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CHAPTER 7

Biosynthesized AgNPs of Endophytic Fungus Arthrinium Gutiae on Plant Growth

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Abstract

In present research *Arthrinium gutiae* endophytic fungi isolated from host plant *Maytenus emarginata* (Willd.) and screened for biosynthesis of extracellular silver nanoparticles (AgNP). The AgNPs of different concentration were used for plant growth promotion in terms of Root, shoot elongation and Seed germination activity in seeds of agricultural crop plants. Biosynthesized AgNPs of *Arthrinium gutiae* showed increase as well as decrease in percentage of seed germination, root and shoot elongation at different concentrations of AgNPs 50µl, 100µl and 200µl in seeds of Chickpea (*Cicer ariantum*), mungbean (*Vigna radiata* (mung), Jowar (*Sorghum bicolor*), pigeon pea (*Cajans cajan*), soybean (*Glycine max*), Safflower (*Carthamus tinctorius*), and sunflower (*Helianthus annus*) as compared to control. Percentage of plant growth promotion varying according to different concentrations of fungal biosynthesized AgNPs. Result showed at different concentrations of AgNP seed germination, root and shoot elongation increased as well as decreased in plants compared to control.

Keywords: Endophyte, *Arthrinium gutiae*, AgNP, seed germination, Root and shoot.

Introduction

A variety of chemical and physical methods to synthesize nanoparticles are in practice but their inherent flaws that include contamination from use of toxic solvents, precursor chemicals and release of hazardous by-products (Sunkar and Nachiyar, 2012). The present research study emphasized on the mycobiosynthesis of silver nanoparticles using on faction of microbes, the endophytic fungus as a green alternative to the chemical method.

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Plant endophytic fungi are novel and important for production of natural bioactive compounds with their potential use in agriculture, industry and medicine. The different important bioactive compounds from entophytic fungi isolated which shows plant growth promoting, antifungal, antibacterial, insecticidal, cytotoxic and anticancer activities (Gangadevi & Muthumary, 2008). (Yiing and Adeline, 2014). (Jingfeng *et al.,* 2013, Tanmayee *et al.,* 2015).

Presently, efforts have been showed that Silver nanoparticles (Ag-NPs) treated seedlings showed an increase production of phytochemical diosgenin and plant growth. AgNPs of *Aspergillus tamaraii* at different concentration N1 showed maximum 66% germination followed by N2 60% it was significant over control. There was significant increase in shoot and root length of *Vigna radiata* ($p > 0.05$) when compared with the control (Priyom and Uma, 2017). (AgNPs) of *Colletotrichum incarnatum* shows the highest seed germination rate 99.4% observed in *Cucumis sativus* (Chandankere *et al.,* 2020).

The present research focuses on plant growth promoting activity of endophyte fungus *Arthrinium gutiae* AgNPs, isolated from medicinal plant *Maytenus emarginata* (Willd.) Ding Hou.

2. Materials and methods

Plant material collection

Plant parts like leaf and stem of *Maytenus emarginata* (Willd.) were collected in sterile polythene bags and brought to the laboratory used for the isolation of endophytic fungi.

Isolation of Endophytic Fungi

Endophytic fungi were isolated by following methods employed by Hallman *et al.* (2007) and Selvakumar *et al.* (2014).

Molecular Identification Endophytic fungus

The identification of endophytic fungi was done using 18s rRNA ITS universal primers.

Biosynthesis of Silver nanoparticles (AgNPs)

For biosynthesis of extracellular silver nanoparticles (AgNPs) the isolated fungi were grown in the Czapadox broth supplemented with streptomycin at 28^0C with shaking at120 rpm for incubation period of 72 hrs. After incubation period fungal biomass was filtered and harvested using Whatman filter paper number 1 and washed several times with sterile distilled water to remove any medium component. Harvested biomass of fungal endophyte was transferred into 100 ml of sterile distilled water and incubated for 48 hours. The cell-free filtrate was used in experiments. SNP was prepared by adding AgNO3 (1 mM) with cell-free extract. The above flask was then incubated at room temperature under dark conditions and observed for change in color. Control was also prepared, containing only the cell filtrate without silver nitrate solution. The formation of AgNPs was confirmed by visual observation of color change from pale white to reddish brown. This appearance of color change from pale white to brown is a clear indication of biosynthesis of silver nanoparticles by reduction of silver in the filtrate extracellularly. (Prabavathy *et al.,* 2015 and Kiran, 2017).

Characterization of Silver nanoparticles (AgNPs)

Characterization of AgNPs was carried out by UV-Visible Spectroscopy and transmission electron microscopy (TEM). The composition and crystalline nature of AgNPs was confirmed by Fourier transform infrared Spectroscopy (FTIR) and X ray diffraction (XRD) analyses.

Effect of biosynthesized silver nanoparticles on plant growth promotion

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To evaluate effect of biosynthesized AgNPs on seed germination, root and shoot elongation on different agricultural crop seeds of Jowar (*Sorghum bicolor*), Chickpea (*Cicer ariantum*), mungbean (*Vigna radiata* (mung), safflower (*Carthamus tinctorius*), Pigeon pea (*Cajans cajan*), soybean (*Glycine max*) and sunflower (*Helianthus annus*) were collected from local market. Ten seeds of each crop plants were initially self-sterilized by dipping in 5% Sodium hypochlorite solution for 30 min and soaked with fungal biosynthesized AgNPs solution.

A Five milliliter of biosynthesized AgNPs solution of endophytic fungus was transferred into a sterilized Petri-plate containing whatman filter paper No. 1 and the treated seeds were kept on the whatman filter paper. $AgNO_3$ and Distilled water were used as negative and positive control, respectively. Lastly, all the Petri-plates of different seed samples were covered and incubated at room temperature for 5 days. Thereafter, the seed germination percentage, shoot and root elongation were calculated (Chandrakane, 2020; Priyom and Uma, 2017).

$$\text{Percentage (\%) Seed Germination} = \frac{\text{Number of germinated seeds}}{\text{Number of inoculated seeds}} \text{ x } 100$$

3. Results and Discussion

The isolated endophyte *Arthrinium gutiae* was screened for biosynthesis of silver nanoparticles. When silver nitrate ($AgNO_3$), upon incubation with the fungal extracellular filtrates, turned brown in colour, while the control flasks showed unchanged. The brown color exhibited by the nanoparticles indicates the formation of silver nanoparticles in the solution.

Similarly, different researcher isolated endophytic fungi and preliminary screened for silver nanoparticles production by observing change in colour. AgNPs by endophytic fungus *Nemania* sp. isolated from *Taxus baccata* L. (*Iranian* Yew) (Mohammad and Saeed., 2018). Silver nanoparticles biosynthesized by endophytic fungus *Aspergillus sp* isolated from *Justicia beddomei* (Prabavathy D.et al., 2015).

AgNPs of *Arthrinium gutiae* shows UV-Visible Spectroscopy absorption peak at 440 nm and size ranges from 10 nm to 30 nm.

Percentage of seed germination, root and shoot elongation was varying according to different concentration of endophytic fungal biosynthesized AgNPs compared to control.

AgNPs of *Arthrinium gutiae* at 50µl concentration shows maximum percentage of seed germination, 100 % safflower, 80 % chickpea, 80 % pigeon pea, and decreased in sunflower. At 100µl the maximum percentage of seed germination was recorded in 100 % jowar, 100% safflower, 90 % chickpea, 90 % mungbean, 80% pigeon pea, 80 % soybean and decreased in sunflower. At 200µl of AgNPs the maximum percentage of seed germination was recorded 100 % jowar, 90 % mungbean, 80 % chickpea, 80 % soybean, 80 % pigeon pea, and decreased in safflower and sunflower compared to control (Table.1 and fig.1).

At 50µl AgNPs of *Arthrinium gutiae* increased root length in mungbean, pigeon pea, safflower, sunflower while, decreased in chickpea, jowar and soybean as compared to control. At 100µl AgNPs increased root length in mungbean, pigeon pea, safflower, sunflower while, decreased in chickpea, jowar and soybean as compared to control. At 200µl AgNPs increased root length in mungbean, pigeon pea, sunflower, safflower, while decreased in chickpea, jowar and soybean as compared to control (Table.2 and fig.2). At 50µl AgNPs increased shoot length in pigeon pea, sunflower, soybean while decreased in chickpea, mungbean, jowar and safflower as compared to control. At 100µl AgNPs increased shoot length in pigeon pea, soybean, sunflower while, decreased in jowar, mungbean, Chickpea, and safflower as compared to control. At 200µl AgNPs increased shoot length in pigeon pea, sunflower while, decreased in jowar, mungbean, chickpea and safflower as compared to control (Table.2 and fig.2).

Different researchers observed the endophytic fungus *Fusarium semitectum* concentration of AgNPs at 100 µl enhanced in the average root and shoot elongation length maximum up to 4.6 cm and 4.7 cm in Mungbean

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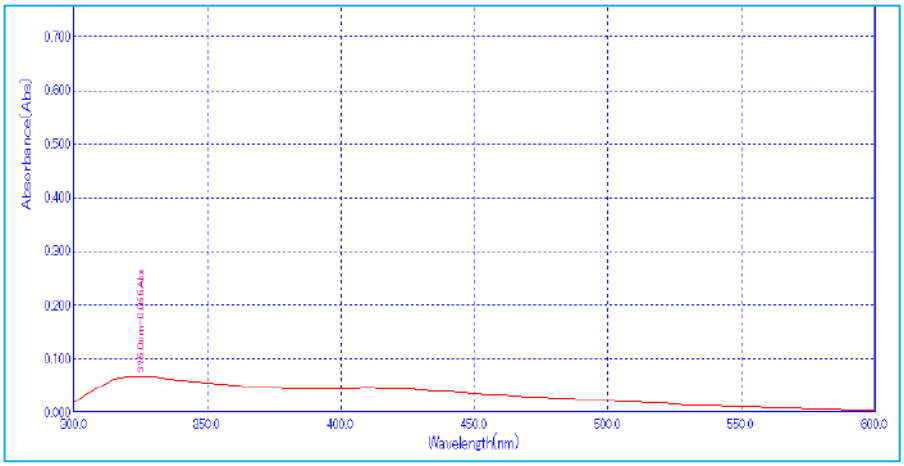
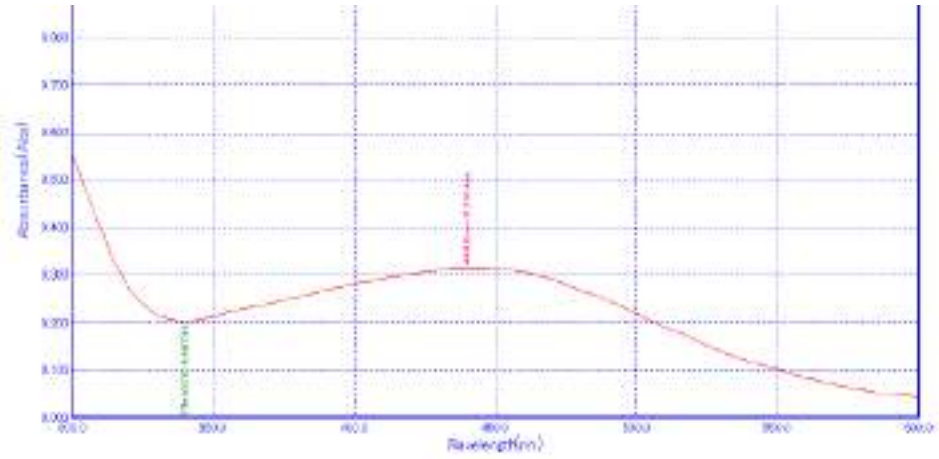
whereas at 200µl, shoot length was 4cm and root length was 4.4 cm respectively. In Chickpea the growth index was maximum 46.51 with 78.5% maximum seed germination and at 100µl of AgNps shoots length of 2cm with maximum root length of 4.9cm (Ashish *et al.*, 2016). Silver Nanoparticles mycosynthesized by an endophytic fungus *Colletotrichum incarnatum* isolated from medicinal plant *Datura metel* shows the maximum 99.4% seed germination and 4.0 cm root elongation observed in *Cucumis sativus*, whereas the lowest 28.5% seed germination and 0.4 cm root elongation was obtained against silver nitrate (Chandankere *et al.*, 2020). *Trichoderma harzianum* and *Aspergillus fumigates* silver nanoparticles (AgNPs) treated seeds showed increase in percentage of seed germination and seedling growth in *Solanum lycopersicum*. More sprouting number found and increase in length in 3 to 12 days of germination, while in control the similar effect was observed on 14-18 days. AgNPs showed significantly increase in growth parameters including fresh biomass, plant height, number of shoots/plants, tomato yield, root weight, and dry biomass (Asma, 2019).

4. Conclusions

Screened endophytic fungus *Arthrinium gutiae* is the good producer of AgNPs. Biosynthesized extracellular AgNPs of *Arthrinium gutiae* enhances seed germination and root shoot elongation. So, it would be used in agriculture as a plant growth enhancer agent in order to increase growth and yield of crop plants. Furthermore, field trial and in vivo study required. The present research finding used as a cheap, simple, and ecofriendly approach of biosynthesis of silver nanoparticles.



Spectra.1. UV-Visible spectrum analysis of silver nanoparticles (Zero hour spectra).



Spectra.2. UV-Visible spectrum analysis of silver nanoparticles synthesized (24 hour spectra).

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Table 1: Seed germination activity of biosynthesized AgNPs produced from *Arthrinium gutiae*

Sr.No	Seed Samples	% Seed germination activity			
		50µl	100µl	200µl	Control
1	Chickpea	80	90	80	70
2	Jowar	90	100	100	90
3	Sunflower	80	90	90	100
4	Soybean	70	80	80	70
5	Safflower	100	100	80	100
6	Pigeon pea	80	80	80	70
7	Mungbean	80	90	90	80

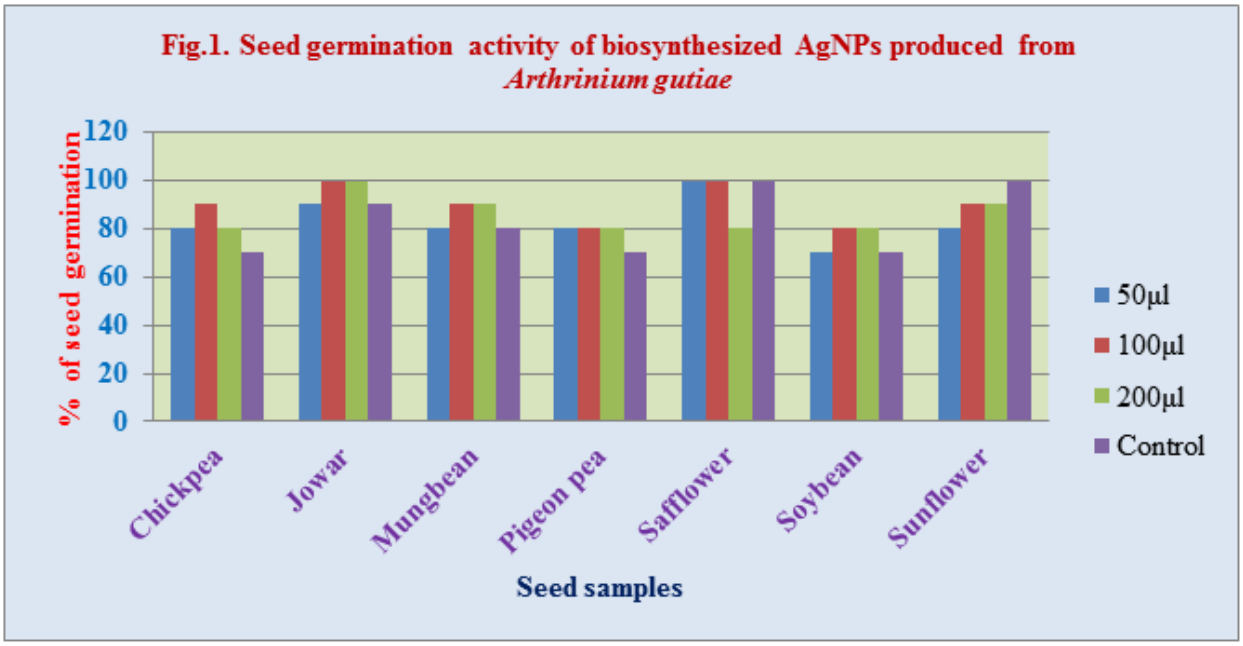

Table 2: Effect of biosynthesized AgNPs produced from *Arthrinium gutiae* on root and shoot length

Seed Samples	Root and shoot length in cm							
	50µl		100µl		200µl		Control	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Jowar	5.1	2	5	2	4.8	1.8	6.3	2.4
Chickpea	4.3	2	4	1.9	3.8	2	4.6	2.2
Pigeon pea	3.9	2.4	4.2	2.7	4.5	2.8	3.8	2.3
Safflower	2.8	1.8	3.4	1.6	4	1.5	2.4	2.1
Mungbean	3	4	3.2	4	3	3.8	2.6	4.6
Sunflower	2.6	1.8	3	2	2.9	1.8	2.4	1.6
Soybean	1.1	1.2	0.8	1	1	0.8	1.3	0.8

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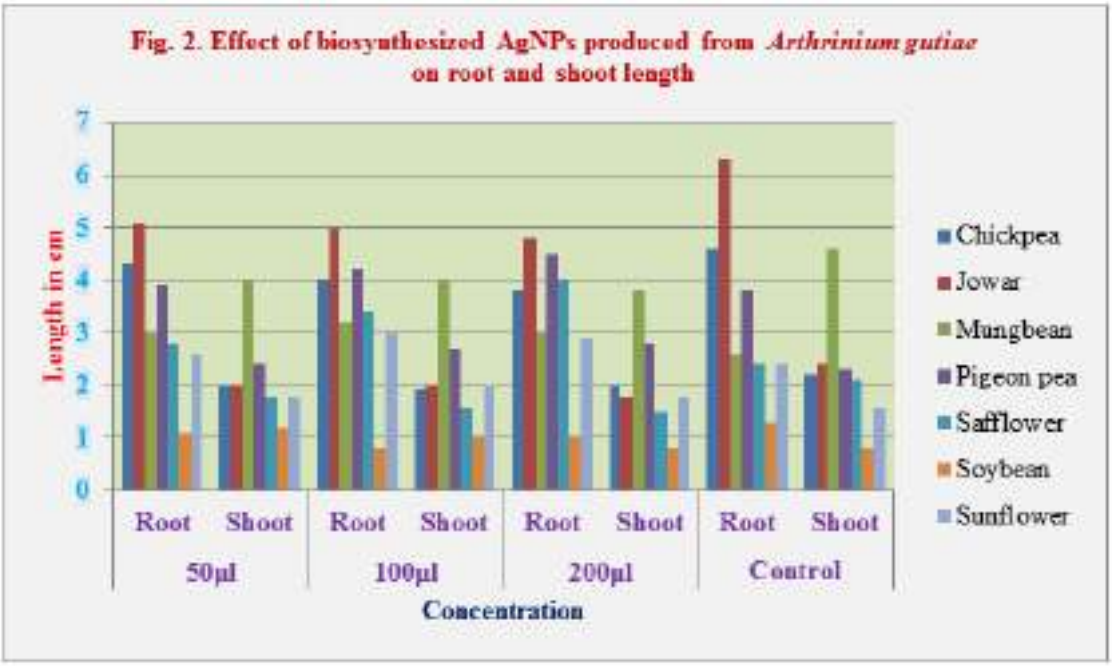

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