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Improvement in Seed Performance and Fruit Yield through Seed Nano-Priming with Silver Nanoparticles in Capsicum (*Capsicum annum* L.)

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ABSTRACT

The present study investigated the effects of seed nano-priming with bio-synthesised silver nanoparticles-SNP on germination, seedling establishment, and fruit yield in capsicum (*Capsicum annum* L.) cv. HC-201. Though nanoparticles are being developed for various potential applications, several concerns are related to their agricultural use. In the present study, seeds were treated by soaking with SNP solutions (25-1000 mg L^{-1}) for 16 h before placing for germination. Seed pre-treatment with 200 mg L^{-1} SNP improved seed germination to 86.0$\pm$3.2 % over the control (66.7$\pm$3.2 %) and the other nano-priming treatments. The rate of germination was significantly ($P \leq 0.05$) higher in the treated (Timson's Index: 43.7$\pm$2.4) than in the control (Timson's Index: 35.1$\pm$2.4) seeds. The pre-germination treatment also enhanced germination potential and emergence index. Seedling development was better in seeds treated with 200 mg L^{-1} SNP (53.3$\pm$3.2 %) than in the control (42.0$\pm$3.2 %). However, the higher concentrations of SNPs inhibited seed germination parameters. Pre-treatment with 75 mg L^{-1} SNP for 16 h resulted in higher fruit yield (170.9$\pm$16.5 g) over the control (141.4$\pm$16.5 g) and other treatments. Thus, the results revealed improved seed germination, seedling growth, and fruit yield in response to pre-treatment with 75-200 mg L^{-1} SNP in capsicum.

Keywords: Capsicum; Germination; Radicle; Seed nano-priming; Silver nanoparticles

1. INTRODUCTION

Capsicum (*Capsicum annum* L.) is an important vegetable crop cultivated around the year under frost-free conditions in different parts of the world. Under low-temperature conditions, the fruit set is affected due to poor pollination. Earlier studies have revealed slow seed germination in response to optimal as well as stressful conditions in capsicum[1-3]. Pre-germination treatments have been used to improve seed performance, growth, and yield in capsicum and other plants[4,5]. The pre-treated capsicum seeds are reported to show faster and more vigorous germination and superior performance even in response to sub-optimal conditions of cold or salt stress[6].

With continuous research in the field of nanotechnology, nanoparticles, and nanomaterials are being utilised in diverse fields such as medicine, pharmaceuticals, electronics, sensors, and agriculture[7-9]. Several chemical, physical, irradiation, and biological approaches are available for the synthesis of silver nanoparticles. The chemical synthesis of nanomaterials generates different hazardous by-products resulting in environmental contamination[10]. The physical methods have constraints due to the requirement of high temperature or pressure, types of equipment, and consumption of high energy. The biosynthetic methods are eco-friendly as the nanomaterials are synthesised using plant, bacteria, or fungal extracts[11]. The biosynthesis of silver nanoparticles using plants is a clean and environmentally safe method with the advantages of low-cost large-scale production capability[12].

Recent studies have revealed the potential of nanomaterials as bio stimulators in improving plant propagation and growth[13-14] and plant resistance to stress[15]. Silver nanoparticles are the most interesting metal nanoparticles, which exhibit strong biological activity[16]. The nanoparticles are reported to improve seed germination, vigour, and shoot proliferation [17].

Advances in the field of seed priming have resulted in the identification and utilisation of nano-materials for pre-germination treatments[18]. The seed-nano priming is being explored as one of the important tools to promote sustainability in agriculture. Recent studies have reported potential of seed nano-priming on improvement in germination, seedling growth, plant productivity, and produce quality[19-21]. The potential of different types of nanomaterials viz. metallic and/or oxide (iron, zinc, silicon, cobalt, calcium, manganese, titanium, silver, gold, copper, platinum, molybdenum) and polymeric (chitosan, lignin) have been assessed for seed nano-priming[18]. Further, metallic bio-genic nanoparticles synthesised using plant extracts as a reducing and stabilising agent are being employed in agriculture [22-23].

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Though the effects of the various seed priming treatments have been earlier reported in capsicum, such studies involving pre-treatment with silver nanoparticles have not yet been reported. Present study, was, hence designed to investigate the effect of seed pre-treatment with silver nanoparticles on germination, growth, and yield in capsicum.

2. MATERIALS AND METHODS

2.1 Synthesis of Silver Nanoparticles

Silver nanoparticles were bio-synthesised using leaves of *Thymus serphyllum*. The plants used in the experiment were grown in the Institute's field. Aqueous extract of leaves was prepared and treated with 1.0 mM silver nitrate ($AgNO_3$) as described in the earlier study[23]. The effect of various concentrations of plant extract and temperature was evaluated with UV-visible spectroscopic analysis. The silver nanoparticles synthesised from 20 % leaf extract with heating at 60 ^oC for 20 minutes were used in the study.

2.2 Seed Pre-Treatment

Capsicum (*Capsicum annum* L.) cv. HC-201 was used in the present study conducted at DIBER Field Station Pithoragarh, Uttarakhand, India. Seeds were pre-treated with 0-200 mg L^{-1} silver nanoparticle for 16 h with agitation at 100 rpm at 25 ^oC. Subsequently, solutions were discarded and the seeds were placed on tissue paper for drying under shade. The shade-dried seeds were placed on moistened tissue paper inside Petri dishes for germination. The un-soaked control seeds did not receive any soaking treatment. Data on seed germination were recorded every day for 13 days from the date of placing the seeds for germination. Similarly, data on vegetative growth parameters such as the number of branches and plant height were recorded 77 days after the date of transplanting. The data on yield attributing traits viz. number of set fruits and fruit weight were recorded 170 days after the date of transplanting the seedling.

The seed germination percentage was computed using the formula,. The speed of germination (Timson's Index) was calculated as reported earlier[24] using the formula: Timson Index = $\sum \frac{G}{t}$ where, G is the percentage of daily seed germination and t is the total germination period in days. A higher Timson's Index indicates a rapid germination process. Mean Emergence Time (MET) was computed as reported earlier[25] using the formula: $\text{MET} = (\sum D \times n)/\sum n$ where n is the number of seeds that emerged on day D, and D is the number of days from the onset of seed germination. MET is the indicator of the emergence performance of a seed lot. The lesser the MET, the better is the emergence performance. Emergence Index (EI) was computed as per the method described by the Association of Official Seed Analysis using the formula:

EI =(number of seeds germinated/days of first count) + …+ (number of seeds germinated/days of final count).

EI indicates the time taken to achieve a certain germination percentage. Thus, the higher the EI, the better the seed performance. A germination test was used to determine the germination potential or seed viability. The germination potential was computed using the formula (Gt/Tt) where Gt = number of germinated seeds on day t, Tt = number of days from the start of the germination. Subsequently, data on seedling development (opening of the cotyledonary leaves) were recorded for up to 20 days from the date of placing the seeds for germination.

2.3 Effect of Pre-Treatment on Plant Growth and Fruit Yield

Seedlings obtained from the pre-treated seeds were transplanted in plastic pots of 10-inch diameter filled with potting mixture for further growth. The pots were kept inside a glass house. Watering was carried out as per requirement. Observations on vegetative growth such as plant height (cm), number of branches, and fruit set were recorded 77 days from the date of transplanting. Data on fruit yield (g) were recorded 170 days after the date of transplanting.

2.4 Effect of Pre-Treatment with Higher Silver Nanoparticle Concentrations

Another seed pre-treatment experiment was performed with higher concentrations of silver nanoparticles (0-1000 mg L^{-1}) for 16 h with agitation at 100 rpm at 25 ^oC. At the end of the treatment, solutions were decanted and the seeds were placed on tissue paper for drying under shade. The shade-dried seeds were placed on moistened tissue paper inside Petri dishes for germination. Data on seed germination were recorded every day for 11 days from the date of placing the seeds for germination. Germination percentage, rate of seed germination, mean emergence time, emergence index, and germination potential were calculated as described in the first experiment. Subsequently, data on seedling development were recorded for up to 20 days from the date of placing the seeds for germination. Data were recorded on plant growth parameters viz. no. of branches and plant height after 50 days of transplanting. The data on yield attributing traits viz. numbers of fruits and fruit yield were recorded 143 days after transplanting of the seedlings.

2.5 Statistical Analysis

CropStat tool developed at IRRI, Philippines was used for Analysis of Variance-ANOVA for the experiment laid out in a completely randomised design. The seed pre-treatment experiments were performed with three replicates comprising 50 seeds each. The mean values were analysed for statistical significance using Duncan's New Multiple Range Test (DNMRT) at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

The seed pre-treatment with 200 mg L^{-1} silver nanoparticles significantly ($P \leq 0.05$) improved the seed germination (86.0 %) as compared to the other treatments and the un-soaked (56.0 %) and soaked (66.7 %) controls (Fig. 1; Table 1).

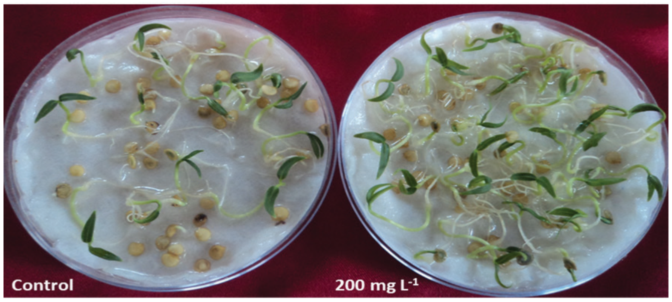

Figure 1. Improvement in germination through seed nano priming with silver nanoparticles in capsicum cv. HC-201. Images were taken 14 days after placing the seeds for germination.

The speed of seed germination was significantly higher in the seeds treated with 150 or 200 mg L^{-1} (Timson's Index: 43.7) than in the other treatments and the un-soaked (Timson's Index: 20.1) and soaked (Timson's Index: 35.1) controls. The higher the Timson's index, rapid the germination process. The treatment with the 150 mg L^{-1} silver nanoparticles significantly reduced the mean germination time (6.72 days) than the un-soaked (7.55 days) or soaked (7.48 days) controls. Similarly, the germination potential was higher in 200 mg L^{-1} silver nanoparticle-treated seeds (3.31) than in both the controls (2.15 and 2.56 respectively) and other treatments. In seeds treated with 150 or 200 mg L^{-1} silver nanoparticles, the emergence index was significantly higher (28.6 and 25.9 respectively) than the controls (14.5 and 19.8 respectively) and the other treatments with 25 to 125 mg L^{-1}silver nanoparticles.

Further, the effect of seed pre-treatment with silver nanoparticles was studied on seedling development. The percent opening of cotyledonary leaves was higher in 200 mg L^{-1}seed treatment (53.3 %) over the un-soaked (32.0 %) and soaked (42.0 %) controls (Fig. 1; Table 2). The speed of opening of cotyledonary leaves computed in terms of modified Timson's Index was also significantly higher in 150 mg L^{-1}seed treatment (31.6) than in the un-soaked (14.1) and soaked (23.1) controls. The treatment with 150 mg L^{-1}silver nanoparticles also significantly reduced the mean emergence time (8.1 days) than the un-soaked or soaked (8.8 days) controls. The results thus suggest enhanced seed performance in terms of better germination and seedling development through pre-treatment with the silver nanoparticles in capsicum. Seed pre-treatment with 75 mg L^{-1}or lower SNPs resulted in higher plant height and number of branches than that of pre-treated with the higher concentrations. In the context of yield attributing parameters, pre-treatment with 75 mg L^{-1}SNP resulted in higher fruit number (7.4 nos.) and fruit yield (170.9 g) than the control (6.9 nos; 141.4 g respectively) and other treatments (Table 3). The pre-treatment with the higher concentration (200 mg L^{-1}) of SNP resulted in reduced fruit yield (126.5 g) than that of pre-treated with 75 mg L^{-1}.

Further, an experiment was performed to investigate the effect of treatment with higher concentrations of silver nanoparticles on seed performance. Seed pre-treatment with 100 mg L^{-1} SNP improved the seed germination (90.0 %) than in the control (78.33 %) and the other treatments (Table 4). The speed of germination computed in terms of Timson's Index was higher in the seeds treated with 100 or 200 mg L^{-1} silver nanoparticles (56.42 and 53.00 respectively) than in the control (48.00) and other treatments. Mean emergence time was lower in seeds treated with 100 mg L^{-1} (7.00 days) or 200 mg L^{-1} (6.96 days) silver nanoparticles than the un-soaked or control treatment. Germination potential was higher in seeds treated with 100 mg L^{-1} silver nanoparticles (3.27) than the control (2.85) and other treatments. In seeds treated with 100 or 200 mg L^{-1}silver nanoparticles, the emergence index was higher (37.21 and 34.53 respectively) than the controls (29.76) and the other treatments. Further, the effect of seed pre-treatment with silver nanoparticles was studied on seedling development. The percent opening of cotyledonary leaves was higher in the 200 mg L^{-1} seed treatment (63.33 %) than in the control (57.50 %) and other treatment (Table 4). The rate of opening of cotyledonary leaves computed in terms of modified Timson's Index was also higher in 200 mg L^{-1} seed treatment (34.64) than the control (31.07). The results thus suggest enhanced seed performance in terms of germination and seedling development through pre-treatment with 100 or 200 mg L^{-1}SNP in capsicum. Higher concentrations however inhibited the seed performance in terms of the different germination parameters. The pre-treatment with higher concentrations also resulted in reduced plant growth and fruit yield (Table 5). Higher numbers of branches (7.9) were recorded in plants obtained from pre-treatment with 100 mg L^{-1}SNP than in the control and other treatments. Among the treatments, pre-treatment with 100 mg L^{-1}silver nanoparticles resulted in a higher number of fruits (5.1) and fruit yield (69.7 g) than that of control and other treatments (Table 5).

Chemical seed pre-treatments have been used to accelerate uniform seed germination, seedling vigor, and yield in many of crop plants including capsicum. Earlier, Patade[5], *et al.* reported the effect of chemical pre-treatments on seed germination in capsicum. The seeds pre-treated with polyethylene glycol, PEG-6000 (16.7 mM) showed a significant increase in speed as well as total germination. Further, the seedlings produced from the pre-treated seed showed improved tolerance to salt or cold stress exposure whereas the seedlings raised from the control seeds failed to survive the exposure to the stresses. Besides, the vegetative growth of the seedlings generated from the pre-treated seeds was superior to those obtained from the control. Recently, Vijai[21], *et al.* reported the stimulatory effect of calcium nanoparticles synthesised from marine molluscan shells of Lima lima. The pre-treatment with calcium nanoparticles at a concentration of 250 mg L^{-1} produced higher radicle growth, seed germination, vigor index, and vegetative growth than the control in green gram.

Table 1. Effect of pre-treatment with silver nanoparticles (SNP) on seed germination in capsicum cv. HC-201

SNP concentration (mg L^{-1})	Radicle protrusion				
	Germination (%)	Timson's index	Mean germination time (Days)	Germination potential	Emergence index
Un Soaked	56.0 d,e	20.1 d	7.55 d,e	2.15 c,d	14.5 c
0	66.7 b,c	35.1 b,c	7.48 d,e	2.56 b,c	19.8 b,c
25	64.7 b,c,d	31.3 b,c	7.63 d,e	2.49 b,c	17.7 b,c
50	60.7 c,d	35.3 b	7.07 b,c	2.33 c,d	21.4 b
75	67.3 b,c,d	34.7 b,c	7.23 c	2.59 b,c	20.4 b
100	51.3 e	27.9 c	6.93 a,b	1.97 d	17.1 b,c
125	58.7 d,e	28.6 b,c	7.70 e	2.26 c,d	16.2 c
150	72.0 b	43.7 a	6.72 a	2.77 b	28.6 a
200	86.0 a	43.7 a	7.46 c,d	3.31 a	25.9 a
SE (n= 3)	3.2	2.4	0.08	0.13	1.7
5 % LSD	9.7	7.2	0.23	0.38	5.1

Observations on seed germination were recorded for 13 days from the date of placing seeds for germination. Mean values (n=3) for a particular trait marked with different alphabets are significantly different as per the least significant difference test ($P \leq 0.05$).

Table 2. Effect of seed pre-treatment with silver nanoparticles (SNP) on seedling development in capsicum cv. HC-201

SNP concentration (mg L^{-1})	Opening of cotyledonary leaves		
	%	Rate (modified timson's index)	Mean emergence time (Days)
Un Soaked	32.0 c,d	14.1 e	8.8 a,b
0	42.0 b,c	23.2 b,c	8.8 a,b
25	32.0 c,d	17.4 d,e	9.1 a
50	31.3 c,d	19.8 c,d	8.3 c,d
75	40.7 c	23.6 b,c	8.5 b,c
100	30.7 d	21.5 c,d	8.3 c,d
125	31.3 c,d	15.0 e	9.1 a
150	51.3 a,b	31.6 a	8.1 d
200	53.3 a	27.4 a,b	8.7 b
SE (n= 3)	3.2	1.6	0.1
5 % LSD	9.6	4.8	0.3

Mean values (n=3) for a particular trait marked with different alphabets are significantly different as per the least significant difference test ($P \leq 0.05$).

Table 3. Effect of seed pre-treatment with silver nanoparticles (SNP) on plant growth and yield attributing traits in capsicum cv. HC-201

SNP concentration (mg L^{-1})	Vegetative traits		Yield attributing traits	
	Plant height (cm)	Branches (Nos)	Fruits (Nos)	Fruit Yield (g)
Un Soaked	82.9 b,c	12.8 b,c	5.3 b,c,d	137.8 a,b
0	93.3 a	13.8 a,b,c	6.9 a,b	141.4 a,b
25	88.1 a,b	13.0 b	6.4 a,b,c	120.0 b
50	88.3 a,b	15.1 a	5.4 b,c,d	134.9 a,b
75	85.8 a,b	12.4 b,c	7.4 a	170.9 a
100	77.5 c	9.3 d	6.0 a,b,c	124.9 b
125	75.9 c	10.9 c,d	5.0 c,d	115.0 b
150	77.8 c	9.9 d	5.5 b,c,d	150.9 a,b
200	74.4 c	8.6 d	4.5 d	126.5 b
SE (n= 3)	2.7	0.7	0.5	16.5
5 % LSD	7.6	2.0	1.4	46.7

Observations on vegetative growth such as plant height (cm), branch (no), and fruit setting were recorded 77 days after the date of transplanting. Data on fruit yield (g) were recorded 170 days after the date of transplanting. Mean values (n=8) for a particular trait marked with different alphabets are significantly different as per the Least Significant Difference test ($P \leq 0.05$).

Table 4. Effect of pre-treatment with higher concentrations of silver nanoparticles (SNP) on seed germination in capsicum cv. HC-201

SNP concentration (mg L^{-1})	Radicle protrusion					Opening of cotyledonary leaves	
	Germination (%)	Rate	Mean emergence time (Days)	Germination potential	Emergence index	%	Rate
0	78.33 b	48.00b,c	7.48 c	2.85 b,c	29.76 c	57.50 d	31.07 c
100	90.00 e	56.42 e	7.00 a	3.27 f	37.21 e	60.00 d,e	32.86 c
200	80.00 b,c	53.00 d,e	6.96 a	2.79 b	34.53 d,e	63.33 e	34.64 c
300	80.83 b,c	47.00 b	7.17 a,b	2.94 c,d	26.44 b	45.00 b	23.45 b
400	85.83 d	51.17 c,d	7.59 c,d	3.12 e	31.87 c,d	50.00 c	21.19 b
500	83.33 c,d	50.58 b,c	7.36 b,c	3.03 d,e	31.97 c,d	45.83 b	25.00 b
1000	69.17 a	29.33 a	7.85 d	2.52 a	16.12 a	24.17 a	13.33 a
SE (n= 3)	1.37	1.22	0.08	0.04	1.01	1.40	1.44
5% LSD	4.21	3.75	0.26	0.13	3.13	4.32	4.43

Observations on radicle protrusion were recorded for 11 days from the date of placing seeds for germination. Mean values (n=3) for a particular trait marked with different alphabets are significantly different as per the Least Significant Difference test ($P \leq 0.05$).

Table 5. Effect of seed pre-treatment with higher concentrations of silver nanoparticles (SNP) on vegetative and yield attributing traits in capsicum cv. HC-201

SNP concentration (mg L^{-1})	Vegetative traits		Yield attributing traits	
	Plant height (cm)	Branches (Nos)	Fruits (Nos)	Fruit yield (g)
0	50.6 a,b	6.7 a,b,c	3.4 b	30.7 b
100	52.6 a,b	7.9 a	5.1 a	69.7 a
200	49.4 a,b	6.3 a,b,c	3.3 b	32.6 b
300	49.1 a,b	6.4 a,b,c	3.6 b	31.8 b
400	46.3 b	5.6 b,c	4.1 b	29.9 b
500	54.9 a	5.4 c	3.7 b	29.9 b
1000	52.9 a,b	5.1 c	4.0 b	41.8 b
SE (n= 3)	2.4	0.6	0.3	5.8
5 % LSD	6.8	1.6	0.9	16.6

Observations on vegetative traits were recorded 50 days from the date of transplanting. Observations on yield attributing traits were recorded 143 days after the date of transplanting. Mean values (n=7) for a particular trait marked with different alphabets are significantly different as per the Least Significant Difference test ($P \leq 0.05$).

Almutairi and Alharbi[26] reported the effect of treatment with different concentrations of silver nanoparticles on germination in corn, watermelon, and zucchini plants. The study reported different dosage responses on germination and growth in the plant species. The ideal concentrations to stimulate the germination and plant growth were different in the different species. The study reported a negative effect of the higher concentrations of nanoparticles on seed germination and growth similar to that observed in the present study. Earlier, Vishwakarma[27], *et al.* also reported concentration-dependent differential phytotoxic impact of SNP (1 and 3 mM) bio-synthesised using *Aloe vera* extract in mustard seedlings. The adverse effects of the SNP were attributed to the induced oxidative stress associated with enhanced accumulation of the SNP. Besides, the accumulation of SNP inside plant cells is reported to alter the plant hormones[28]. The positive or negative biological responses depend on the physico-chemical properties of the nanoparticles like size, concentration, and zeta potential[29]. Detailed investigations are further required to study the interactions of SNP with plants.

4. CONCLUSION

The present study provides new insights on enhanced germination, seedling development, vegetative growth, and fruit yield through seed pre-treatment with 75-200 mg L^{-1} silver nanoparticles in capsicum. The findings also indicated concentration-dependent positive and negative effects of the seed nano priming on seed performance, plant growth as well as fruit yield.

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