shrinkage of the cytoplasm membrane or its detachment from the cell wall. As a result, DNA molecules become condensed and loose their ability to replicate upon the infiltration of Ag ions.

The silver ions also interact with the S-H bounds of the proteins, blocking and inactivating them [4]. It has also been reported that bactericidal action of silver ions is basically caused due to the interaction of silver ions with ribosome and the suppression and expression of enzymes and proteins necessary for ATP production [5]. Ag⁺ ions are known uncouple the respiratory chain from oxidative phosphorylation or collapse the proton-motive force across the cytoplasmic membrane [6]. Uncontrolled generation of free radicals from the surface of Ag can attack membrane lipids that lead to a breakdown of membrane function [7]. Kim *et al.* [8] used N-acetylcystein as antioxidant to deactivate the free radical generated by Ag nanoparticles and observed that both Ag⁺ anoparticles and silver nitrate showed similar growth inhibitory effect against *E. coli*. However, such inhibitory effect was abolished Various methods of preparing the nanoparticles include mechanical grinding, co-precipitation, spraying, sol-gel manufacture, electrolysis, inverse microemulsion, etc: [9, 10]. Keep at mind that nano particles have high tendency for agglomeration however use of stabilizer agent for preventing from this phenomena

is undeniable. Every matter or condition that can abolish this property could cause particle adhering together and become large. When nano particles become larger than 100 nm they are not nano particles and anti fungal or antimicrobial potential became very low or abolish. Anti fungal property of these particles was noticeable and mentioned at different papers. At this research we investigated this potential for controlling on the very common and damaging groups of fungi (powdery mildew) on the *Euonymus sp* as important ornamental plants. This disease caused by *Erysiph sp* [11] this fungus is one of the most common and widespread fungal diseases of *Euonymus spp*.

MATERIAL AND METHODS

Silver Nano Particle Preparation and Analyzing:

Silver nano particle is prepared from Nano-nasb pars COM, Iran. This particle was 10-47 nm at liquid phase with 4000ppm concentration. Before use particle size, shape and distribution size investigated by spectrophotometer (S2100UV, unico-USA) and scanning tunneling microscope (Nanoscope IIIA, Digital Instruments, Santa Barbara, CA, USA). For spectrophotometer analyze, distilled water selected as blank sample and quartz cell used. For STM analyze one drop of diluted AgNPs distilled at the HOPG (Highly Ordered Pyrolytic Graphite) and allowed to become dry. By dilution the original stock with deionized water dosages 10, 50, 100 and 200ppm were prepared and used.

In vitro Anti Fungal Tests: Cut branches of Celestial plants that showed symptom of powdery mildew selected and tested at two different conditions. 1) Cut branches put in to the vials containing 45ml of sterilized water and sprayed with mentioned dosages. 2) Cut branches put in glass vials containing 45ml of mentioned dosages and kept at Lab. for investigating the effect of components that may extract from cut branch against nano particles. After 24h. one ml of each vial analyzed by spectrophotometer and compared with original stock curve.

In vivo Anti Fungal Test: Infected plants garden at university campus (Ferdowsi university of Mashhad) selected for *in vivo* test. Five infected bushes were sprayed with mentioned dosages. For control just used sterilized water instead of silver nano particles. Plastic cover tops during the tests were used to protect plants from any possible extra material such as rain. Reducing in disease symptoms was daily observed visually up to one month.

RESULTS AND DISCUSSION

Spectrophotometer Analyzes: Shape of curve at spectrophotometer analyze is congruous with the standard curve of silver nano particles. On the other hand, the strong surface plasmon resonance centered at ca. 419 nm. (Fig. 1), width of curve shown that AgNPs have ordinary size distribution, but this width after 24 h become expanded for AgNPs that were affected by possible components extracted from cut branches in the vials. This phenomenon shown that some of particles become agglomerated and larger. However curve shifted to longer wavelength (Fig. 1).

Stm Analyzes: This picture shows individual silver particles as well as a number of aggregates. The morphology of the nanoparticles is variable, with spherical and occasionally elliptical nanoparticles observed in the micrograph. Some agglomerated particles are visible at surface of HOPG (Fig. 2).

Laboratory Test Results: After three weeks any positive effect was not observed for reducing or controlling the disease on the cut branches (Fig. 3). This result was common for both conditions. Two reasons for lack of effectiveness at the second tested.

The second tested condition may be related to particle agglomeration and or lack of ability of particles movement from vial to leaves for approaching the fungus mycelium through the stem.

In vivo Tests Results: After AgNPs spray, daily visual probe carried out for one month but we did not observe any disease reduction. Also any phyto toxicity or abnormality did not observe at any of dosages.

The antifungal effect of silver NPs has received only marginal attention and just a few studies on this topic have been published [12-14]. Silver nanoparticles of size 3 nm at 2 ppm prevented the mycelium growth for *Candida albicans* [8]. When silver nano particle 1-5 nm at 10 ppm was sprayed on the leaf of the Rose plant the white rose powdery mildew fades out above 95% after 2 days and was not recurred for a week [8]. Silica-silver nanoparticles 1-5 nm when sprayed to infected leaf, could control pumpkin powdery mildew at 0.3 ppm in both field and greenhouse tests. These particles under Lab. Conditions at 3 ppm in PDA had a higher inhibitory effect on the growth of *Botrytis cinerea* than those of test groups including 20 nm sized silver and 100 nm sized silver, further more it could prevent spore germination of this fungus to [15]. When compared the result of this

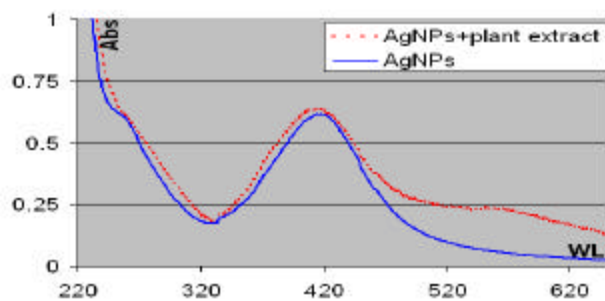


Fig. 1: Comparison between the curve of AgNPs at start of anti fungal test (line) and 24 h after host plant extract diffused in to vial containing the AgNPs(dash-line)

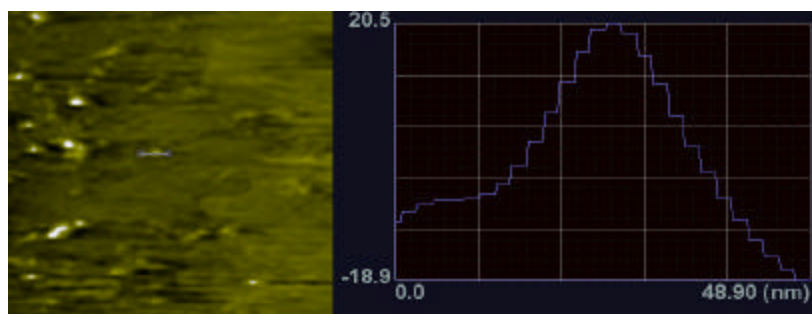


Fig. 2: STM micrograph shows the size and shape of selected AgNp. Some agglomerated particles are visible at the image (white colors). Sample bias=0.7v, Tunneling current = 0.1 nA, 430nm*325 nm. Taken by NAMA-ss1 (Natsyco Com,Iran)

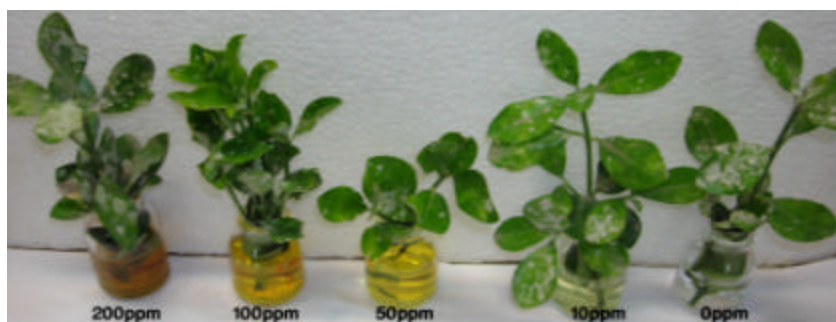


Fig. 3: Infected cut branches put in to the vials containing different dosages of AgNps. For investigate the AgNps ability for movement through the stem and approach the fungus on the leaf.



Fig. 4: Plants with symptom of powdery mildew that were sprayed by different dosages of AgNps in field study

research to mentioned study that have done about antifungal properties of AgNps we find out that it varies to the variety of species or genus of fungi. Our hypothesis is that they relate to AgNps variety itself. There are many chemical and physical methods for AgNps synthesis. Usually different companies use different methods and material for their products. However, different brands of AgNps have variety in particle size, shape, distribution, stability, pH, etc. When this factors combining the experimental condition such as temperature, light, component media, extraction of Micro or macro organism, variety of micro organism (taxon below species such as race, cultivar, form special, strain, Etc) diversity at tests and interpretation of their results, etc..., comparison and decision will become more complicated. Indeed, attention to these parameters may not be reasonable in comparing the results of different anti microbial or toxicity tests [1]. Therefore, it is necessary to use standard protocol on the basis of which both biology and nanotechnology parameters are considered.

CONCLUSION

In this study we concluded that AgNPs did not show any visual effect for reducing of powdery mildew (*Erysiphe sp*), while reported 10 ppm of these particle can reduce rose powdery mildew about 95% [8] and also, composite of silver- silica could control pumpkin powdery mildew at 0.3 ppm in field tests [15]. We can report that at least this brand of AgNps could not control this disease even at 200ppm of AgNps. Our hypothesis for reason of this variety may be due to nano particles property. We believe that determining a standard protocol for anti microbial and toxicity test of AgNps is vital. Finally, for using this particle at agricultural usage, vivo test is essential though environmental parameters may limit or complicate the tests and results.

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