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Ratan Das
Department of Horticulture and Postharvest Technology, Institute of Agriculture, Visva-Bharati, Sriniketan, WB, India

Prahlad Deb
Division of Vegetable Crops, Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru, Karnataka, India

Veere Gowda R
Division of Vegetable Crops, Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru, Karnataka, India

Correspondence
Ratan Das
Department of Horticulture and Postharvest Technology, Institute of Agriculture, Visva-Bharati, Sriniketan, WB, India

Effect of silver Nano-particle on seed germination and seedling vigour of onion (*Allium cepa* L.)

Ratan Das, Prahlad Deb and Veere Gowda R

Abstract
An experiment was conducted to evaluate the effects silver nanoparticles (AgNPs), on different germination parameters of onion varieties Arka Pragati. AgNPs of 100 nm size in five different concentrations, viz. 0, 25, 50, 75 and 100 ppm were used as a treatment. Result reviled that 25ppm concentration of AgNPs has significantly higher percent of seed germination over all other treatments. Further, the same dose also had a positive response on seedling growth. But all other treatment had a toxic effect on seed germination and seedling growth, while the degree of toxicity was positively correlated with the strength of the suspension. AgNPs with strength of 100ppm had the lowest percentage of seed germination but there was no significant difference between 75ppm and 100ppm. Thus, 25ppm concentration of AgNPs was best as seed treatment of onion for better seed germination and seedling growth than others.

Keywords: Potato, Weed, Fenoxaprop–p-ethyl, tuber yield and Economics of Returns

Introduction
Onion (*Allium cepa* L.) belongs to the family Allium and is one of the most important vegetable crops in India. It also known as queen of kitchen is one of the oldest known and an important vegetable crop grown in India. It contains a lachrymatic agent, a strong antibiotic, fungicidal, bacterial, anti-cholestrol, anti-cancer and antioxidant components such as quercetin (Baghizadeh *et al*. 2009) [3]. In addition, it has been reported to be rich in phytochemicals especially flavonols which has medicinal value (Javadzadeh *et al*. 2009) [7]. In the World, India ranks second in area and production of onion next only to China. In total export of vegetables from India, onion has the largest entry of export accounting to 76.2 per cent. But productivity is much lower than many of the country. There are many reign for the low productivity. The unavailability of quality onion seed is one of the important reasons for its lower yield. The seed quality parameters especially seed size and seed weight affect the final yield in onion production (Gamiely *et al*., 1991) [13].

Furthermore, high quality seed is considered as a critical input in onion on which all other inputs have to be managed for potential yield in onion. In India onion is grown in an area of 1.01 m ha with a production of 16.8m tonnes having productivity at 16.6 t ha^{-1}.The major onion growing states are Maharashtra, Gujarat, Uttar Pradesh, Orissa, Karnataka, Tamil Nadu and Andhra Pradesh. Onion seed quickly loss its viability due to the production of free radicals by lipid peroxidation during storage which result in low germination percentage. Present technologies are not efficient enough to overcome this problem in large scale but nanotechnology may bring a ray of hope to overcome such problem. Nanoparticles show different effects on seed germination of different plants (Rico, *et al*. 2011) [14]. Toxicity studies, both positive and negative effects was observed when some higher plants were treats with of several nano particles viz. TiO2, ZnO, Mg, Al, Pd, Cu, Si, C60 fullerenes, and multiwall carbon nanotubes (Monica and Cremonini, 2009) [12]. (Senthilkumar, 2011) [18] and (Sridhar, 2012) [19] observed that metal oxide nano-particles are efficient enough to improve the germination of aged seeds of black gram and tomato up to 30 per cent respectively. This could be probably due to the quenching of reactive oxygen species (ROS) generated during seed storage. Zhang *et al*., 2006 [20] reported that applications of nanotechnology improve seed germination, emergence and growth of seedlings. Hence the present investigation was made to study the effect of AgNPs on seed germination of onion seed.

Materials and Methods

AgNPs having pertical size less than 100 nm was used. Seed of onion (Arka Pragati) was selected for the experiment, which was obtained from Indian Institute of Horticultural Research (IIHR) Bangaluru. A strength of 25ppm (T_1) 50ppm (T_2) 75ppm (T_4) and 100ppm (T_5) AgNPs was prepared in dabble distilled water by using sonicator as a treatment. Completely Randomized Design (CRD) with four replications was followed to carry out the experiment. Fifty no of seed was placed in each replications for the respective treatment. Seeds were immersed in a 5% sodium hypochlorite solution for 10 min to ensure surface sterility (USEPA, 1996) [16], followed by rinsing with double-distilled water several times. 200 seeds were socked for about 3 hour in each treatment solution containing AgNPs and in control, seeds was socked for the same duration in dabble distilled water. The container was shaken frequently so to ensure uniform adsorption of AgNPs. Borosil Petri plates with 100 x 15 mm diameter were floored with whatman no. 1 filter paper and 2.5 ml of treatment solution was poured on it and control was treated with 2.5 ml of double-distilled water. Then those petri plates were placed in a germination room having 25 ± 2 0C and 95 ± 3 per cent RH. The germination was recorded daily and other observations was taken once in 6 days and the final observations on 14 days (ISTA, 2005) [6].

Parameters measured in this study were:

Germination Percentage (GP %) = (Gs/n) × 100

Where Gs is the total number of germinated seeds and n is the total number of seed used in the test.

Cumulative germination percentage was also calculated by counting the cumulative germination against each day.

Germination Speed Index (GSI)

The germination speed index was calculated by the sum of the number of seeds germinated each day, divided by the number of days elapsed between the seeding and germination, according to the Maguire formula (1962).

GSI = G1/N1+G2/N2 ... +G1/N1 where

GSI = seedlings' germination speed index;

G = number of seeds germinated each day;

N = number of days elapsed from the seeding until the last count.

Mean germination Time (MGT)

Mean germination time was calculated by the formula given by (Ellis, 1981) [5]

MGT = n1 x d1 + n2 x d2 + n3 x d3 + --------/ Total number of days

Where, n= number of germinated seed

d = number of days

Mean daily germination (MDG)

Mean daily germination can be calculated by the following formula given by (Czabator, 1962) [4]

MDG = Total number of germinated seeds/ Total number of days

Vigour index

Vigour index was computed by adopting the method suggested by (Abdul-Baki and Anderson, 1973) [1] and expressed as whole number.

Vigour index = Germination percentage × Seedling length.

Tolerance Index (TI) = mean length of longest root in AgNPs solution/length of longest root in control solution × 100. (Saeed *et al.* 2016) [15].

Root and shoot length: Root length of all the normal seedlings from the germination test was measured from collar reasons to the root tip and the mean was expressed in centimetre. Shoot length of all the normal seedlings from the germination test was measured from collar region to the shoot apex once on 6th day and another on 14th day and the mean was expressed in mm. Similarly root and shoot ratio was calculated. The fresh mass was quantified through weighing in precision scale with an electronic weighing balance and expressed in mg. The fresh seedling was placed at a temperature of 70 °C, in an oven until constant weight and the weight was expressed in mg.

Result and desiccation

In this study, we detected a significant increase in germination percentage (GP) in T_1 (25ppm of AgNPs) over all other treatments (Table 1). Gradual higher concentration of silver nanopertical effect the GP and lowest GP was observed in 100ppm of AgNPs in both the observation. Maximum germination speed index was observed in 25ppm whereas T_3 and T_4 was at par but significantly less then control (table 1). Cumulative germination percentage (CGP) represent that germination started on 3rd day for all the treatments but for T_4 1st germination was observed on 4th day. It may be concluded from the cumulative germination percentage that T_2, T_3 and T_4 were partially toxic to the seedling hence a little increase in CGP was

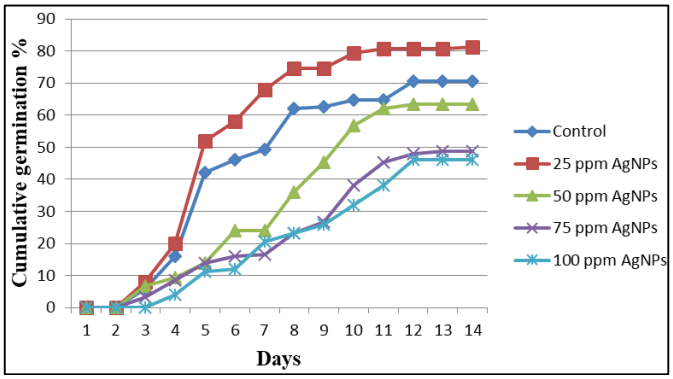

Fig 1: Cumulative germination percentage of AgNPs treated seeds.

Observed in initial days where as a rapid increase in CGP was observed in T_1 and control (Fig 1). It may also notice that seed of T_2 could manage them self to pick the germination after 7 days. On the other hand mean germination time was non-significant. Among all the treatment.

Table 1: Mean performance of onion seed for germination and vigour parameters.

Treatment	Germination %		Germination speed Index	Mean germination time	Mean daily germination		Vigour index	
	6th day	14th day			6th day	14th day	6th day	14th 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]
T_1	58.00[a]	81.33[a]	7.98[a]	2.36	4.83[a]	2.90[a]	235.86[a]	871.16[a]
T_2	24.00[c]	66.00[b]	4.87[c]	2.46	2.00[c]	2.26[c]	62.48[c]	500.78[c]
T_3	16.00[d]	50.00[c]	3.6c[d]	2.03	1.33[d]	1.73[d]	17.07[d]	250.42[d]
T_4	12.00[d]	46.00[c]	3.12[d]	1.99	1.00[d]	1.64[d]	7.25[d]	216.42[e]
CD (0.05)	6.30	5.05	1.81	NS	0.52	0.19	19.96	28.71
SEm	2.00	1.60	0.28	NS	0.14	.06	6.33	9.11

mean daily germination was significantly higher on 6th day as well 14th day but on 6th day mean daily germination was higher (4.83) then 14th day (2.90) which represent that 25ppm AgNPs stimulate for early germination whereas data represent that 50ppm, 75ppm and 100 ppm of AgNPs inhabit germination in early days (Table 1) Treatment with 25ppm of AgNPs indicated maxima vigour index (VI) on 6th and 14th days and it was significantly higher over control. The VI was gradually decreased with incrassating concentration of AgNPs i.e. 50, 75 and 100ppm respectively. Lowest concentration of VI was observed on T_4 (Table 1). It is also.

Table 2: Mean performance of onion seed for tolerance index, shoot and root ratio, seedling fresh weight and seedling dry weight.

Treatment	Tolerance index		Shoot and root ratio		Seedling fresh weight (mg)	Seedling dry weight(mg)
	6th day	14th day	6th day	14th day		
Control	100.00[b]	100.00	2.46[a]	3.72[b]	20.27[b]	1.50
T_1	140.61[a]	120.57	1.97[b]	3.64[b]	25.89[a]	1.71[a]
T_2	105.85[b]	94.47	1.50[c]	3.51[b]	21.40[b]	1.09[d]
T_3	76.39[c]	51.90	0.45[d]	5.04[a]	16.47[c]	1.52[b]
T_4	58.13[d]	52.37	0.27[d]	4.67[a]	17.50[c]	1.30[c]
CD (0.05)	11.64	10.32	0.27	0.63c	1.29	0.17
SEm	3.27	3.21	0.2	0.08	0.4	0.05

Important to draw the attention that treatment T_2, T_3 and T_4 had very little VI compare to T_1 or control in 6th day, which reflect the minimum growth in the initial days.

On the other hand highest tolerance index (TI) is observed in case of T_1 and least was observes in T_4.(table 2) The decrease in TI and VI are due to greater inhibition of seed germination percentage followed by root length and shoot height in response to the composite adverse effects of metal toxicity like AgNPs by oxidative stress. Metal toxicity effect of AgNPs on plants, was previously investigated, which high accumulation of enzymatic and non-enzymatic antioxidant components *viz*, peroxidase, catalase, superoxide dismutase, malondialdehyde, and free amino acids content under AgNPs was revealed (Mehrian *et al*, 2015) [11]. Those may be the factor for the same.

Seedling fresh weight was significantly higher over all other treatment in T_1 (25.89 mg) where as lowest Seedling fresh weight was recorded in T_3 but it was at par to T_4. Similarly highest dry weight was recorded in T_1 but surprisingly lowest dry weight was recorded in T_2. It was noticeable that shoot length was very less in T_4 and T_3 compare to other treatment (Table 3) however maxima shoot length was observed in T_1 which was.

Table 3: Mean performance of onion seed for shoot length and root length.

Treatment	Shoot length (mm)		Root length (mm)	
	6th day	14th day	6th day	14th day
Control	24.00 [b]	71.54 [b]	9.77[b]	19.213[b]
T_1	27.03 [a]	83.91[a]	13.72[a]	23.187[a]
T_2	15.59 [b]	63.52c	10.33[b]	18.133[b]
T_3	3.37 [c]	49.73d	7.43[c]	9.947[c]
T_4	1.48 [d]	46.84 [e]	5.70[d]	10.06[c]
CD (0.05)	2.10	2.61	1.16	2.10
SEm	0.66	0.82	0.34	0.66

Significantly higher than all other treatment. Similar result was observed in root length. Highest root length was observed in T_1 and minimum root length was observed in T_3 but it was statistically at par with T_4 (Table 3.) Yin *et al*, 2011 [17] reported that root and shoot growths are more affected by the Ag treatment than seed germination. When *Phaselous radiatus*, *Sorghum bicolor* and *Lolium multiflorum* were subjected to silver nanoparticles resulted in reduced root growth, root length and biomass were observed (lee *et al*, 2012) [9].

Conclusion

All the parameters clearly indicate that low concentration (25ppm) of AgNPs helps in seed germination and also has a positive effect on the different physiological activity but higher concentration of AgNPs are toxic to the seedling. Among all the treatment T_4 was most toxic to the seedling. 100 ppm of AgNPs reduced the almost all the parameter considerably. The toxic effect of AgNPs could be due to present of nanoparticles and secondly release of Ag^+ ions from nanoparticles and generation of free radicals during the AgNPs suspension (Asharani *et al*, 2008) [2]. Researchers also demonstrated that AgNPs could disrupt cell division process causing chromatin bridge, stickiness and cell disintegration (Kumari *et al*., 2009) [8].

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