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Effect of biosynthesized nanoparticles on seed quality parameters of chilli

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Abstract

Experiment was conducted to evaluate the effects biosynthesized silver nanoparticles (AgNPs) and copper nanoparticles (CuNPs) on different seed quality parameters of chilli variety Arka Lohit. Nanoparticles with different concentration, *viz.* 10, 25, 50, 75, 100, 200, 400 ppm along with raw silver solution, plant sample 2%, Silver nitrate 0.1mM and Bavistin-2g/lit. Results revealed that 400ppm of silver has significantly higher percent of seed germination (73.66%), seedling vigour index (1363), seedling dry weight (0.31 g), shoot (6.33 cm) and root length (12.16 cm) over all other treatments but there was no significant difference between silver nitrate, Bavistin, plant sample and raw silver solution. CuNPs results reviled that 50ppm of copper concentration has significantly higher percent of seed germination (75.33%), seedling vigour index (1327), seedling dry weight (0.32 g), shoot (5.96 cm) and root length (11.66 cm) over all other treatment. But at higher concentration treatment had a toxic effect on seed germination and seedling growth Thus, 400ppm concentration of AgNPs and 50ppm concentration of CuNPs was best as seed treatment of chilli for better seed germination and seedling growth than others.

Keywords: Chilli, AgNPs, CuNPs, seed quality, germination, shoot length, root length, vigour, seedling dry weight

1. Introduction

Chilli (*Capsicum annuum* L.) belongs to family *Solanaceae* has a chromosome number of 2n= 24 and it is often cross-pollinated crop. There are five cultivated species of peppers including *Capsicum annuum, C. frutescents, C. chinense, C. pubescens. and C. Baccatum*. (Heiser and smith, 1957) [5]. The most important cultivated species of pepper is *C. annuum*. Green synthesis of nanoparticles has emerged as a simple and viable alternative eco-friendly method for chemical and physical methods of synthesis.

Quality seed play an important role in the agricultural production. It acts as a catalyst for realizing the potential of all other inputs in agriculture. Modern agriculture demands that each and every seed must germinate readily and produce a vigorous seedling, thus ensuring high yield. Among the different characteristics of a quality seed, seed viability and vigour are the vital factors that determine the stand of the crop in field and ultimately the yield. Seed vigour is a vital character of seed lot and is of greater importance for successful establishment of plant in the field especially under stressed situations.

The seed treatment with nano particles has gaining much importance with respect to seed research. Nanomaterials have gained increasing attention because of their novel properties, including a large specific surface area and high reaction activity **(**Khorsand *et al*, 2011) [7]. Nanomaterials have also been used for various fundamental and practical applications. Nanoparticles represent a new generation of environmental remediation technologies that could provide cost effective solution to some of the most challenging environmental clean-up problems (Chinnamuthu and Murugesa, 2009) [3]. The use of nanoparticles in the growth of plants and for the control of plant diseases is a recent practice studied the effect of mixtures of nano-SiO2 and nano-TiO2 on soybean seed and they found that the mixture of nanoparticles increases nitrate reductase in soybean increasing its germination and growth (Prasad *et al.*, 2009) [10]. Nanotechnology has the potential to protect plants, monitor plant growth, detect plant and animal diseases, increase global food production, enhance food quality, and reduce waste for "sustainable intensification" (Locke J.M. *et al.*, 2000) [8].

In order to exploit the potential of nano based seed treatment the present study is "Effect of biosynthesized nanoparticles on seed quality parameters of chilli"

2. Martial and Methods

The laboratory studies were carried out to find out the effect of seed treatment with nanoparticles on seed quality of chilli. For seed treatment, chilli seeds (cultivar Arka Lohit) procured from the Indian Institute of Horticultural Research (IIHR), Hesarghatta Bangalore were used to conduct experiment.

2.1 Green Synthesis of Silver nanoparticles

The flask containing 100 mL of aqueous solution (1 mM) of $AgNO_3$ was added with 10 mL of the aqueous extract of *K. alvarezii* for 30min under continuous stirring at 35 °C and then allowed to stand at 30^0 C temperatures for another 24hrs. The solution colour changed to Dark brown from colorless. The Solution was centrifuged at 11000 rpm for 30 min and the dark brown solid product was collected and dried at room temperature. The resulting dried sample was crushed into powder and stored in an air tight container for further analysis.

2.2 Characterization of Silver nanoparticles

The synthesized nanoparticles exhibited maximum absorption at 430 to 440nm which is UV-SPR for silver nanoparticles. The silver nanoparticles were characterized based on SEM and whose size was found to be in the ranges from 60 to 90nm. The XRD analysis of synthesized silver nanoparticles showed four peaks at 38.36, 44.48, 64.62 and 77.62 angles. Also, at 38.36 = 2θ, the curve showed the highest peak indicated the crystal shape of nanoparticles. In particle size analysis mean particle size observed that 36 nm.

2.3 Green synthesis of Copper nanoparticles

The flask containing 50 mL of aqueous solution (1 mM) of $CuSO_4$ was added with 10 mL of the aqueous extract of *K. alvarezii* for 30min and added 40 ml of 4 mM PEG 600 under continuous stirring at 70 °C and then allowed to stand at 80 ^{0}C temperatures for another 1hr, after that solution was maintained alkaline (pH-10) condition by adding NaOH. The solution colour changed to Dark Black from colourless.
The Solution was centrifuged at 11000 rpm for 30 min and the dark brown solid product was collected and dried at room temperature. The resulting dried sample was crushed into powder and stored in an air tight container for further analysis.

2.4 Characterization of Copper nanoparticles

The synthesised nanoparticles exhibited maximum absorption at 650 to 670nm which is (SPR) for Copper nanoparticles. The Copper nanoparticles were characterized based on Scanning Electron Microscopy (SEM) and whose size was found to be in the ranges from 60 to 90nm. The XRD analysis of synthesized Copper nanoparticles showed four peaks at 38.36, 44.48, 64.62 and 77.62 angles. Also, at 38.36 = 2θ, the curve showed the highest peak indicated the crystal shape of nanoparticles. In particle size analysis mean particle size observed that 68 nm.

2.5 Treatment of seeds with nanoparticles

Arka lohit hybrid seeds were soaked in prepared solution of silver and copper nanoparticles for overnight at room temperature (Fig. 1) and seeds shaken without the nanoparticles served as control. Treatment details are listened in Table1. This treated seed is taken for germination test, seeds were placed in a germination room having 27 ± 2 ^{0}C and 95 ± 3 per cent RH. The germination was recorded daily and other observations was taken once in 7 days and the final observations on 14 days as per ISTA (2010) [6]. At final count, shoot height and root length were measured. The effect of different concentrations of CuNPs and AgNPs viz. 10, 25, 50, 75, 100, 200 and 400 ppm were evaluated on growth of *C. annuum* and compare with control (untreated). Various parameters of germination such as Germination percentage (GP), Shoot length, Root length, Seedling vigour index I and seedling dry weight were also studied.

Fig 1: Overnight soaking of chilli seeds with different concentration of nanoparticles.

Table 1: Treatment details

Treatment details- SILVER	
T-1	Plant sample- 2% *(K. alvarezii)*
T-2	Silver nitrate- 0.1mM
T-3	Bavistin-2g/ltr
T-4	10 ppm Biosynthesized Silver Nano
T-5	25 ppm Biosynthesized Silver Nano
T-6	50 ppm Biosynthesized Silver Nano
T-7	75 ppm Biosynthesized Silver Nano
T-8	100 ppm Biosynthesized Silver Nano
T-9	200 ppm Biosynthesized Silver Nano
T-10	400 ppm Biosynthesized Silver Nano
T-11	Raw Silver solution
T-12	Control

Treatment details – COPPER	
T-1	Plant sample- 2% (*K. alvarezii)*
T-2	Copper Sulphate- 1mM
T-3	Bavistin-2g/ltr
T-4	10 ppm Biosynthesized Copper Nano
T-5	25 ppm Biosynthesized Copper Nano
T-6	50 ppm Biosynthesized Copper Nano
T-7	75 ppm Biosynthesized Copper Nano
T-8	100 ppm Biosynthesized Copper Nano
T-9	200 ppm Biosynthesized Copper Nano
T-10	400 ppm Biosynthesized Copper Nano
T-11	Raw Copper solution
T-12	Control

2.6 Estimation of seed quality parameters

2.6.1 Germination (%)
The germination of the seeds was assessed with 100 seeds in 4 replications by between paper method as per ISTA (2010) [6]. The numbers of normal seedlings were counted on 14 the day (final count) of germination from all the replications. The average was expressed as percentage (%).

2.6.2 Shoot length
Shoot length was measured from collar region to the apex in ten randomly selected 14 days old normal seedlings and the mean was recorded as shoot length in centimeter (cm).

2.6.3 Root length
The root length between collar region and the tip of the root in ten randomly selected 14 days old normal seedlings was measured and mean was calculated and expressed in centimeters (cm).

2.6.4 Seedling vigour index I
For determination of seedling vigour, 10 normal seedlings were selected randomly from each treatment and replication at the end of germination test. The shoot and root length of each of the 10 seedlings were measured in centimeters and average seedling length was calculated.
SVI–I = Germination (%) x Average seedling length (cm)

2.6.5 Seedling dry weight
Ten normal seedlings after measurement of root and shoot length were kept in butter paper and dried in a hot-air oven maintained at 80 °C temperature for 24 hours. Later they were removed and allowed to cool in a desiccator for 30 minutes before weighing in electronic balance. The mean dry weight of the seedlings was recorded.

3. Result and Discussion
In the present investigation an attempt was made to cognizant of the influence of nanoparticles on seed quality parameters. The seed treatment of nanoparticles significantly influenced the seed quality attributes and consistently maintained the viability and vigour of the seeds compared to the untreated control. Nanoparticles treated seeds significantly outperformed over control in terms of seed germination, seedling shoot length, seedling root length, seedling vigour index and seedling dry weight.
Among the tested nanoparticles the seed treatment with silver nanoparticles at the rate of 400ppm is outperformed over all other nanoparticles including control. (Fig.2) Seed germination percentage (73.66%), longest shoot length (6.33 cm), longest root length (12.16 cm), seedling dry weight (0.31 gm) and higher seedling vigour index I (1363) show significant difference over all other treatments. Table 2. Gradual lower concentration of silver nanoparticle affects the germination (%) and lower germination (%), seedling vigour index, seedling dry weight, shoot and root length was observed in control. The reason for good germination is could be due to the penetration of nanoparticles into the seed coat facilitating the influx of water inside the seed and activated the enzymes in early phase, thereby enhancing the speed of germination These results are in conformity with the findings of Sridhar, 2012 [3]. Almutairi and Alharbi, 2015 [1] found that, Ag NPs at 2000 ppm had increased germination speed (1.59 seeds/ day) for watermelon over the control (0.85 seeds/day). Pandey *et al*., 2014 who observed the maximum root length (6.50 cm) due to the application of Ag NPs (100 ppm) on *Brassica Juncea*, the application of Ag NPs (1000 ppm) on onion seeds showed increased shoot length (7.50 cm) over the control (5.40 cm) (Anandaraj and Natarajan, 2017) [9]. The higher seedling vigour index I was recorded with standard Ag NPs at 400ppm over the control. The results are in good agreement with those published by Shyla and Natarajan, 2014 [12] who reported that, the application of Ag NPs on groundnut seeds increased seedling vigour index by about 40.35% against control.

Control 400 Ppm Comparison of seedling between control and 400 PPM treatment

Fig 2: Image showing effect of Ag NPs on seed germination and seedling growth of *Capsicum annuum***.**

The effect of Cu nanoparticle on seed germination, shoot length, root length, seedling dry weight and seedling vigour index I is presented in Table 2. Significantly higher germination (75.33%), shoot length (5.96 cm), root length (11.66 cm), seedling dry weight (0.32 gm) and seedling vigour index I (1327) was recorded in 50ppm concentrations of NPs (Fig 4). Seed treatment with 10ppm, 25ppm, raw silver solution, plant sample 2%, copper sulphate-1mM, Bavistin and control has on par with each other. Increase in NPs concentration germination, shoot length, root length, seedling dry weight and seedling vigour index also was found to decline. Similar findings were also reported by (Doshi *et al*. 2008, Shah & Belozerova 2009) [4, 11] toxic effects of Cu-NPs in various studies reporting that concentration was higher than 200 ppm. For mung bean seedling the best growth response for radicle and plumule was observed at lowest concentration.

Table 2: Effect of biosynthesized nanoparticles on seed germination, shoot length, root length, seedling dry weight and VI-1 of chilli.

Treatments	Germination (%)		Shoot length (cm)		Root length (cm)		Seedling dry weight (g)		SVI-1	
	Silver	Copper	Silver	Copper	Silver	Copper	Silver	Copper	Silver	Copper
T_1	65.33	67.66	4.17	4.63	7.90	7.87	0.23	0.26	790	845
T_2	64.33	61.00	3.73	4.47	7.23	7.50	0.21	0.25	705	733
T_3	62.33	60.66	3.40	4.30	6.33	6.97	0.20	0.25	605	682
T_4	67.00	57.67	5.10	3.33	9.23	5.10	0.24	0.22	959	560
T_5	68.66	59.66	5.40	3.57	9.77	6.17	0.26	0.20	1040	631
T_6	69.00	75.33	5.43	5.97	9.93	11.67	0.27	0.32	1059	1327
T_7	70.66	74.00	5.53	5.73	11.07	11.37	0.28	0.32	1173	1264
T_8	71.00	72.66	5.60	5.67	11.30	10.17	0.30	0.30	1200	1150
T_9	72.33	70.33	5.83	5.30	11.90	9.27	0.30	0.29	1283	1023
T_{10}	73.66	69.33	6.33	5.07	12.17	8.90	0.32	0.29	1363	968
T_{11}	66.33	68.66	4.77	4.80	8.37	8.23	0.24	0.28	872	895
T_{12}	58.00	57.33	2.67	2.77	4.50	4.83	0.17	0.14	416	518
S.Em. (±)	1.87	2.45	0.22	0.22	0.31	0.3	0.02	0.03	40.64	34.8
C.D. (0.01)	7.44	9.75	0.89	0.87	1.24	1.20	0.07	0.12	162.02	138.74

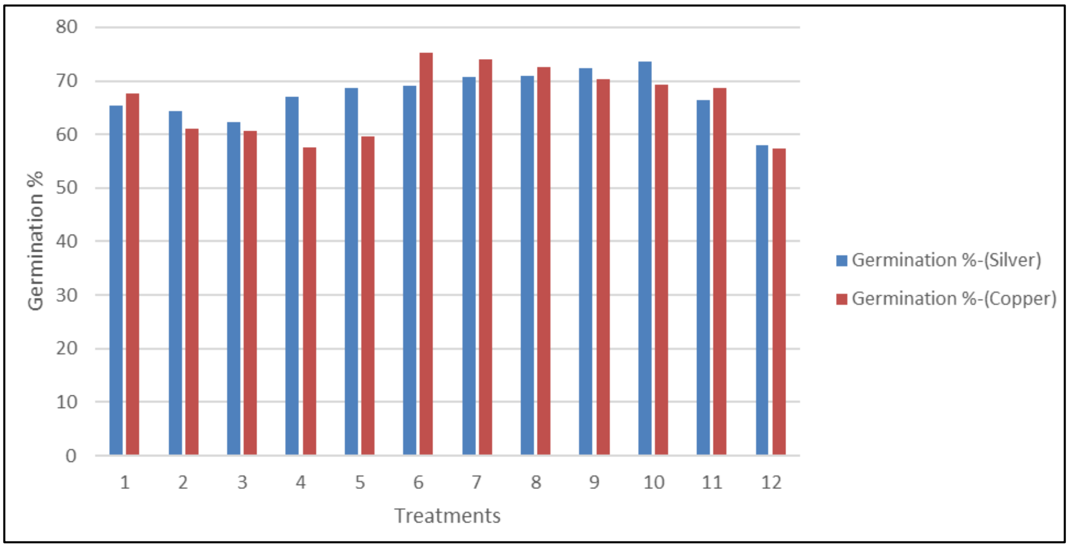

Fig 3: Effect of silver and copper nanoparticles on germination (%) in chilli

Control 50 PPM

Fig 4: Image showing effect of Cu NPs on seed germination and seedling growth of *Capsicum annuum*

At 400 ppm of CuNP observed burning symptoms in seedling.

Fig 5: Image showing negative effect of Cu NPs on seed germination and seedling growth of *Capsicum annuum* at higher concentration.

4. Conclusion

The present work demonstrated the effect of silver and copper nanoparticles on seed quality parameters. Different concentration increased seed germination, vigour, seedling dry weight, shoot and root length. Among various concentration used in the study, 400ppm of AgNPs and 50ppm of CuNPs was the most effective treatment for the improvement in seed germination and seedling growth of chilli.

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