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Evaluation of biosynthesized silver nanoparticles against fungal pathogens of mulberry *Morus indica*

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ABSTRACT

Biologically synthesized silver nanoparticles were subjected for *in vitro* studies against fungal pathogens *Cerotelium fici, Cercospora moricola* and *Phyllactinia corylea* of mulberry. Biologically synthesized silver nanoparticles showed promising antifungal activity against assayed fungus.

Key words: Nanotechnology, silver nanoparticles, fungal diseases, mulberry.

INTRODUCTION

Sericulture has been rightly adjudged as one of the tools for rural development by the planners (Datta, 1994). Introduction of new technology of sericulture has made the industry a highly remunerative crop as reflected in increasing acreage being brought under mulberry cultivation of the step-up in the raw silk output being witnessed every year (Anonymous, 2000; Datta, 2000; Arundhati Choudhury *et al.,* 2004).

Mulberry silkworm, *Bombyx mori* L. sustains its nutrition from mulberry, *Morus indica*. Quality of mulberry leaves plays an important role in the success of sericulture industry and this directs its economics. Foliar diseases of mulberry plays a critical role in silkworm rearing. Leaf rust, leaf spot, powdery mildew caused by *Cerotelium fici, Cercospora moricola* and *Phyllactinia corylea* are some of the major pathogens of fungal diseases of mulberry. They reduce the leaf yield to the extent of 10-15% in terms of premature defoliation and 20-25% by destruction of leaf lamina. Nutritional values of leaf also get depleted by reducing its total proteins, total sugars, chlorophyll and moisture contents. Therefore, disease management in mulberry is one of the prerequisites for successful silkworm rearing. In the present investigation an attempt was made on the nanotechnology application for the management of fungal borne diseases of mulberry. Therefore, the goals of this study were in investigate the influence of biologically synthesized silver nanoparticles on the control of fungal pathogens of mulberry, *Morus indica*.

MATERIAL AND METHODS

Investigation made on the effect of biologically synthesized silver nanoparticles role on the control of fungal species *Cerotelium fici, Cercospora moricola* and *Phyllactinia corylea* of mulberry *M.indica*. Field study was conducted at Karasamangalam, which is 15km away from Thiruvalluvar University campus. Fungal infected part of the mulberry field was identified for the study. The biologically synthesized silver nanoparticles was sprayed on the mulberry garden @ 5lt/ha. The treatment with a water spray was kept as control.

Synthesis of silver nanoparticles was mediated using *Spirulina platensis* as described by Singaravelu and Ganesh Kumar, (2006). Briefly one gram of *S.plantensis* was dissolved in 50ml of 10^{-3}M concentration of aqueous $AgNO_3$ solution. After completion of reaction the nanoparticles solution was characterized using UV-vis spectrophotometer and Transmission electron microscope (Figure 1).

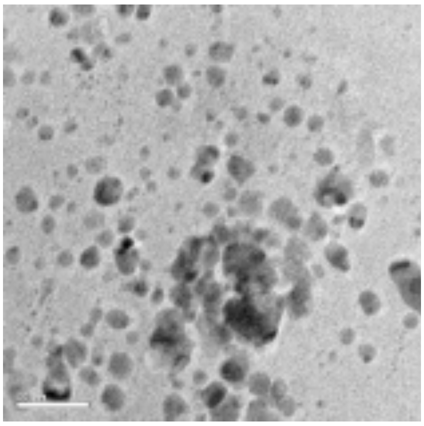

Figure.1 Transmission electron microscopic Photograph showing the *Spirulina platensis* mediated silver nanoparticles.

One gram of the diseased leaves were weighed in a sterile container and homogenized in a pre-sterilized homogeniser. The homogenized samples were streaked separately on mycological agar plates and all the inoculated plates were incubated at room temperature (28^0 C± 2^0C) for 2 to 4 days. After incubation period, the fungal colonies were picked up from the agar plate and stored for

Table 1. Effect of biologically synthesized silver nanoparticles on the control of fungal pathogens of mulberry *Morus indica*.

Silver nanoparticles	Fungal pathogens	Zone of inhibition (mm)
20µl	*Cerotelium fici*	18.12
	Cercospora moricola	17.23
	Phyllactinia corylea	21.15
50µl	*Cerotelium fici*	23.00
	Cercospora moricola	25.00
	Phyllactinia corylea	27.23
100µl	*Cerotelium fici*	25.12
	Cercospora moricola	27.00
	Phyllactinia corylea	28.00

identification. The spores slant culture was mixed up with one or two drops of lacto phenol cotton blue which was kept over the clean glass slide. A cover slip was put over the culture and observed under the microscope to identify the fungal organism. Fungicide activity of biosynthesized silver nanoparticles was studied at different concentrations. Fungal species collected from the infected mulberry leaves were subjected for microbial culture. Well Diffusion assay was employed to assess the inhibitory effect of biosynthesized silver nanoparticles at a 20µl, 50µl and 100µl concentrations.

Results

The results on the *in vitro* studies on the efficacy of biosynthesized silver nanoparticles against *Cerotelium fici, Cercospora moricola* and *Phyllactinia corylea* are presented in Table I. The diameter of inhibition zones around the subjected three fungal strains clearly indicates the effect of biosynthesized silver nanoparticles. The antifungal activity of biosynthesized silver nanoparticles seems to be same among the three different species, however the inhibitory effect was high in the concentration of 100µl. Table 2 depicts the results of field evaluation of biosynthesized silver nanoparticles on the control of fungal pathogens of mulberry *M.indica* . The subjected fungal infected part of the mulberry garden get free of fungal infection in 10 days after the application of biosynthesized silver nanoparticles. It maintains till 30 days.

Discussion

The use of chemical pesticides may achieve a measure of control of those mulberry diseases but there remains the problem of residual toxicity in the treated plants. The toxicity of nematocide furadan results in reduced palatability of the leaves to the feeding silkworm larvae, reduction in growth of the larvae and also in silk production (Paul *et al.,* 1995). The problems reported due to the application of pesticide includes reduced palatability, reduction in growth, oviposition behaviour and economical parameters of silkworm *Bombyx mori* L. Foliar diseases reduce the leaf yield and quality, thus affecting the silkworm rearing. Leaf yield will be reduced by the premature defoliation due to diseases and by way of reducing the total consumable area in those leaves, which do not defoliate, but are diseased. Similarly, diseases also reduce the protein and moisture content in the leaves. The commercial characters were greatly reduced when the larvae were fed by the leaf affected with rust, leaf spot and powdery mildew.

Nanoscience is a relatively new branch of science dedicated to the improvement and utilization of devices and structures ranging from 1 to 100nm in size, in which new chemical, physical and biological properties, not seen in bulk materials can be observed (Roco, 1998). Nanomaterials have received considerable attention because of their potential for application in a wide spectrum of areas that include biology and medicine (Albers *et al*., 2001; Park *et al*., 2002). Much of this interest stems from the fact that nanomaterials possess interesting optoelectronic properties and is also due to their size compatibility with a variety of biologically active molecules. Nanoparticles could also serve as excellent delivery vehicles for a variety of biomolecules such as proteins, DNA and drugs (Hrushikesh *et al*., 2006). Reducing the particle size of materials is an efficient and reliable tool for improving their biocompatibility. In fact, nanotechnology helps in overcoming the limitations of size and can change the outlook of the world regarding science (Mirkin and Taton, 2000). Furthermore, nanomaterials can be modified for better efficiency to facilitate their applications in different fields such as bioscience and medicine.

The usefulness of silver as an antimicrobial agent has been known for a long time. It is an effective agent with low toxicity, which is especially important in the topical antibacterial treatment of burn wounds, where transient bacteremia is commonly cited (Mozingo *et al.,*1997). Combination of different antibiotics with silver nanoparticles exhibit synergistic antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (Shahverdi *et al*.,2007). The mechanism of inhibitory action of silver ions on microorganisms is partially known. It is believed that DNA loses its replication ability and cellular proteins become inactivated on Ag^+ treatment (Feng *et al*.,2000). In addition it was also shown that Ag^+

Table 2: Impact of fungal pathogens on the fungal disease burden under field condition.

Fungal Pathogens	Duration of observation on the fungal disease burden					
	10th day		20th day		30th day	
	Control	Experimental	Control	Experimental	Control	Experimental
Cerotelium fici	+++	-	+++	-	+++	-
Cercospora moricola	+++	-	+++	-	+++	-
Phyllactinia corylea	+++	-	+++	-	+++	-

+++ - Heavy incidence   - Absence of pathogen

binds to functional groups of proteins, resulting in protein denaturation (Spadaro *et al*.,1974). The obvious question is how nanosized silver particles act as biocidal material against *E.coli*. There are reports in the literature that show that electrostatic attraction between negatively charged bacterial cells and positively charged nanoparticles is crucial for the activity of nanoparticles as bactericidal materials (Stoimenove *et al*.,2002; Hamouda and Baker,2000).

With the emergence and increase of microbial organisms resistant to multiple antibiotics, and the continuing emphasis on health-care costs, many researchers have tried to develop new, effective antimicrobial reagents free of resistance and cost. Such problems and needs have led to the resurgence in the use of silver (Ag)-based antiseptics that may be linked to broad-spectrum activity and far lower propensity to induce microbial resistance than antibiotics (Jones *et al*.,2004)

The antibacterial effects of Ag salts have been noticed since antiquity (Silver and Phug, 1996) and is currently used to control bacterial growth in a variety of applications, including dental work, catheters, and burn wounds (Catauro *et al*.,2004 and Crabtree *et al*.,2003). In fact, it is well known that Ag ions and Ag-based compounds are highly toxic to microorganisms, showing strong biocidal effects on as many as 12 species of bacteria including *E. coli* (Zhao and Stevens,1998). Aymonier *et al*.,(2002) showed that hybrids of Ag nanoparticles with amphiphilic hyperbranched macromolecules exhibited effective antimicrobial surface coating agents. Results of the present investigation clearly indicate the antifungal activity of biologically synthesized silver nanoparticles. The study also confirms the non-toxic nature of biologically synthesized silver nanoparticles. Since the normal growth of the silkworm, which fed with biosynthesized silver nanoparticles treated leaves not affected by any way.

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