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Growth promoting impact of biosynthesised silver nanoparticles (AgNPs) on *Gossypium hirsutum* and *Vigna radiata*

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ABSTRACT

To check the impact of biosynthetic agglomerate form of AgNPs on plant growth, synthesised AgNPs was characterized by using UV- Visible spectroscopy. Antifungal assay was performed by poisoned food method and plant growth was evaluated by using shoot length (cm), Leaves (no./plant), shoot dry wt./plant (g), % seed germination and photosynthetic properties.AgNPs exhibit absorption spectra at 420 nm and showed antifungal activity against *A. niger* and *F. oxysporium*. Significant positive effect was found over *Gossypium hirsutum.*The biosynthesised AgNPs exert significant antifungal activity against *F. oxysporium*. AgNPs show positive effect over shoot length (cm), Leaves (no. /plant), shoot dry wt./plant (g), % seed germination and photosynthetic properties of both plants. The produced AgNPs was in agglomerate form and low in concentration, so they were safe for plant and environment.

Key words:*Green synthesis of silver nanoparticles, Antifungal activity, plant growth promotion activity.*

1. INTRODUCTION

Silver was an ancient element used in the treatment of infections[1] The first use of silver as medicine was reported in 5000 B.C. by Caraka. The use of silver was traced back in 18th century after its use in ulcer treatment [2]. This silver showed its inhibitory action as in ionic or nanoparticle form. The silver exhibit its activity by 3 possible mechanisms as making a puncture in cell membrane, by generating reactive oxygen species and by disturbing DNA replication cycle. [3]Because of its antimicrobial property the silver was widely used in agriculture and medical sector to inhibit the growth of harmful microorganisms. In the last decade the 5000 new applications of silver containing material was investigated and most of them were nanoparticles based. [4] The production of silver nanoparticles was mainly carried

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out by using chemical and physical methods, which make a large amount of hazardous by products and hence are the major concern for environmental contamination. The used techniques are expensive and inadequate to produce it. Hence alternative green, eco friendly tools are required to fulfil the demand. [5]The microbes are found as to be an effective tool for production of nanoparticles. The microbe secreted an extracellular protein, convert elemental form of metal into its nano form and these extra cellular synthesised nanoparticles were found as to be effective as nanoparticles synthesised by other methods. [6]

In this work, we useda simple and eco friendly method for extracellular biosynthesis of silver nanoparticles. *Pseudomonas species* isolated from *Gossypium hirsutum* rhizosphere, were screened for its silver reduction property and the isolate which exhibit, was further identified as per morphological, biochemical and 16S rRNA gene sequencing.An isolate *Pseudomonas fluorescence*AJ B2produced AgNPs was initially confirmed by UV Visible spectroscopy. The effect of produced AgNPS was evaluated against two plant pathogenic fungi *A. niger* and *F. oxysporium*. The consortium-based plant growth promotion activity was checked against *Gossypium hirsutum* and *Vigna radiata* plant, it was found to be significant in presences of AgNPs.

2. MATERIALS AND METHODS

2.1 Isolation of *Pseudomonas* species

Serial dilution of soil samples were carried out and an appropriate dilution 10 -2 (0.5 ml) was spread over Nutrient agar plates. [7] The plates were incubated at 37^0C for 24 hours.The grown isolates were studied for its cultural and morphological characterization and only Gram negative, aerobic, motile rods were transferred over King's B agar medium for further study [8,9] The isolates were further characterized by using 16S rRNA gene sequencing. The genomic DNA was isolated and amplified by using 16S universal primers F: 5' AGA GTT TGA TCC TGG CTC AG 3' and R: 5' AAG GAG GTG ATC CAG CCG CA 3'.

2.2 Screening of isolates for AgNPs synthesis

The isolates were screened for extra cellular biosynthesis of silver nano particles production. For screening purpose the isolates were inoculated in king's B medium and incubated at 150 rpm, 37^0C for 48 hours. After incubation the broth was centrifuged at 8000 rpm for 10 minutes. The 2 ml supernatant was added in 1 ml (1mM) Silver nitrate ($AgNO_3$) solution. The mixture was incubated at 30^0C for 24 hours and analysed by UV Visible spectroscopy for primary confirmation. [10]

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2.3 Characterization of AgNPs

For primary confirmation of AgNPs broth was scanned from 200 to 650 nm to determine its absorption maxima which primary confirms the presences of silver nanoparticles in broth solution.[11]

2.4 Antifungal activity of AgNPs

The antifungal activity of metabolites was carried out by poisoned food method. [13] In sterilized soft molten PD agar tubes 1 ml testing compound was added and molten tubes were poured in empty sterilized perti plates. The plates were allowed to solidify. $1x10^6$ spore/ml fungal spore containing suspension was used for the antifungal assay. A loopful culture was placed over the agar plates and plates were incubated at 30^0C for 48 hours. After 48 hours growth was measured, and inhibition was calculated by formula:

$$\text{Antifungal activity (\%)} = [(Dc-Ds)/Dc] \times 100$$

Dc= Diameter of fungi in control plate, Ds= Diameter of fungi in sample plate

2.5 Plant Growth Promotion activity

The surface sterilization of *Gossypium hirsutum* seeds was carried out as[14] following, Initially Bt *Gossypium hirsutum* seeds were added in 5 ml concentrated Sulphuric acid (H_2SO_4) for 30 -60 seconds. After incubation the seeds were washed for 2 – 3 times with 500 ml distilled water, followed by running tap water for 2 hours. Then seeds were treated with 100 ml 70% ethanol and 1 ml Tween 20 mixture for 1 minute and washed with sterile distilled water for 2 – 3 times and flooded by 10% bleach for 2 minutes. Again rewashed the seeds with sterilized distilled water 2 – 3 times and overnight soaked the seeds in sterilized distilled water. The seeds soaked in sterilized distilled water were considered as control. Such soaked Bt *Gossypium hirsutum* seeds was used for pot assay.[15] while the surface sterilization of *Vigana radiata* was carried out by using 70% ethanol with repeated washing by sterilized distilled water. [16]Approximately 1000 gm of soil was added in every pot and labeled them accordingly. In each pot 3 seeds were added and kept them in a room with moderate sunlight. With 1 day interval approximately 20 ml distilled water was added in every pot. The seeds were observed initially for germination and periodically analyzed for root-shoot length, photosynthetic pigments and dry mass of plant. [17,18,19]

3. RESULTS

3.1 Isolation and characterization of AgNPs producing *Pseudomonas* species

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Out of 20 isolates only 2 isolates were able to synthesize AgNPs, hence were selected and identified as*Pseudomonas fluorescence* by usingBergey's Manual of Systematic Bacteriology.Out of two only one strain *Pseudomonas fluorescence* AJ B 2 showed significant activity, this was used for further study. (NCBI Accession no.MG230469). [20]

3.2 Characterization of AgNPs

The broth was scanned from 200 to 650 nm to determine its absorption maxima and it was recorded at near to 420 nm.[21] The impurities may change the absorption maxima. The absorption maxima primary confirms the presences of silver nanoparticles in broth solution.[22,23]

3.4 Antifungal activity of AgNPs

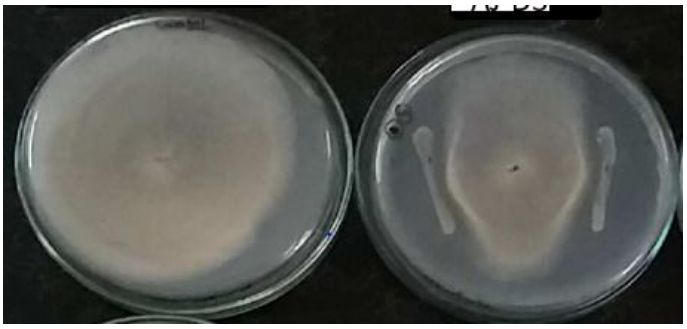

Figure 1: Antifungal activity of *Pseudomonas fluorescence*

Table 1: Antifungal activity of AgNPs

Sr. No.	Incubation	*Fusarium oxysporium* Control = 4.5cm	*Aspergillus niger* control = 8.5 cm
		% of inhibition	% of inhibition
1	AgNPs after 24 Hrs	100 ± 0	68.2 ± 1.2
2	AgNPs after 48 Hrs	83.3 ± 0 .7	60.9 ± 1.6
3	AgNPs after 72 Hrs	31.6 ± 1.1	54.9 ± 1.9

The effect of AgNPs was evaluated against *F. oxysporium* and *A. niger*. The combination showed high activity (Avg.71.66%) against *F. oxysporium*and low activity (Avg.61.39%) against *A. niger*. (Fig. 1, Table 1)

3.5 Plant Growth Promotion activity

The seed germination and plant growth promotion activity of AgNPs was evaluated against *Gossypium hirsutum* and *Vigna radiata.* AgNPs exert effect over *Vigna radiata*seed germination as compared to *Gossypium hirsutum* (Table 2 &3).after exposure of AgNPs a slight deviation in chlorophyll pigmentation was found. (Table 4 & 5)

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Table 2: Effect of AgNPs on *Gossypium hirsutum* seed germination and growth

AgNPs (mg/l)	Shoot length (cm)	Leaves (no./plant)	Shoot dry wt./plant (g)	% Seed germination
10 days	12	4	0.95	100
20 days	25.33	10.33	1.32	100
30 days	32.33	12.5	2.88	100
40 days	40.33	15.00	3.26	100
sd	± 12.0	± 4.79	± 1.13	± 0
Control	10	3	0.76	80

Table 3: Effect of AgNPs on *Vigna radiata* germination and growth

AgNPs (mg/l)	Shoot length (cm)	Leaves (no./plant)	Shoot dry wt./plant (g)	% Seed germination
10 days	8.00	3	0.47	80
20 days	14.12	5.7	0.98	100
30 days	18.1	6.8	1.64	100
40 days	20.33	7.4	2.06	100
Sd	± 5.4	± 1.94	± 0.70	± 10
Control	6.5	2	0.35	75

Table 4: Effect of AgNPs on photosynthetic pigments of *Vigna radiata*

AgNPs (mg/l)	Chl a	Chl b	Carotenoids	Total pigments
10 days	1.169	0.56	0.34	2.069
20 days	1.152	0.608	0.357	2.117
30 days	1.251	0.55	0.389	2.19
40 days	1.241	0.568	0.398	2.207
sd	± 0.1	± 0.03	± 0.03	± 0.1
Control	1.145	0.54	0.22	1.715

Table 5: Effect of AgNPs on photosynthetic pigments of *Gossypium hirsutum*

AgNPs (mg/l)	Chl a	Chl b	Carotenoids	Total pigments
10 days	1.29	0.66	0.361	2.311
20 days	1.352	0.72	0.392	2.464
30 days	1.351	0.747	0.352	2.45
40 days	1.241	0.724	0.368	2.333
sd	± 0.1	± 0.04	± 0.02	± 0.1
Control	1.089	0.442	0.28	1.811

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4. DISCUSSION

This current study was based over the impact of biosynthesised AgNPs on *Gossypium hirsutum* and *Vigna radiata*. AgNPs showed significant positive effect over seed germination of Bt cotton plant.

The isolate was screened for extracellular AgNPs synthesis and characterized. [24] The change in medium color and maximum absorption spectra at 420 nm was related with extracellular AgNPs synthesis and previously reported for another *Pseudomonas*[26,27]

Reports on the antifungal mechanism of AgNPs have shown the effect of Ag on DNA replication, cellular proteins and electron transport chain of mitochondria. [28] while the antimicrobial activity of AgNPs as inactivation of sulfhydryl groups in the fungal cell wall and disruption of membrane-bound enzymes and lipids resulting in lysis of cell was also oberved.[29] Our finding also highlights the antifungal activity of AgNPs and showed significant effect over *F. oxysporium* as compared to *A. niger*. The similar result for AgNPs was also reported by Villamizar [30] and postulate about resistivity of AgNPs that, Aspergillus may produce polyketides (PKs), ribosomal and nonribosomal peptides (NRPs), and terpenoids which may interfere the results.

Positive significant change in shoot length (cm), Leaves (no. /plant), shoot dry wt./plant (g), % seed germination and photosynthetic properties was observed when compared with control. Similar, results were also found[31,32] with change in photosynthetic properties because of alterations in nitrogen metabolism. Qian [33]over his work Arabidopsis thaliana suggests that the concentration of AgNP's more than 3mg/Litre may induce apoptosis process in it, while the review of Tripathi et al. 2017 conclude that lower sized AgNP's(10 -50 nm) have higher toxic effects than high sized AgNP's. In this study the produced AgNP's have size > 100 nm and concentration was less than 3mg/L, showed good effect in direct plant growth promotion, plant pathogen growth inhibition and safe for environment.

5. CONCLUSION

The present work demonstrated the effect of silver nanoparticles on cotton and Mung plant. The biosynthesised AgNPs exert significant antifungal activity against *F. oxysporium*. The produced AgNPs show positive effect over shoot length (cm), Leaves (no. /plant), shoot dry wt./plant (g), % seed germination and photosynthetic properties of both plants. The produced

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AgNPs was in agglomerate form and low in concentration it can be conclude that it was safe for plant and environment.

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7. CONFLICT OF INTEREST

No conflict of interest.

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