Effect of silver nanoparticles on the growth and development of *Indian Brassica* and *Cicer arietinum*

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Abstract

Agriculture fulfils the daily needs of humans directly or indirectly. As population on earth is increasing, necessity to use advanced technologies is also increasing like nano-biotechnology in agriculture to enhance the yield and protect the crops from several diseases. In this study we have isolated and screened some rhizospheric microflora and checked their activity for the biological synthesis of silver nanoparticles (*AgNPs*). *The effect of synthesised AgNPs was then analysed for the growth of two crop plants Cicer arietinum and Indian brassica. Among the microbial strains Bacillus cereus was identified by 16sRNA gene sequencing technique for the biological synthesis of silver nanoparticles.*

Synthesised AgNPs were confirmed by UV- visible spectra (λ=430nm) and Transmission Electron Microscopy 7-50nm. The effect of silver nanoparticles (10mM) was checked on growth of crops Cicer arietinum and Indian brassica and germination percentage was calculated. In case of Cicer arietinum, the seeds that were soaked in nanoparticles solution for overnight were found to have highest germination percentage 100% as compared to non-treated seeds. Whereas, in case of Indian brassica the germination percentage of seeds that were treated with bacterial culture was enhanced by 80% and the GP of silver nanoparticles treated seeds was inhibited. This study illustrated the effect of biologically synthesised silver nanoparticles on the growth and development of two crops Cicer arietinum and Indian brassica and can be stretched to other important crops.

Keyword: Biosynthesis, microflora, silver nanoparticles, *Cicer arietinum*, *Indian brassica*.

Introduction

Nanotechnology is the branch of science which deals with the study of nano-materials and helps in overcoming the restriction of size one of them are silver nanoparticles.[7,13] Silver nanoparticles are known for antimicrobial activity and are widely used in the fields of agriculture, pharmaceutical, biomedical and textile industries. Synthesis of AgNPs can be done by physical as well as chemical methods but these cannot be used in day to day life for the synthesis because they cause harm to the environment due to the requirement of high energy and by the use of some hazardous and toxic chemicals.

Biological synthesis of AgNPs has drawn much attention because they are eco-friendly, non-toxic, require room temperature, pressure and pH.[3,19] There are various methods for the biological synthesis of silver nanoparticles by using different microorganisms and plant extracts as reducing agent for faster synthesis of silver nanoparticles.[1,12] The size and shape of silver nanoparticles are responsible for antimicrobial activity, the smaller is the size, the more is its antimicrobial activity. Therefore, particular microbe can be used for the synthesis of AgNPs with controlled shape and size.[5,9]

There are various microorganisms that help in the synthesis of AgNPs either intracellularly or extracellulary or by both.[15] The effect of silver nanoparticles on plants depends on various factors like the species and age of plant, the shape, size and concentration of AgNPs, the process and time of exposure, temperature, pressure, pH etc. The growth and development or inhibition in growth of plants depend on the measured quantity of silver nanoparticles. As silver nanoparticles possess high surface area and fraction of surface atoms, so they have high antimicrobial activity as compared to bulk silver. This antimicrobial property of silver has been widely used against human pathogens [5,6,11,14] but its full potential is still unexplored in the field of agriculture.

Therefore, there is increasing awareness of how to use the antimicrobial property of silver nanoparticles in plant growth promotion.[17] Some current study showed the effect of silver nanoparticles on plants. Silver nanoparticles synthesis from white radish has been reported in the size ranging from 6-38 nm.[16] Silver nanoparticles synthesised from culture supernatant of *Serratia* species BHU-S4 were found to have vigorous action against *Bipolaris sorokiniana*, a spot blotch disease of wheat. [17] Nano based fertilizers can be used in order to provide full nutrients to plants. These fertilizers are applied to crops in different ways either to soil or through leaves.[2]

In this study we have focused on the effect of biologically synthesised silver nanoparticles on two crop plants *Cicer arietinum* and *Indian brassica*. Here, we analysed the dose response of the two crop plants for AgNPs which is likely to enhance our capability to utilize them to increase crop production to control several plant disease control and plant growth promotion.

Material and Methods

Silver nanoparticles were synthesized via a biological route using bacteria isolated from soil collected from the fields of Lucknow. 10mM concentration of silver nitrate was used for the biological synthesis of silver nanoparticles. The synthesized (AgNPs) were characterized by UV-visible spectroscopy and Transmission electron microscopy.

The two stress tolerant bacterial strains i.e. CD^4 and CD^{10} responsible for the synthesis of silver nanoparticles were used for further experiments to check its effect on the growth and development of two crop plants i.e. *Cicer arietinum* and *Indian brassica*.

Collection of seeds: For this experiment the seeds of both the crops i.e. *Cicer arietinum* (Pusa 362) and *Indian brassica* (Pusa Mustard 25) were procured from Indian Agriculture Research Institute (IARI), New Delhi.

Preparation of solutions for treatment

i. AgNPs synthesised by bacterial strain CD^4: Sterilized nutrient broth medium was used to inoculate the stress tolerant bacterial strain CD^4 and kept for incubation at 37ºC for 48 hours in shaker (150 rpm). After 48 hours of incubation, cultures were centrifuged at 5000 rpm for 4 minutes and supernatant was transferred to fresh sterile vessel and supplied with 10mM concentration of silver nitrate. The vessel was then kept for further incubation at 37ºC under shaking condition at 150 rpm until colour change was observed.

ii. AgNPs synthesised by bacterial strain CD^{10}: Sterilized nutrient broth medium was used to inoculate the stress tolerant bacterial strain CD^{10}, then and kept for incubation at 37ºC for 48 hours under shaking condition (150 rpm). After 48 hours of incubation, culture was centrifuged at 5000 rpm for 4 minutes and supernatant was transferred to fresh sterile vessel and supplied with 10mM concentration of silver nitrate. The vessel was then kept for further incubation at 37ºC under shaking condition at 150 rpm until colour change was observed.

iii. Preparation of silver nanoparticles synthesised by bacterial strain CD^4 + bacterial culture CD^4: The AgNPs were synthesized from bacterial strain CD^4 as mentioned above.

For the preparation of bacterial culture of $CD,^4$ nutrient broth medium was prepared and sterilised, the culture was inoculated in the broth and kept for incubation at 37ºC for 48 hours under shaking condition (150 rpm). After 48 hours of incubation, good growth was observed that was sufficient for the treatment. Both the treatment were given in combination.

iv. Preparation of silver nanoparticles synthesised by bacterial strain CD^{10} + bacterial culture CD^{10}: The AgNPs were synthesized from bacterial strain CD^{10} as mentioned above.

This method was used to prepare silver nanoparticles from bacterial strain. For the preparation of bacterial culture $CD,^{10}$ nutrient broth medium was prepared and sterilised, the culture was inoculated in the broth and kept for incubation at 37ºC for 48 hours under shaking condition (150 rpm). After 48 hours of incubation, there was good growth found in the broth, the solution was used for the treatment. Both the treatment were given in combination.

v. Preparation of bacterial culture CD^4: For the preparation of bacterial culture $CD,^4$ nutrient broth medium was prepared and sterilised the culture was inoculated in the broth and kept for incubation at 37ºC for 48 hours under shaking condition (150 rpm). After 48 hours of incubation, there was good growth found in the broth, therefore the solution is ready for the treatment.

vi. Preparation of bacterial culture CD^{10}: For the preparation of bacterial culture $CD,^{10}$ nutrient broth medium was prepared and sterilised, the culture was inoculated in the broth and kept for incubation at 37ºC for 48 hours under shaking condition (150 rpm). After 48 hours of incubation, there was good growth found in the broth, therefore the solution is ready for the treatment.

Seed nanopriming with AgNPs: For this experiment, the seeds of both the crops i.e. *Indian brassica* and *Cicer arietinum* were first submerged in 2% sodium hypochlorite solution for 10-15 minutes for surface sterilisation, then washed several times with double distilled water.

The pot experiment was carried out in two different ways.

Each experiment was setup in duplicate and given same treatment. Control was also kept for both the crops. Total 38 pots were setup for the experiment.

a) Seeds directly sown- In this experiment seeds of *Indian brassica* and seeds of *Cicer arietinum* were directly sown in soil.
b) Seeds first soaked then sown- In this experiment seeds of *Cicer arietinum* were first soaked in silver nanoparticles solution of both the bacterial strains CD^4 and CD^{10} separately for overnight and then sown in soil.

Treatment was given thrice during the experiment- first the treatment was given just after seeds were sown in the soil, then after fifteen days and the last after one month. The seeds of *Indian brassica* were sown during the first week of November and matured in February whereas the seeds of *Cicer arietinum* were sown during the first week of October and matured in March.

The growth and development of crop were monitored and data was collected on the basis of germination, plant growth, branching, plant height, budding, flowering, fruiting as compared to control.

Results and Discussion

Among the microbial strain was identified as *Bacillus cereus* by 16sRNA gene sequencing technique, used for the biological synthesis of silver nanoparticles. Synthesised silver nanoparticles were confirmed by UV- visible spectra (λ=430nm) and Transmission Electron Microscopy 7-50nm. The effect of silver nanoparticles (10mM) was checked on growth of crop plants *Cicer arietinum* and *Indian brassica* and germination percentage was also calculated.

The microbial strain was found to be salt tolerant and was checked up to 10% NaCl concentration.

Preparation of different solutions for the treatment of crops Chickpea and Mustard:
The six types of solutions for the treatment of crops are prepared:

a. Treatment of nanoparticles solution synthesized by bacterial strain CD^4.
b. Treatment of nanoparticles solution synthesized by bacterial strain CD^{10}.
c. Treatment with bacterial strain CD^4 + nanoparticles solution.
d. Treatment with bacterial strain CD^{10} + nanoparticles solution.
e. Treatment with the bacterial strain CD^4.
f. Treatment with the bacterial strain CD^{10}.

Each treatment was given thrice to crops: first treatment was given just after sowing the seeds in the soil, second treatment was given after 15 days and last treatment was given after one month.

Seed Germination Measurement: The final germination percentage was calculated based on the total number of seeds germinated at the end of the experiment. The measurement was carried out according to International Rules for Seed Testing.[4] Germination percentage was calculated using the formula:

Germination Percentage (G.P. %) = (Gf/n) × 100
where Gf is the total number of germinated seeds at the end of the experiment and n is the total number of seeds used in the experiment.

Effect of AgNPs on the growth and development of *Cicer arietinum* (Chickpea): The seeds of *Cicer arietinum*, Pusa 362 (Chickpea) were procured from Indian Agriculture Research Institute (IARI), New Delhi. The seeds were submerged in 2% sodium hypochlorite solution for 10-15 minutes for surface sterilization and washed several times with distilled water.

Pot experiment was carried out, seeds were sown in pot in unsterile soil and grown in desired condition by providing different treatments. The pots were kept in field to provide natural environment for its growth and development. For this experiment 10mM concentration of silver nitrate was used for the biological synthesis of silver nanoparticles.

60 seeds of *Cicer arietinum* were taken and divided in two equal parts: 30 seeds were soaked for overnight in silver nanoparticles solution obtained from bacterial strain CD_4 and rest 30 seeds were soaked for overnight in silver nanoparticles solution obtained from bacterial strain CD_{10}. These seeds were then sown in soil after. Another set of 60 seeds were taken and they were directly sown in soil without soaking in silver nanoparticles solution. Both the experiments were then given same treatment except control. Table represents the effect of different treatment given to chickpea growth and development.

It is clear from the above data that the seeds that were nanoprimed with biologically synthesised AgNPs showed maximum growth rate as compared to normal seeds. For control seeds, the germination percentage was found to be 90% with shoot length 16.7cm, root length 12.4cm, shoot dry weight 0.21g, root dry weight 0.08g.

For seeds treated with CD^4 ➔ NP: (normal seeds) G.P. 60%, S.L. 9.5cm, R.L. 5.8cm, SDW 0.13g, RDW 0.03g. (AgNPs nanoprimed seeds) G.P. 95%, S.L. 19.2cm, R.L. 14.2cm, SDW 0.23g, RDW 0.09g.

For seeds treated with CD^{10} ➔ **NP** (normal seeds) G.P. 60.3%, S.L. 9.8cm, R.L. 6.0cm, SDW 0.14g, RDW 0.03g. (AgNPs nanoprimed seeds) G.P. 95%, S.L. 19.3cm, R.L. 14.9cm, SDW 0.23g, RDWght 0.09g.

For seeds treated with CD^4+NP: (normal seeds) G.P. 40%, S.L. 5.5cm, R.L. 3.4cm, SDW 0.09g, RDW 0.002g. (AgNPs nanoprimed seeds) G.P. 90%, S.L. 18.2cm, R.L. 13.6cm, SDW 0.22g, RDW 0.07g.

For seeds treated with CD^{10}+NP: (normal seeds) G.P. 40%, S.L. 5.6cm, R.L. 3.2cm, SDW 0.08g, RDW 0.002g. (AgNPs nanoprimed seeds) G.P. 90%, S.L. 18.1cm, R.L. 12.9, SDW 0.19g, RDW 0.08g.

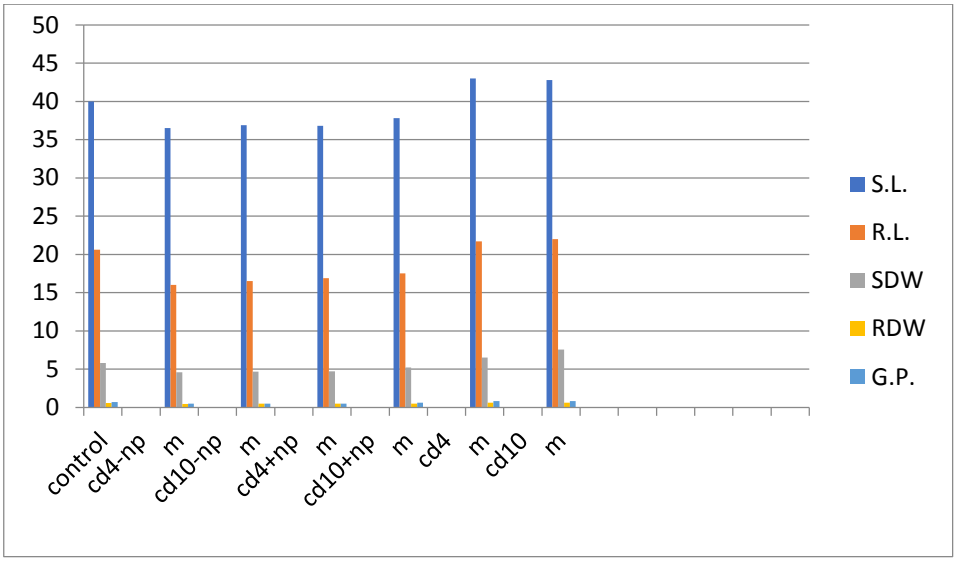
Table 1
Salt (NaCl) tolerant bacteria isolated from rhizospheric soil

		Sodium Chloride Concentration				
S.N.	**Bacterial isolate**	**5%**	**6%**	**7%**	**8%**	**10%**
1	Cd^4	++	++	++	+	+
2	Cd^{10}	++	+	+	+	+

Table 2
Difference between the germination percentage of chickpea seeds in control, directly sown seeds and silver nanoparticles soaked seeds.

***Cicer arietinum* (Chickpea)**											
S.N.	**Exp. type**		**Seeds sown in soil**	**Trt. 1**	**Trt. 2**	**Trt. 3**	**Shoot length (cm)**	**Root length (cm)**	**Shoot dry mass (g)**	**Root dry mass (g)**	**G.P.**
			day 1	day 2	day 15	day 30					
1.	Control		seeds sown	-	-	-	16.7	12.4	0.21	0.08	90%
2.	CD^{4} NP										
	C.P.		seeds sown	1^{st} Trt.	2^{nd} Trt.	3^{rd} Trt.	9.5	5.8	0.13	0.03	60%
	SS C.P.		seeds sown	1^{st} Trt.	2^{nd} Trt.	3^{rd} Trt.	19.2	14.2	0.23	0.09	95%
3.	CD^{10} NP										
	C.P.		seeds sown	1^{st} Trt.	2^{nd} Trt.	3^{rd} Trt.	9.8	6.0	0.14	0.03	60.3%
	SS C.P.		seeds sown	1^{st} Trt.	2^{nd} Trt.	3^{rd} Trt.	19.3	14.9	0.23	0.09	95%
4.	CD^{4}+NP										
	C.P.		seeds sown	1^{st} Trt.	2^{nd} Trt.	3^{rd} Trt.	5.5	3.4	0.09	0.002	40%

	SSC.P.		seeds sown	1st Trt.	2nd Trt.	3rd Trt.	18.2	13.6	0.22	0.07	90%
5.	CD^{10}+NP										
	C.P.		seeds sown	1st Trt.	2nd Trt.	3rd Trt.	5.6	3.2	0.08	0.002	40%
	SSC.P.		seeds sown	1st Trt.	2nd Trt.	3rd Trt.	18.1	12.9	0.19	0.08	90%
6.	CD^{4}										
	C.P.		seeds sown	1st Trt.	2nd Trt.	3rd Trt.	-	-	-	-	0%
	SSC.P.		seeds sown	1st Trt..	2nd Trt.	3rd Trt.	10.7	8.0	0.11	0.03	60%
7.	CD^{10}										
	C.P.		seeds sown	1st Trt.	2nd Trt.	3rd Trt.	3.0	2.1	0.04	0.001	30%
	SSC.P.		seeds sown	1st Trt.	2nd Trt.	3rd Trt.	10.6	8.3	0.13	0.04	60%



Graph 1: Graph representing the effect of 10mM AgNPs on shoot length, root length, shoot dry weight, root dry weight, germination percentage of *Cicer arietinum*.

For seeds treated with CD^4: there was 0 G.P. recorded. (AgNPs nanoprimed seeds) G.P. 60%, S.L. 10.7cm, R.L. 8.0cm, SDW 0.11g, RDW 0.03g.

For seeds treated with CD^{10}: (normal seeds) G.P. 30%, S.L. 3cm, R.L. 2.1cm, SDW 0.04g, RDW 0.001g. (AgNPs nanoprimed seeds) G.P. 60%, S.L. 10.6cm, R.L. 8.3cm, SDW 0.13g, RDW 0.04g.
Silver nanoparticles solution when supplied to this crop in different form showed different results, the seeds that were soaked in nanoparticles solution for overnight and then sown in soil and provided with different treatments showed maximum germination rate whereas there was inhibition found in shoot length, root length, shoot dry weight and root dry weight of seeds that were directly sown in soil and then provided with different treatments.

Effect of various treatments on the growth and development of *Indian brassica* (Mustard): The seeds of *Indian brassica* (Pusa Mustard 25) were procured from Indian Agriculture Research Institute (IARI), New Delhi. The seeds were then submerged in 2% sodium hypochlorite solution for 10-15 minutes for surface sterilization, then washed several times with distilled water.

Uniform seeds were sown in pot in unsterile soil, collected from the local field of Lucknow and grown in desired condition by providing different treatments. The pots were kept in field to provide natural environment for its growth and development. For this experiment 10mM concentration of silver nitrate was used to obtain biologically synthesised silver nanoparticles.

Seeds were sown in unsterile soil and then supplied with different treatments except control. The seeds were sown in soil without soaking it in nanoparticles solution. The experiment was set up in duplicate for each treatment type.

Treatment was given thrice, first just after sowing seeds, second after fifteen days and last after one month. The differences in the growth and development of silver nanoparticles treated plants and non-treated plants were recorded.

The above data obtained from the experiment represents that the mustard seeds that were treated with the bacterial culture solution CD^4 and CD^{10} have the highest germination percentage (80%) as compared to control which showed 70% G.P.

For control seeds, the germination percentage was found to be 70%, shoot length 40.0cm, root length 20.6cm, shoot dry weight 5.8g, root dry weight 0.57g.

For seeds treated with CD^4 NP → G.P. 50%, S.L. 36.5cm, R.L. 16cm, SDW 4.6g, RDW 0.43g.

For seeds treated with CD^{10} NP→ G.P. 50%, S.L. 36.9cm, R.L. 16.5cm, SDW 4.68g, RDW 0.47g.

For seeds treated with CD^4+NP: G.P. 50%, S.L. 36.8cm, R.L. 16.9cm, SDW 4.7g, RDW 0.49g.

For seeds treated with CD^{10}+NP: G.P. 60%, S.L. 37.8cm, R.L. 17.5cm, SDW 5.2g, RDW 0.5g.

For seeds treated with CD^4: G.P. 80%, S.L. 43.0cm, R.L. 21.7cm, SDW 6.5g, RDW 0.61g.

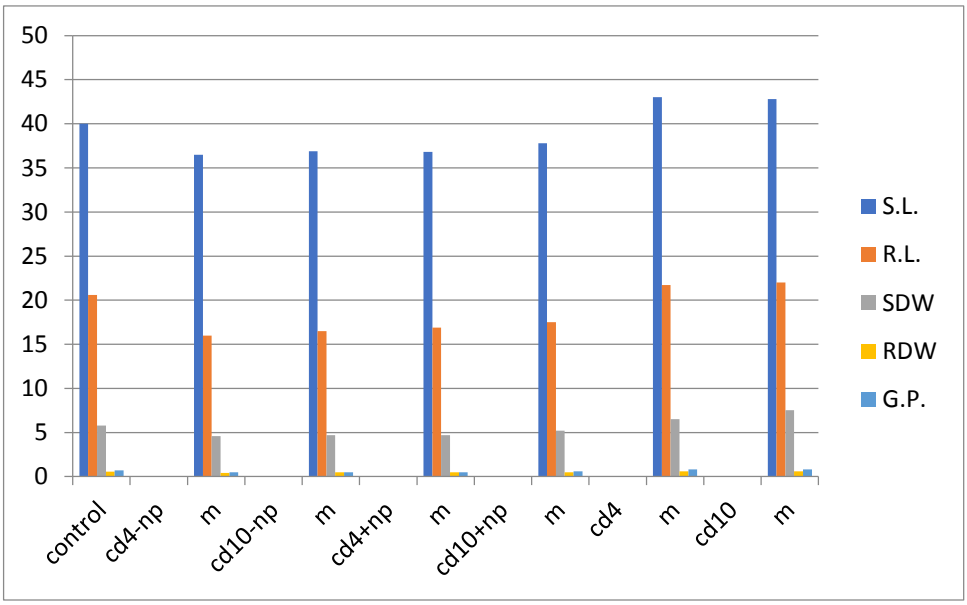
For seeds treated with CD^{10}: G.P. 80%, S.L. 42.8cm, R.L. 22.0cm, SDW 7.54g, RDW 0.60g.

When 10mM concentration of silver nanoparticles solution was supplied to mustard seeds, it showed less growth and development of crop as compared to seeds that were treated only by bacterial culture solutions.

Table 3
Germination percentage of *Indian brassica* (Mustard) in treated and non-treated Condition.

***Indian brassica* (Mustard)**											
S.N.	**Exp. type**		**Seeds sown in soil**	**Trt. 1**	**Trt. 2**	**Trt. 3**	**Shoot length (cm)**	**Root length (cm)**	**Shoot dry mass (g)**	**Root dry mass (g)**	**G.P. %**
			Day1	D-2	D-15	D-30					
1.	control		Seeds sown	-	-	-	40.0	20.6	5.80	0.57	70%
2.	CD^4 → NP										
	M		Seeds sown	Trt.1	Trt.2	Trt.3	36.5	16.0	4.60	0.43	50%
3.	CD^{10}→NP										
	M		Seeds sown	Trt.1	Trt.2	Trt.3	36.9	16.5	4.68	0.47	50%
4.	CD^4+NP										
	M		Seeds sown	Trt.1	Trt.2	Trt.3	36.8	16.9	4.70	0.49	50%
5.	CD^{10}+NP										
	M		Seeds sown	Trt.1	Trt.2	Trt.3	37.8	17.5	5.20	0.50	60%
6.	CD^4										
	M		Seeds sown	Trt.1	Trt.2	Trt.3	43.0	21.7	6.50	0.61	80%

7.	CD^{10}										
	M		Seeds sown	Trt.1	Trt.2	Trt.3	42.80	22.0	7.54	0.60	80%



Graph 2: Graph representing the effect of 10mM AgNPs on shoot length, root length, shoot dry weight, root dry weight, germination percentage of *Indian brassica*.

This showed that the growth of crop treated with silver nanoparticles was inhibited by germination percentage, shoot length, root length, shoot dry weight and root dry weight.

Biological method is considered to be more convenient for the synthesis of silver nanoparticles rather than physical and chemical methods due to its eco-friendly nature. Physical and chemical methods require the use of harmful chemicals and expensive instruments. Here in this study we have biologically synthesised silver nanoparticles using bacteria isolated from soil. These environment friendly methods of synthesizing silver nanoparticles play an important role as antimicrobial agents in agriculture.

To improve the production of crops, it is necessary that the planted seeds germinate completely, so nowadays silver nanoparticles are used in the field of agriculture. In the present study, the two crops chickpea and mustard showed different growth response after treatment with silver nanoparticles and bacterial isolates.

Based on the earlier studies, it can be possible that a nanoparticle helps in the nutrition uptake of plants like it may be helpful in proper uptake of water, fertilizers, can improve seed antioxidant system.[10,21] Nanoparticles may also be helpful in the diminution of anti oxidant stress by reducing H_2O_2, manolyldialdehyde content and superoxide radicals and promote some enzymes like superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase and catalase activity which help in seed germination, growth and development of some plant species.[8,18,20]

Conclusion

So here we conclude that the chickpea seeds that were first soaked in 10mM concentration of silver nanoparticles solution synthesised by both the bacterial strains and then planted and given treatment were found to have enhanced germination percentage and growth rate as compared to control and the seeds that were directly planted and treated. Whereas, in the case of mustard, there was inhibition in germination percentage and growth rate of seeds planted and exposed with nanoparticles solution. While the growth rate and germination percentage of seeds that were planted and then treated with bacterial culture solution of both the bacterial strains CD^4 and CD^{10} were enhanced. In case of chickpea it is possible that when the seeds are exposed to nanoparticles solution, it perforates into the seed coat and helps in germination. However, there are also some toxic effects of nanoparticles on growth and development of some plant species.

As it is seen in case of mustard, the crop was harmed by AgNPs as indicated by decrease in the seed germination. The seeds that were treated with the bacterial culture solution CD^4 and CD^{10} showed maximum germination percentage as

compared to silver nanoparticles treated seeds. The toxicity of silver nanoparticles may result in the mere occurrence of nanoparticles in cells or accumulation in the cell wall hampering the uptake of essential nutrients and thus developing oxidative stress. Our findings thus showed that nanoparticles can be beneficial as well as toxic for different plant species.

Future Perspectives

To modernize and to maximize agriculture production, Government is adopting various new technologies, one of which is nanotechnology. Crop production can be increased by using nanofertilizers, nanopesticides, nanoherbicides etc. According to the previous studies, how different plant species and environmental factors respond to nanoparticles uptake is still to be investigated. Use of nanoparticles for the delivery of essential nutrients to plants will be in high demand in the coming future.

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