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Integrated Management of Linseed Blight by Green Silver Nanoparticles Synthesized from Fruit and Leaves Extract of *Morinda citrifolia* L.

Shital B. Koparde *, D.K. Gaikwad

Plant Physiology Laboratory, Department of Botany, Shivaji University, Kolhapur. 416004, (M.S.) India

Address for Correspondence: Shital B. Koparde, shitalkoparde@gmail.com

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ABSTRACT: In the present work, an attempt has been made to synthesize green silver nanoparticles by using medicinal plant *Morinda citrifolia*. Biologically synthesized nanoparticles have been used widely in the various fields. *Morinda* fruit and leaves extract was used to reduce silver nitrate to synthesized silver nanoparticles. The peak showed at 425nm for fruits and 425 to 450nm for leaves sample by Uv-vis spectrophotometer and FESEM analysis reveales the spherical shaped nanoparticles with size ranging between 25-60 nm and 40-60nm respectively. These synthesized nanoparticles showed inhibitory activity against *Alternaria lini* a linseed blight pathogen. The 3% carbendazim showed inhibition of growth of fungi *A.lini*, Whereas Carbendazim combined with silver nanoparticles significantly inhibit the growth of *A.lini.* This will help to reduce the percentage of carbendazim and also help to minimize the fungicide residues. Thus these nanoparticles synthesized from *M. citrifolia* fruits and leaves might be used in agricultural industries for the development of efficient fungicide. This will helps to reduce the amount of fungicide and residual biomagnifications in food chain.

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INTRODUCTION

Synthesis of metal nanoparticles through biological method is an important aspect of current nanotechnology. The process of biological synthesis of nanoparticles provides a vast range of environmentally acceptable methodology, low cost production and minimum time requirement. Silver nanoparticles are reported to influence antibacterial [4], antifungal [6], antiviral [12], and anti- inflammatory activity [9]. The use of plant leaves and fruit extract for the synthesis of silver nanoparticles was reported by several researchers such as *Solanum xanthocarpum* [1], *Elettaria cardamomom* [3], *Coleus forskohlii* [5], *Nitraria schoberia* [11], *Nelumbo nucifera* [14]. *Morinda citrifolia* is important medicinal plant belongs to family Rubiaceae. This plant have various pharmaceutical uses. *Linum usitatissimum* L. commonly known as flax or linseed belongs to family Linaceae. Linseed plant is the economically important oil yielding plant. At the time of cultivation of crop plants farmers facing various problems like disease management. Linseed Blight caused by *Alternaria lini* Dey. is one of the major limiting factor for linseed cultivation. Thus attempt has been made to biological synthesis of silver nanoparticles from *M. citrifolia* and assess its antifungal potential against linseed blight.

MATERIALS & METHODS

Preparation of Fruit and Leaves extract

Fruits and leaves of the *M. citrifolia* were collected from the botanical garden, Department of Botany, Shivaji University Kolhapur. The material washed with tap water and then distilled water. 10g of fruit and leaf sample was mixed in 100 ml distilled water and kept in boiling water bath for 15

minutes. The extract was cooled and filtered using whatman no. 1 filter paper and used for the further reaction.

Silver nanoparticles synthesis

For the synthesis of silver nanoparticles *M. citrifolia* fruit and leaves extract was reacted with silver nitrate solution. 10 ml extract added to the 90 ml silver nitrate solution. This reaction mixture containing conical flask was kept in dark condition at room temperature.

The bioreduction of silver ions by fruit and leaves extract was scrutinized by measuring the Uv-vis spectra of the reaction medium at 200-800nm. Complete bioreduced sample was centrifuged at 10000 rpm for 15 minutes, redispersed the pellet in double distilled water and again centrifuged. This process was repeated for 2-3 times for obtaining silver nanoparticles free from any unwanted residue [8 and 13].

FESEM analysis

The morphology and size of the synthesized silver nanoparticles was studied by using FESEM images. Used 20 kv voltage for imaging nanoparticles in FESEM. [Model-MIRA 3 LMH].

Antifungal activity

Infected plant material of linseed collected from field. These material was poured on CDA medium for obtaining culture of *A. lini*. This culture was used for studying inhibitory activity of silver nanoparticles. Well diffusion method was used for the study of antifungal activity. The agar plates were inoculated by spreading the spores of the *Alternaria lini* over the entire agar surface. Then 6 mm well was punched and the different concentrations (10µl, 20 µl, 30 µl) of the nanoparticles were loaded in each plate. After incubation, zone of inhibition was recorded.[7and 10].

RESULTS AND DISCUSSION

The colour change in 1 mM AgNO3 solution from colourless to blakish brown (Fig- 1) was noticed after reaction with fruit extract and reddish brown (Fig-2) noticed after reaction with leaves extract of *M. citrifolia*, which indicates the nanoparticles synthesized in the reaction mixtures. The characteristics surface plasmone absorption peak was observed at 425nm (Fig-3) and 425-450nm (Fig-4) by Uv- vis spectrophotometer. Field Emission scanning Electron Microscope analysis revealed that the synthesized nanoparticles were spherical in shape and 25-60nm in size (Fig-5) for nanoparticles synthesized from fruit sample and 40-60nm in size for nanoparticles synthesized from leaves sample (Fig-6) which confirm the synthesis of nanoparticles.

Silver nanoparticles synthesized from fruit and leaves extract have potential to inhibit the growth of fungal strain such as *Alternaria lini*. (Table-1) 10µl, 20 µl, 30 µl concentrarions of nanoparticles showed inhibitory activity but 10µl, 20 µl, 30 µl concentrarions of carbendazim was ineffective. The isolate of *A. lini* was sensitive to 3% carbendazim, Whereas Carbendazim combined with silver nanoparticles significantly inhibit the growth of *A.lini.* This will help to reduce the percentage of carbendazim and also help to minimize the fungicide residues. In India the productivity of linseed crop is very lowest because of various diseases. The disease caused by *Alternaria lini* reduced the yield of plant and its creats annual loss of 28-60% in the yield [2]. Thus the nanoparticles synthesized from fruit and leaves extract are useful in management of the pathogen and maintaining the crop yield of linseed.

M. citrifolia has been used in medicine, this work confirmed the medicinal values of this plant and also evaluate simple, ecofriendly route for nanoarticles synthesis and their ability of the antifungal efficacy. Synthesized AgNPs increases the therapeutic potential and build up the medicinal values of *M. citrifolia.* Silver nanoparticles synthesized from plant extract are toxic to multidrug resistant micro-organisms including plant pathogens.

Table. 1: Antifungal activity

Test sample	Zone of inhibition in millimeter		
	Concentration of AgNPs (µl)		
	10(µl)	20(µl)	30(µl)
Fruit SNPs	9	10.66	14
Leaves SNPs	9	10	12
Carbendazim	-	-	-
Carbendazim+ Fruit SNPs	11		
Carbendazim+ leaves SNPs	10.66		

Figure 1(1) AgNO3 solution (2)Fruit extract (3) Synthesized AgNPs

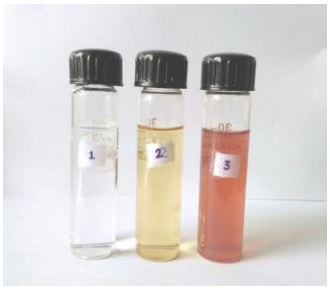

Figure 2(1) AgNO3 solution (2) Leaves extract (3) Synthesized AgNPs

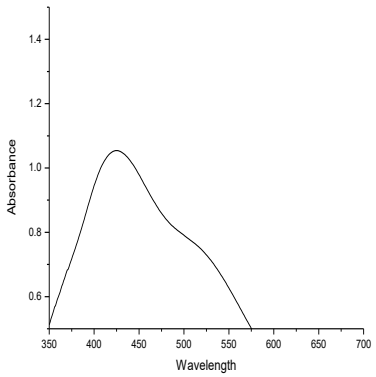

Figure 3 UV- Vis absorption spectra fruit sample

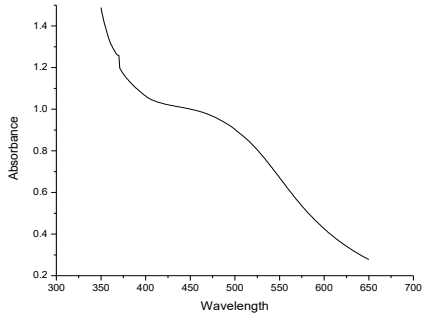

Figure 4 UV- Vis absorption spectra leaf sample

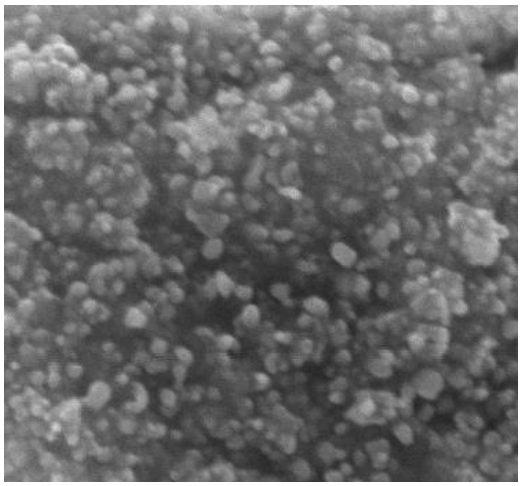

Figure 5 FESEM micrograph of Fruit silver nanoparticles.

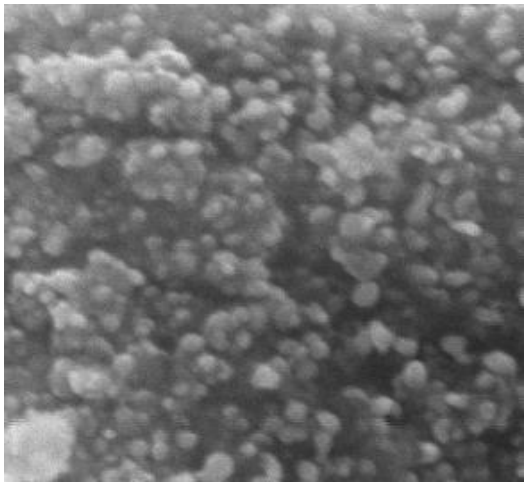

Figure 6 FESEM micrograph of Leaf silver nanoparticles.

CONCLUSION

The present study demonstrated the synthesis of silver nanoparticles from fruit and leaves extract of *M. citrifolia* and resulted in the spherical shaped nanoparticles. These nanoparticles have been potential to inhibit the growth of fungal pathogens. This property of silver nanoparticles synthesized from *M. citrifolia* which is helpful in managing linseed blight caused by *Alternaria lini.* Thus the silver nanoparticles synthesized from *M. citrifolia* are useful in managing the pathogen and maintaining the crop yield. This will reduces the residues of fungicides and also useful in the development of chemical free organic farming.

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