Biocompatible Synthesis of Silver Nano Particles from *Plumbago zeylanica* and Its Antifungal Capability Against *Alternaria alternata* Causing leaf spot Rose.

M.B. Waghmare

Department of Botany, The New College, Kolhapur, Maharashtra 416012, India.

ABSTRACT

In Present days nano technolology is one of the sound research field of material science and technology. Green synthesis of silver nanoparticle has great potential against various plnat pathogens. In the plant disease management , as compared to the physical and chemical methods it is eco friendly and cost effective. An attempt was made to synthesis of biocompatible silver nanoparticles from the *Plumbago zeylanica* leaf extract. The synthesized nanoparticles were characterized using ultraviolet-visible spectroscopy (UV-Vis) and Scanning electron microscopy (SEM).Spectroscopy confirmed the formation of nanoparticles. The synthesized nanoparticles from selected plant were spherical in shape and 55nm in size. The antifungal activity was evaluated against the *Alternaria alternata* causing flower blight of rose by food poisoning technique. Green synthesized nanoparticles shown significant effect against the pathogen. All tested concentrations of AgNPs shown very good antagonistic effect against the *Botrytis cinerea.*

Keywords: *Alternaria alternata*, *Plumbago zeylanica*, Rose, Silver nanoparticles.

INTRODUCTION

Plumbago zeylanica is a perennial and herbaceous medicinal plant belonging to the family Plumbaginaceae. It is widely found in West Bengal, Uttar Pradesh and Maharashtra. In Maharashtra it is commonly called as is chitraka (Yuwraj et al., 2011). Some phytochemicals like alkaloids, flavonoids, glycoside, saponins, steroids, triterpenoids, coumarins, phenolic compounds, tannins, carbohydrates are present in this plant. Kumar et al., 2009 reported the anti-bacterial, anti-plasmodial, anti-tumour, anti-fungal, anti-inflammatory, anti-hyperglycemic, anti-cancer and anti-atherosclerotic activity.

Rose is one of the most important ornamental and medicinal plant belongs to the family rosaceae. In use the cut flowers of rose are in first rank. It increased the economic status of our country. Such economically important plant suffered from number of fungal diseases. Among the fungal diseases, leaf spot caused by *Alternaria alternata* is very harmful to the plant. For getting the high yield there is a need of management of this disease by various methods.

In order to manage the plant diseases cultivars have been used different fungicides, due to indiscriminate use of agrochemical increased the resistance in the pathogens (Arora et al., 1993; waghmare et al., 2012). Moreover , such agrochemicals can be lethal to the beneficial soil micro organisms they may enters and acuminate in the human body as undesirable chemical residues (Bartlett et al.,2002) .

Nanotechnology is a multidisciplinary field now a days it has been very efficiently using in the field of engineering, physics, chemistry and biology (M. Wilson et al., 2002). Green synthesized AgNPs displaying antibacterial activity are known to have various advantageous in medical applications in medicine and biological fields

We are very much familiar that Nano particles exhibit new properties due to its specific characteristic. Generally, nano materials synthesized by using physical and chemical methods but the by-products from these methods are toxic in nature and the process is also costly. Chemically synthesized silver nano particles also showed poor antibacterial activity compared to biologically synthesized nano particles. This could be due to the protein coating of NPs obtained from the biological extract (Salunkhe et al., 2014).

To overcome this problem there is an urgent need for eco friendly methods of synthesis of nano particles and green synthesis of nanopartcles is the best alternative for other physical and chemical method (Logeswari et al., 2013).

MATERIAL AND METHODS

Plant material and Preparation of Leaf extracts for the synthesis of Nano particles

Fresh and diseased free leaves of *Plumbago zeylanica* were collected from the western Ghats and brought to the New College Botany research laboratory in the sterilized condition . Washed thouraly with distilled water. Fresh leaves were cut into small pieces, 20 g of fresh leaves was finely chopped transferred into 500ml beaker containing 100 ml of distilled water. Mixed and stirred well and boiled at 60°C for 1 h. allowed the mixture to cool later on filtered the mixture through Whatman 1 filter paper. Filtrate was collected in clean sterilized Erlenmeyer flask and stored in BOD incubator for the future study this is followed by (Narayanswami et al., 2015).

Synthesis of silver nano particles Nanoparticles from the selected plant

Prepared 1 mM concentration of silver nitrate by adding 16.987g of silver nitrate in 1000 ml of distilled water and used for further study.

5 ml leaf extract of selected plant was added into 95 ml of aqueous solution of 1 mM silver nitrate. Reduction of silver nitrate to silver ions was confirmed by change in colour, the appearance of colourless to brown colour confirmed the formation of silver nano particles .

The AgNPs formed from selected plant (*Plumbago zeylanica* -AgNPs) were harvested by centrifugation at 5000 rpm for 25 minutes at 25°C. After centrifugation the upper supernatant liquid was removed and obtained the pellets again transferred into deionized water. Repeat three to four times centrifugation process because to washout other unwanted substances from the surface of the nano particles. Dried the synthesized nano particles in an oven at 60°C for prospective observations.

Characterization of silver nano particles.

In the nanotechnology the physicochemical study is important to know the properties of the synthesized nanopaticles , there are various techniques are present to determine the properties of the nano particles.

UV- Visible Spectroscopy analysis

The reduction of the Ag^{+} ions by the supernant of the *Plumbago zeylanica* leaf extracts in the solution and formation of silver nano particles were characterized by UV- visible spectroscopy.

Silver ion bio reduction was monitored by sampling of aliquots (1mL). To determine the maximum production of the silver nano particles the absorption spectra of the sample was taken at 300 to 700 nm using UV – Vis spectrophotometer. Distilled water was used to adjust the baseline.

Scanning electron microscope (SEM) analysis:

SEM is a surface imaging technique, for SEM a thin films of sample was prepared on a carbon coated copper grid by dropping very small amount of sample on the grid, film on the SEM grid was allowed to dry by putting it under a mercury lamp for 5 minutes. Obtained the detail morphology of synthesized nano particles through the SEM.

Isolation of the pathogen

Naturally infected leaf spot of rose (Rosa floribunda) were surveyed and collected in the sterilized glass bottles and brought to the laboratory. In the Laboratory washed the collected sample with sterile distilled water and made the small pieces with sterile blade, Sterilized the small cut pieces with sodium hypo chloride solution of 5% concentration for 5 minutes. Again 2 times washed the infected samples with sterile distilled water and removed the water traces from the plant material with the help of blotting paper. Later on transferred the infected tissues on the sterile solidified CDA plates and kept on incubation at 25-30 ^{0}C in the BOD incubator. The fungal mycelial growth was observed after 8 days of the incubation. Identified the pathogen as *Alternaria alternata* followed by (Barnett and Hunter, 1972; Subramanian,]1971). Obtained the pure culture and maintain in the BOD incubator at $5^{0}C$ for further study. The pathogen was confirmed by the Koch postulates.

In Vitro assay

It was performed on CDA (Czapek Dox Agar) growth medium. In vitro assay was followed by Sang et al (2012). Took 5ml of different concentrations (i.e., 10, 25, 50, and 100 ppm) of silver nano particles, mixed with 15ml of sterilized growth medium and poured into pre sterilized Petri dishes of 90mm in diameter. Allowed the Media for the solidify. After 48 hours 8 mm actively growing 7 days old culture of *Alternaria alternata* was placed on the centre of the solidified culture media contained different concentrations nano particles and allow growing the pathogen. After 8 days of the inoculation radial growth of the mycelium was measured. The plate without nano particles considered as control.

Data analysis

After incubation of fungi on different culture medium containing silver nanoparticles, radial growth of fungal mycelium was recorded. Radial inhibition was calculated when growth of mycelia in the control plate reached the edge of the Petri dish. The following formula was used for calculation of the inhibition rate (%).

The percent inhibition of the mycelium growth over the control was calculated by (Vincent, 1947)

$$PI = \frac{C-T}{C} \times 100$$

Where PI= Percent Inhibition
C = Control (Radial growth of the Fungus in control plate)
T- Treated (Radial growth of fungal mycelia on the plate treated with AgNPs.).

RESULT AND DISCUSSION

In the biosynthesis of nanoparticles, a colour change in the reaction mixture is the first step that indicates that nanoparticles have been synthesized (Moideen and Prabha 2014).

In this study the after the formation of the silver nano particles the colourless mixture of plant extract and silver nitrate solution turns brown in colour due the reduction of silver ions. Silver nanoparticles exhibits yellowish brown colour in aqueous solution due to the phenomenon of Surface Plasmon Resonance (Shivshanker 2004). As per the UV- VS spectroscopy the nanoparticals synthesized from the *Plumbago zeylanica* shown the maximum absorbance at 445nm. The Scanning Electron Microscopy shown the synthesized silver nano particles were of relatively spherical in shape.

In the present study author investigated the effect of silver nanoparticles from *Plumbago zeylanica* L on the growth of *Alternaria alternata* causing leaf spot of rose. In the result it is found that all tested different concentrations of AgNPs (25,50,75 and 100 ppm solutions) shown the significant potential against the *Alternaria alternata* causing leaf spot of rose. 100ppm concentrated solution of AgNPs of *plumbago zeylanica* shown 88.88 percent inhibition rate (Table 1) while 25 pmm concentrated solution shown 80.00 percent inhibition. Similarly Sang et al., (2012) evaluated the antifungal efficacy of silver nanoparticles

against various plant pathogens pathogens. Marzy et al., 2014 studied the antifungal effect of magnesium oxide nanoparticles on *Fusraium oxysporum* F.Sp. *lycopersici*. Role of silver nanoparticles against several phyto-pathogenic fungi like *Alternaria alternata, Macrophomina phaseolina, Sclerotinia sclerotiorum, Botrytis cinerea, Rhizoctonia solani*, and *Curvularia lunata* (studied by (Krishnaraj et al., 2012; Monteiro , 2012).

CONCLUSION

Flourishing awareness towards green chemistry and implementation of plant extracts for metal nanoparticles synthesis lead to the development of environment-friendly techniques. A benefit of silver nanoparticles synthesis by utilizing the plant extracts is - economical, energy efficient , cost-effective, protects human health and environment. Green synthesis of silver nanoparticles from plant extarcts play a blooming role in the area of nanotechnology. Therefore utilization of plant extract for the synthesis of silver nanoparticles has gaining an significant potential in the field of plant disease management.

Tbale 1. Effect of silver nanoparticals synthesized from the *Plumbago zeylanica L.* against the *Alternaria alternata* causing leaf spot of rose.

Sr. No.	concentrations of silver nano particles	Percent of Inhibition
1	25ppm	80.00
2	50ppm	84.44
3	75ppm	86.66
4	100 ppm	88.88
5	control	00.00
		S.Em = 67.99±3.09

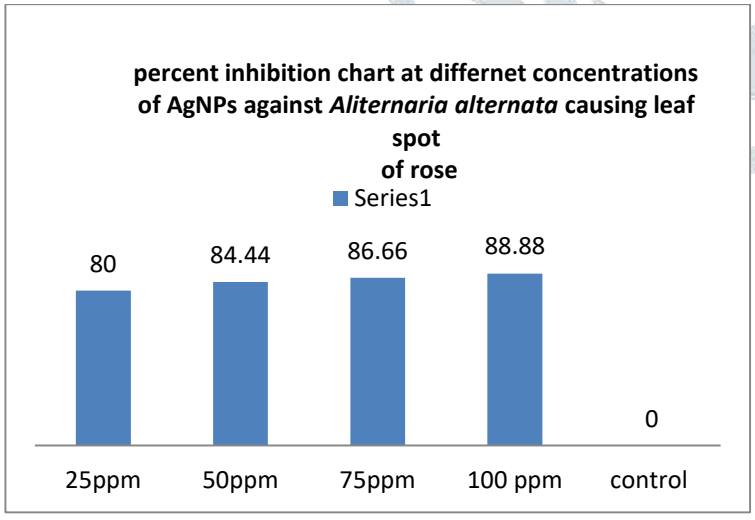
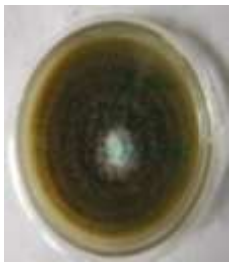


Fig 1. Green synthesized silver nano particles from leaf extracts of *Plumbago zeylanica* and its effect on the growth of *Alternaria alternata* causing leaf spot of rose.

25 ppm Ag NPs

100 ppm Ag NPs



Control

Spores of Alternaria

Leaf spots on Rose plant

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