

Influence of Silver Nanoparticles on onion (*Allium cepa* L.) seed germination and seedling Vigor

Abstract

During this study on the influence of silver nanoparticles (AgNPs) on growth of L-883 onion varieties *Allium cepa* L. AgNPs 100 nm size were used in four different concentrations (25, 50, 75, and 100 ppm) and T₀ was kept as control. The seeds of onion were brought from NHRDF Indore. The findings revealed that 25ppm of silver nanoparticle led to the highest seed germination percentage as compared to other concentrations. On the other hand, all the other concentrations were found to have a harmful effect on both seed germination and seedling growth as the level of toxicity are strongly associated with concentration of the suspensions. AgNPs of 100ppm concentration was found to have lowest rate of seed germination, however non-significant difference was found in 75ppm and 100ppm concentrations. Therefore, a concentration of 25ppm of AgNPs proved to be the most effective treatment for promoting optimal seed germination and seedling growth in onions.

Keywords: Onion, Nanoparticles, seed germination and seedling growth

Introduction

Onion (*Allium cepa*) is an important bulb crop and a key vegetable in India. It belongs to *Alliaceae* family which includes several more economically substantial crops. Onions are the second most extensively consumed vegetable in the world and they are the oldest to be produced (Benitez *et al.*, 2011)

Onion has anti-bacterial, anti-fungal properties and many medicinal properties such as anti-cancer properties and work as an anti-cholesterol agent. Onion also has an antioxidant component quercetin in it (Baghizadeh *et al.*) In addition to trade of vegetables in India, onions have the biggest section of Ledger account of 76.2 per cent yet its efficiency is lower than numerous of the nation. Insufficiency of high-quality onion's germplasm is one of the main reasons for less yield. Seed quality parameters such as seed size and seed weight also influence the ultimate reduction in the yield of onion (Gamiely *et al.*, 1991).

In expansion, it has been detailed to be wealthy in phytochemicals particularly flavanols which has therapeutic esteem (Zadeh *et al.*, 2009). Within the World, India ranks second after China in terms of range and production.

During storage, onion seed rapidly misfortune its reasonability because they generate free radicals by lipid peroxidation which result in low germination rate. As current technologies are insufficiently effective in tackling these issues on a large scale. nevertheless, nanotechnologies hold promise for finding a solution. Nanoparticles appear diverse impacts on seed germination of distinctive plants (Rico, *et al.* 2011).

According to Noshad *et al.*, (2019), AgNP_s dramatically boosted *Solanum lycopersicum* seed germination and seedling growth. In tomato AgNP_s increased the length

and quantity of roots in seedlings, as well as the amount of nitrogen and phosphorus in the leaves (Guzman-Baez *et al.*, 2021).

Materials and Methods

AgNPs with different concentration was used in this experiment. Theseeds of onion (L-883) was brought from National Horticultural Research and Development Foundation (NHRDF) Madhya Pradesh. AgNPs of strengths of 25 ppm (T₁), 50 ppm (T₂), 75 ppm (T₃), and 100 ppm (T₄) were prepared in distilled water using a water bath as treatments.

Procedure

The experiment was conducted in completely randomized design (CRD) (Maxwell 2005) with three replicates. For each treatment, 50 germplasm were placed in each replicate. To ensure surface sterility, seeds were soaked in 5% sodium hypochlorite solution for 20 minutes. (USEPA, 1996) and subsequently washed many times with distilled water. 150 seeds were submerged in the solution containing AgNPs for around 3 hours. As supplementary control, seeds were kept in distilled water for the same period. The vessel was frequently shaken for uniform adsorption of AgNPs. Whatman no. 1 filter paper was placed on the Petri plate with a diameter of 100 x 15 mm and 2.5 ml of solution was poured onto it, and in control 2.5 ml of double-distilled water was poured. The Petri dishes were then placed in a germination chamber at 26 ± 5 °C and $96 \pm 2\%$ relative humidity. Germination were recorded daily and further observations were taken on 6th days, and the last observation was recorded on the 14th day.

Parameters measured in the study:

$$\text{Germination Percentage} = (\text{Gs}n^{-1}) \times 100$$

Where Gs = number of germinated germplasm

n = Total number of seed used in the test.

By counting the cumulative germination against each day Cumulative germination percentage is calculated.

Germination rate index (GSI)

The germination index was calculated as the total number of germplasm germinated per day divided by the number of days from sowing to germination, according to the Maguire equation (1962).

Mean Germination Time (MGT)

Mean germination time was calculated by the formula given by (Robert, 1981)

$$\text{MGT} = \frac{n_1 \times d_1 + n_2 \times d_2 + n_3 \times d_3 + \dots}{\text{Total number of days}}$$

Where, n = Number of seed which have germinated

d = Number of days

Mean daily germination (MDG)

Mean daily germination was calculated by the formula given by (Czabator, 1962)

$$\text{MDG} = \frac{\text{Number of germinated seeds}}{\text{Total number of days}}$$

Vigour index

The vigour index was calculated using the approach recommended by Anderson (1973).

Vigour index = Germination percentage × Seedling length.

Tolerance Index (TI) = Average root length in AgNPs solution divided by longest root length in solution containing double distilled water x 100. (Saeed & Associates, 2016)

Root and shoot length

The measurement of root length from the base of the collar to the tip of a root was done for all normal seedlings, and the average value was in centimetres. The average value for the shoot length of seedlings from the germination test was measured on the 6th and 14th days, respectively, from around the collar area to the tip of the shoot. A similar approach was taken to determine root-to-shoot ratios. Fresh mass was weighed with an electronic balance and was expressed in mg. seedlings were placed in an oven at a temperature of 65°C until the weight became constant and the weight was expressed in mg.

Result and Discussion

In the study, there was a significant increase in germination percentage (GP) in T1 (25 ppm AgNPs) compared to other treatments (Table 1). lowest germination % was observed in 100ppm of AgNPs in both the observations as high concentration of silver nanoparticle effects germination %. Germination speed index was observed maximum in 25ppm whereas T3 and T4 was at par but significantly lesser than control (table 1). A cumulative germination percentage (CGP) means that germination has started on 3th day for all treatments except in T4(100 ppm) where, the first germination was observed on 4th day. From the cumulative germination rates, it can be concluded that T2, T3, and T4 were partially toxic to the seedlings. It may also notice that seed of T₂ could manage themselves to pick the germination after 7 days. whereas, mean germination time was non-significant among all the treatment.

Table1: Germination and vigour parameters of onion.

Treatment	Germination %		Germination speed Index	Mean germination time	Mean daily germination		Vigour index	
	6 th day	14 th day			6 th day	14 th day	6 th day	14 th day
Control	46.00 ^b	70.66 ^b	6.60 ^b	2.23	3.83 ^b	2.52 ^b	155.34 ^b	641.35 ^b
T1	58.00 ^a	81.33 ^a	7.98 ^a	2.36	4.83 ^a	2.90 ^a	235.86 ^a	871.16 ^a
T2	24.00 ^c	66.00 ^b	4.87 ^c	2.46	2.00 ^c	2.26 ^c	62.48 ^c	500.78 ^c
T3	16.00 ^d	50.00 ^c	3.6 ^d	2.03	1.33 ^d	1.73 ^d	17.07 ^d	250.42 ^d
T4	12.00 ^e	46.00 ^c	3.12 ^e	1.99	1.00 ^e	1.64 ^e	7.25 ^e	216.42 ^e
CD at 0.05	6.28	5.04	1.82	NS	0.53	0.20	19.97	28.75
SEm	2.03	1.62	0.27	NS	0.15	0.05	6.34	9.10

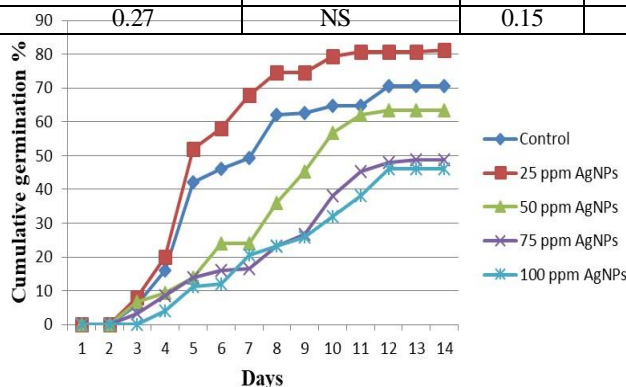


Figure 1: Cumulative germination percentage of germplasm treated with AgNPs.

The average daily germination was higher on days 6 and 14, but the average daily germination on day 6 was higher (4.83 %) than on day 14 (2.90). This means that 25 ppm AgNP_s stimulate early germination and data represents that 50 ppm, 75 ppm, and 100 ppm AgNP_s of populate early germination (Table 1). Treatment with 25 ppm AgNP_s showed the maximum vitality index (VI) on days 6 and 14, which was significantly higher as compared to control. It was that vigour index gradually decreases as concentration increases as 50, 75 and 100ppm has less vigour index. Lowest vigour index was observed in T4 (Table 1). AgNP_s incorporate with seed coats and helps in water uptake inside seeds which promotes starch metabolism and seed germination. It is also applied as a Nano-priming agent to enhance rice-aged seeds and starch metabolism. (Khan et al 2023)

Table 2: Performance of ion seed for tolerance index, shoot and root ratio, seedling fresh weight and seedling dry weight.

Treatment	Tolerance index		Shoot and root ratio (cm)		Seedling fresh weight (mg)	Seedling dry weight (mg)
	6 th day	14 th day	6 th day	14 th day		
Control	100.00 ^b	100.00	2.46 ^a	3.72 ^b	20.27 ^b	1.50
T1	140.61 ^a	120.57	1.97 ^b	3.64 ^b	25.89 ^a	1.71 ^a
T2	105.85 ^b	94.47	1.50 ^c	3.51 ^b	21.40 ^b	1.09 ^d
T3	76.39 ^c	51.90	0.45 ^d	5.04 ^a	16.47 ^c	1.52 ^b
T4	58.13 ^d	52.37	0.27 ^d	4.67 ^a	17.40 ^c	1.30 ^c
CD(0.05)	11.68	10.30	0.26	0.61 ^c	1.28	0.16
SEm	3.26	3.22	0.3	0.06	0.5	0.04

It was also noticed that treatment T₂, T₃ and T₄ had very little VI compare to T₁ or control on 6th day, which indicates that in initial days there was minimum growth. Maximum tolerance index (TI) was observed in T₁ and minimum was observed in T₄ (table 2). The decline in TI and VI was brought on by a higher proportion of seed germination inhibition, which was followed by a decrease in shoot height and root length as a result of the combined negative impacts of metal poisoning from AgNPs via oxidative stress. The treatment T₁ had the highest seedling fresh weight (25.89 mg) compared to all other treatments, while T₃ had the lowest seedling fresh weight (however it was comparable to T₄).

Similarly, highest dry weight was recorded in T₁ and lowest dry weight was recorded in T₂. It was notice that shoot length was less in T₄ and T₃ compare to other treatment (Table 3). maximum shoot length was observed in T₁. Maximum and minimum root length was observed in T₁ and T₃ respectively (Table 3.) Pandey *et al.*, (2014) reported that root and shoot growths are more affected by the Ag treatment than seed germination. The roots length was retarded to 1% and 0.5% in *Oryza sativa* and *Brassica campestris*; *Vigna radiata*, respectively when AG nanoparticles were used at higher concentration. Mazumdar *et al.*, (2011)

Conclusion

All parameters indicate that AgNPs at lower concentrations (25 ppm) will promote germination and have a positive effect on physiological activities, but higher concentrations of AgNPs are toxic for seedlings. Among all treatments, T₄ was most toxic to seedlings. 100 ppm AgNPs significantly reduced all parameters. The toxic effect of AgNPs is may be due to increased production of ROS during interaction between nanoparticle and plant (Marslin et al.,2017). Less concentration induced plantlets while higher concentration caused inhibition (Mandal et al.,2006) AgNPs at 0-, 10-, 20-, and 30-mM increased plant growth and maintains the ionic balance of cells (Na⁺, K⁺, and Na⁺/K⁺ ratio) by decreasing the uptake of Na and Cl and oxidative stress by salt-stressed plants (khan et al 2023).

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

- 1.
- 2.
- 3.

References

- Abdul-Baki AA, Anderson JD.** Vigour deterioration of soybean germplasm by multiple criteria. *Crop Sci.* 1973; 13:630-633.
- Asharani PV, Wu YL, Gong Z, Valiyaveetil S.** Toxicity of silver nanoparticles in zebrafish models. *Nanotechnology.* 2008; 19(25):255102.
- Baghizadeh A, Baniasadi F, Bonjar GHS, SirchiGRS, Massumi H, Jorjandi M** Biocontrol of *Botrytis allii* Munn the causal agent of neck rot, the post-harvest disease in onion, by use of a new Iranian isolate of *Streptomyces*. *American Journal of Agriculture and Biological Sciences.* 2009; 4:72-78.
- Benitez V, Molla E, Martin-Cabrejas MA, Aguilera Y, Lopez-Andreu FJ, Esteban RM.** Effect of sterilization on dietary fiber and physicochemical properties of onion by-products. *Food Chem.* 2011;127(2):501–507. doi: 10.1016/j.foodchem.2011.01.031

Czabator FJ. Germination value: An index combining speed and completeness of pine seed germination. *Forest Science*. 1962; 8:386-395.

Ellis RH, Roberts EH. The quantification of ageing and survival in orthodox germplasm. *Seed Sci. Tech.* 1981; 9:373-409.

Gamiely S, Randle, William, Mills, Harr, Smittle DA et al. Onion Plant Growth, Bulb Quality, and Water Uptake following Ammonium and Nitrate Nutrition. Hort Science: a publication of *the American Society for Horticultural Science*, 1991, 26.

Guzman-Baez, G.A., Trejo-Tellez, L.I., Ramírez-Olvera, S.M., Salinas Ruíz, J., BelloBello, J.J., Alcantar-Gonzalez, G., Hidalgo-Contreras, J.V., Gomez-Merino, F.C., 2021. Silver nanoparticles increase nitrogen, phosphorus, and potassium

ISTA. International Rules of Seed Testing. *Seed Sci. & Technol.* 2005; 27:27-32.

Javadzadeh A, Ghorbanihaghjo A, Bonyadi S, Rashidi MR, Mesgari M, Rashtchizadeh Preventive effect of onion juice on selenite-induced experimental cataract. *Indian Journal of Ophthalmology*. 2009; 57:185-189.

Khan.S, Zahoor.M, Khan.R, Ikram.M, and Islam (2023) The impact of silver nanoparticles on the growth of plants: The agriculture applications

Kumari M, Mukherjee A, Chandrasekaran N. Genotoxicity of silver nanoparticles in *Allium cepa*. *Sci Total Environ*. 2009; 407:5243-5246.

Khan S., Zahoor M, Khan R.S, Ikram M and Islam N (2023)The impact of silver nanoparticles on the growth of plants: The agriculture applications

Lee WM, Kwak JI, An YJ. Effect of silver nanoparticles in crop plants *Phaseolus radiatus* and *Sorghum bicolor*: media effect on phytotoxicity. *Chemosphere*. 2012; 86:491.

Maguire JD. Speed of germination-aid in selection and evaluation for seedling emergence and vigour, *Crop Sci*. 1962; 2:176-177.

Marslin G, Sheeba CJ, Franklin G. Nanoparticles alter secondary metabolism in plants via rosburst. *Front Plant Sci*. 2017; 8:832.

MAXWELL SC (2005) Completely Randomized Design. Encyclopedia of Statistics in Behavioral Science

Mandal D., Bolander M.E., Mukhopadhyay D., Sarkar G., Mukherjee P. The use of microorganisms for the formation of metal nanoparticles and their application. *Appl. Microbiol. Biotechnol.* 2006;69:485–492. doi: 10.1007/s00253-005-0179-3.

Monica BC, Cremonini R. Nanoparticles and higher plants. *Caryologia*. 2009; 62:161-165.

Mazumdar H., Ahmed G.U. Synthesis of silver nanoparticles and its adverse effect on seed germinations in *oryza sativa*, *vigna radiata* and *Brassica campestris*. *Int. J. Adv. Biotechnol. Res.* 2011; 2:404–413.

Noshad, A., Hetherington, C and Iqbal, M., 2019. Impact of AgNPs on seed germination and seedling growth: a focus study on its antibacterial potential against *Clavibactermichiganensis* subsp. *michiganensis* infection in *Solanum lycopersicum*.

Pandey C., Khan E., Mishra A., Sardar M., Gupta M. Silver nanoparticles and its effect on seed germination and physiology in Brassica juncea L. (Indian mustard) plant. *Adv. Sci. Lett.* 2014; 20:1673–1676.

Rico CM, Majumdar S, Gardea MD, Peralta Videa JR, Jorge L, Torresdey GL. Interaction of nanoparticles with edible plants and their possible implications in the food chain. *J Agric. Food Chem.* 2011; 59(8):3485-98.

Saeed KM, Reza H, Fatemeh R, Solmaz N. Effect of Chemical Synthesis Silver Nanoparticles on Germination Indices and Seedlings Growth in Seven Varieties of *Lycopersiconesculentum* Mill (tomato). *Plants. J Clust Sci.* 2016; 27:327-340.

USEPA. Ecological effects test guidelines: Seed germination/root elongation toxicity test, OPPTS 850, 4200, EPA 712-C-96-154, Washington DC, 1996.

Yin L, Cheng Y, Espinasse B, Colman PB, Auffan M, Wiesner M The effects of silver nanoparticles on *Loliummultiflorum*. *Environ. Sci. Technol.* 2011; 45:2360.

Das R. Effect of silver Nano-particle on seed germination and seedling vigour of onion (*Allium cepa* L.). *IJCS.* 2018;6(3):427-30.