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Sakshi Kademani
Research Scholar, Department of Seed Science and Technology, College of Agriculture, Dharwad University of Agricultural Sciences, Dharwad, Karnataka, India

Chandrashekhar SS
Associate Professor, Department of Seed Science and Technology, College of Agriculture, Dharwad, University of Agricultural Sciences, Dharwad, Karnataka, India

Hunje Ravi
Professor (SS & T) & SOS (Seeds), Department of Seed Science & Technology College of Agriculture, Dharwad UAS, Dharwad, Karnataka, India

Motagi BN
Senior Scientist, Genetics and Plant Breeding and Head, Agricultural Research Station, Annigeri, University of Agricultural Sciences, Dharwad, Karnataka, India

Corresponding Author:
Sakshi Kademani
Research Scholar, Department of Seed Science and Technology, College of Agriculture, Dharwad University of Agricultural Sciences, Dharwad, Karnataka, India

Effect of green synthesized silver and copper nanoparticles to enhance seed quality parameters of groundnut (*Arachis hypogaea* L.)

Sakshi Kademani, Chandrashekhar SS, Hunje Ravi and Motagi BN

Abstract
Groundnut (*Arachis hypogaea* L.) is referred as 'King of Oilseeds'. Time immemorable, silver and copper have received substantial attention to enhance the seed quality through seed nano priming. The laboratory experiment was conducted to investigate the effect of green synthesized silver and copper nanoparticles on seed quality parameters in groundnut JL-24 and Dh-256 varieties during 2022-23 at the Department of Seed Science and Technology, University of Agricultural Sciences, Dharwad. The experiment was laid out in Factorial completely randomized design with three replications and nine treatments *viz.*, For T_1, T_2 and T_3 - seeds were primed with silver nanoparticles of concentration 200, 300, and 400 ppm respectively for 1 h. T_4, T_5, and T_6 – seeds were primed with copper nanoparticles of concentration 100, 200, and 300 ppm respectively for 1 h. T_7 and T_8 - Seeds primed with 1mM $AgNO_3$ and 1mM $CuSO_4$, T_9 – Hydroprimed. Results revealed that among treatments seeds primed with silver nanoparticles 400 ppm recorded higher germination (%) (95.83%), Seedling Vigour Index-II (415), Seedling Dry Weight and lower Electrical Conductivity and seeds primed with 300 ppm silver nanoparticles recorded higher Seedling Vigour Index I, shoot and root length compared to control. Overall, Dh-256 recorded higher seed quality parameters. For germination percent, seedling length, Seedling Vigour Index I, Seedling Vigour Index II, Seedling dry weight and Electrical conductivity interaction between treatments and genotypes was significant.

Keywords: AgNPs, CuNPs, groundnut, seed nano-priming, seed quality

Introduction
Nanotechnology is a rapidly advancing field with significant implications for the agricultural sector. Phytosynthesis, or the production of nanoparticles using plants, is gaining prominence since it provides a one-step biosynthesis process. As a result, employing green materials offers a number of benefits, including low energy use, cost effectiveness and moderate operating conditions (such as pressure and temperature) without the hazardous chemicals (Mie *et al*., 2014) [9]. Through green chemistry, synthesis of nanoparticles is mediated by plant extracts for the bio reduction of metal ions to nanoparticles. Seeds primed with nanoparticles enhance physiological parameters like germination, seedling growth and vigour because direct effect of elemental composition.

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop and is referred to as 'King of Oilseeds'. However, as being an oilseed crop, viability is lost quickly due to the production of free radicals by lipid peroxidation during storage. Low yield in groundnut under present scenario is attributed to several factors of biotic and abiotic stress conditions.

Guava leaves contain a variety of bioactive compounds including tannins, flavonoids, phenolics, ascorbic acid, proteins, and polysaccharides. These compounds include quercetin, gallic acid, Guaijavarin, Catechin, Epicatechin, rutin, gallic acid, kaempferol and glycosides. (Lorena *et al*., 2022) [8]; (Barbalho *et al*., 2012) [5]; (Rahman *et al*., 2018) [11]. This can be used to stabilize and reduce metal nanoparticles. Silver and copper are a substantial element as antimicrobials. Silver nanoparticles (Ag NPs), a type of metal nanoparticle, are often used in a number of research fields, including medicine, catalysis, antibacterial agents and in diagnostics. Silver nanoparticles have remained focus of most researchers due to their numerous biomedical applications. The application of Ag and Cu nanoparticles have proved to enhance the seed quality through nano priming, according to the findings of various research. The present work is designed through a novel, rapid and cost-effective method for synthesis of AgNPs and CuNPs by guava leaf extract and to evaluate effect of Ag and CuNPs on seed quality parameters.

Materials and Methods

The laboratory experiment was conducted to investigate the effect of green synthesized Ag and Cu nanoparticles on seed quality parameters in groundnut varieties JL-24 and Dh-256 during 2022-23 at the Department of Seed Science and Technology, UAS Dharwad. The experiment was laid out in Factorial completely randomized design with 3 replications and 9 treatments *viz*., For T_1, T_2 and T_3 – seeds primed with AgNPs of concentration 200, 300, and 400 ppm respectively for 1 h and T_4, T_5, and T_6 – seeds primed with CuNPs of concentration 100, 200, and 300 ppm respectively for 1 h and T_7, T_8: Seeds primed with 1mM $AgNO_3$ and 1mM $CuSO_4$, T_9 – Hydroprimed. The different concentrations of Ag and CuNPs suspension were prepared with 400 ppm stock solution of $AgNO_3$ and $CuSO_4$ respectively through distilled water. The seed to solution ratio used for nano-priming treatment was 1:2. Later seeds were shade dried to bring down to original moisture content under ambient condition. Observations on seed quality parameters were recorded. Germination test was conducted in 3 replications of 50 seeds each by adopting between paper towel methods as described by ISTA procedures (Anon., 2019) [4]. Germination percentage was recorded on 10th day on the basis of normal seedlings. Ten normal seedlings were randomly selected from each replication, shoot and root length measured on 10th day of standard germination test and expressed in centimeter using metric scale. Vigour Index was computed by following formula and expressed in number (Abdul-Baki and Anderson, 1973) [2]. The ten normal seedlings used for measuring root and shoot length were put in a butter paper packet and kept them in hot air oven maintained at 80 ± 1 °C for 24 hours. The dry weight of the seedlings was recorded and expressed in grams (g) per 10 seedlings. The electrical conductivity of the seed leachate was measured in the digital conductivity bridge (ELICO) with a cell constant 1.0 and the mean values were expressed in Deci Simons per meter (dSm^{-1}) (Milosevic *et al*., 2010) [10].

Results and Discussion

Among the treatments significantly higher mean germination (%) was recorded in T_3 (AgNPs 400 ppm) primed seeds (95.83%) compared to control *i.e.*, T_9 (hydropriming) (Table. 1). Enhanced α - amylase activity, through nanopriming will produce more soluble sugar, which would accelerate the growth of seedlings. In addition, aquaporin genes were stimulated to increase in expression in germination-ready seeds by nanopriming. In the germinating seeds subjected to nanopriming treatment, a higher level of ROS production was observed in comparison to the unprimed control and other primed treatments. This observation highlights the significance of both ROS and aquaporins in facilitating seed germination.

Between the varieties Dh-256 (93.63%) recorded significantly higher mean germination (%) as compared to JL-24 (93.19%). This is due to genetic components of Dh-256 variety. A significant interaction between treatments and the varieties was observed in relation to the germination percentage. Higher germination percent was recorded with AgNPs (400 ppm) primed seeds (96.33%) in Dh-256 (Table.1). Nanoparticles contribute positively in enhancing seed quality by implying in process that involves oxidation and reduction during germination. This is achieved by generating superoxide ion radicals, which effectively neutralize free radicals present during seed germination. Enhanced oxygen produced in this process leads to higher respiration and this further encourage germination. These results are in compliance with the findings (Shyla and Natarajan, 2014) [12].

Significantly higher mean shoot (cm) length was recorded in T_2 (AgNPs 300 ppm) primed seeds (9.54 cm) as compared to control *i.e.*, T_9 (hydropriming) (7.59 cm). Between the varieties significantly higher mean shoot length (cm) was recorded in Dh-256 (9.06 cm) as compared to JL-24 (7.51 cm). This is due to genetic components of Dh-256 variety. The interaction effect between treatments and varieties was found significant with shoot length. Significantly higher shoot length (cm) was recorded with AgNPs (300 ppm) primed seeds (10.50 cm) in Dh-256 (Table. 2).

Higher mean root length among treatments was recorded in T_2 (AgNPs 300 ppm) primed seeds (19.13 cm) as compared to control *i.e.*, T_9 (hydropriming) (16.50 cm). Significantly higher mean root length was recorded in Dh-256 (18.38 cm) compared to JL-24 (16.92 cm). The interaction between treatments and varieties were significant for root length. Higher root length was recorded with AgNPs (300 ppm) primed seeds (20.00 cm) in Dh-256 (Table. 3). The formation of pores helps in nanoparticles to penetrate through the seed coat and stimulate the genes for aquaporin. This promotes the transport of H_2O_2, water into cells and enhances α - amylase activity there by enhancing seedling emergence. Nanopriming will produce more soluble sugar, which would assist the growth of seedlings. Based on the studies conducted by (Acharya *et al.*, 2019) [3] in aged onion seeds, it was discovered that the same activity improved the starch metabolism needed for seedling growth. Similar findings reported in (Hojjat and Hojjat, 2015) [6] and Jassem *et al*., 2018 [7].

Significantly higher mean seedling dry weight was recorded in T_3 (AgNPs 400 ppm) primed seeds (4.33 g) as compared to control *i.e.*, T_9 (hydropriming) (2.73 g). Between the varieties significantly higher mean seedling dry weight was recorded in Dh-256 (3.21 g) as compared to JL-24 (3.16 g). The interaction effect among treatments and varieties was found significant with seedling dry weight. Significantly higher seedling dry weight was recorded with AgNPs (400 ppm) primed seeds (4.40 g) in Dh-256 (Table.4). Suriyaprabha *et al.* (2012) [13] reported that the content of organic substances in the primed seeds, including protein, chlorophyll, and phenols, was increased by the application of nanoparticles to the seed. Due to increase in seedling length (cm) through nanopriming, primed seeds recorded higher seedling dry weight. Abbasi *et al*. (2016) [1] also noted comparable outcomes after applying silver nanoparticles to *Thymus Kotschyanus*.

Significantly higher mean Seedling Vigour Index-I was recorded in T_2 (AgNPs 300 ppm) primed seeds (2705) as compared to control *i.e.*, T_9 (hydropriming) (2196). Higher mean Seedling Vigour Index-I between varieties was recorded in Dh-256 (2572) followed by JL-24 (2277). The interaction effect between treatments and varieties was significant with Seedling Vigour Index I. Significantly higher Seedling Vigour Index I was recorded with AgNPs (300 ppm) primed seeds (2887) in Dh-256 (Table.5).

Higher mean seedling vigour index – II among treatments was recorded in T_3 (AgNPs 400ppm) primed seeds (415) as compared to control *i.e.*, T_9 (hydropriming) (248). Between the varieties significantly higher mean seedling seedling vigour index – II was recorded in Dh-256 (301). The interaction effect among treatments and varieties was found significant with seedling vigour index – II. Significantly higher seedling vigour index – II was recorded with AgNPs (400 ppm) primed seeds (424) in Dh-256 (Table. 6)

Compared to the untreated control, the seed treatment with nanoparticles considerably changed the seed quality parameters and consistently preserved the viability and vigour of the seeds. In terms of seed germination (%), seedling shoot length (cm), seedling root length(cm) and Seedling Vigour Index – I seeds treated with nanoparticles significantly increased as compared to control. The seed treatment with AgNPs outperforms other treatments examined, including the control.

Leachates of seed as influenced by the seed's nano primed with silver and copper nanoparticles varied significantly. lower mean electrical conductivity was recorded in T_3 (AgNPs 400 ppm) primed seeds (0.064) which is on par with T_4 (CuNPs 100 ppm) primed seeds (0.070) and T_2 (AgNPs 300 ppm) primed seeds (0.074) as compared to control *i.e.*,T_9 (hydropriming) (0.250). Between the varieties significantly lower mean electrical conductivity was recorded in Dh-256 variety (0.106) which is on par with JL-24 variety (0.109). The interaction due to treatments and variety was found significant with electrical conductivity Significantly lower electrical conductivity was recorded in Dh-256 variety with T_3 (AgNPs 400 ppm) (0.045) (Table. 7) AgNPs at 400 ppm, 300ppm and copper nanoparticles at 100 ppm showed lower electrical conductivity (dSm^{-1}) as compared with control and their interaction effect recorded lower electrical conductivity (dSm^{-1}) in Dh-256 variety with T_3, T_4 and T_2. Among varieties both JL-24 and Dh-256 showed lower electrical conductivity (dSm^{-1}). Nanoparticle treatment increased the enzymatic activity and free radical removal system by quenching the free radicals and reduced oxidative damages. This ultimately increased the viability and vigour of seeds.

Table 1: Effect of seed priming with Ag and Cu nanoparticles on germination (%) of groundnut

Treatments	Germination (%)		
	V_1 - JL-24	V_2 - Dh-256	Mean
T_1 - Seeds primed with AgNPs at 200 ppm for 1 h	93.00 (74.66) *	93.67 (75.43)	93.33 (75.05)
T_2 - Seeds primed with AgNPs at 300 ppm for 1 h	94.00 (75.82)	94.67 (76.66)	94.33 (76.24)
T_3 - Seeds primed with AgNPs at 400 ppm for 1 h	95.33 (77.54)	96.33 (78.98)	95.83 (78.26)
T_4 - Seeds primed with CuNPs at 100 ppm for 1 h	93.67 (75.43)	94.67 (76.66)	94.17 (76.05)
T_5 - Seeds primed with CuNPs at 200 ppm for 1 h	93.00 (74.66)	93.67 (75.43)	93.33 (75.05)
T_6 - Seeds primed with CuNPs at 300 ppm for 1 h	92.67 (74.30)	93.67 (75.43)	93.17 (74.86)
T_7 - Seeds primed with 1mM $AgNO_3$	92.67 (74.30)	93.00 (74.66)	92.83 (74.48)
T_8 - Seeds primed with 1mM $CuSO_4$	92.33 (73.93)	92.67 (74.30)	92.50 (74.11)
T_9 - Hydroprimed (Control)	92.00 (73.57)	90.33 (71.89)	91.17 (72.73)
Mean	93.19 (74.91)	93.63 (75.49)	
	S.Em (±)	C.D (1%)	
Varieties	0.114	0.44	
Treatments	0.243	0.94	
Varieties × Treatments	0.343	1.33	

* Values in the parenthesis are arcsin transformed

Table 2: Effect of seed priming with Ag and Cu nanoparticles on shoot length (cm) of groundnut

Treatments	Shoot length (cm)		
	V_1 - JL-24	V_2 - Dh-256	Mean
T_1 - Seeds primed with AgNPs at 200 ppm for 1 h	7.33	9.10	8.21
T_2 - Seeds primed with AgNPs at 300 ppm for 1 h	8.58	10.50	9.54
T_3 - Seeds primed with AgNPs at 400 ppm for 1 h	7.57	9.42	8.49
T_4 - Seeds primed with CuNPs at 100 ppm for 1 h	7.58	9.50	8.54
T_5 - Seeds primed with CuNPs at 200 ppm for 1 h	7.42	9.13	8.28
T_6 - Seeds primed with CuNPs at 300 ppm for 1 h	7.33	9.17	8.25
T_7 - Seeds primed with 1mM $AgNO_3$	7.30	8.50	7.90
T_8 - Seeds primed with 1mM $CuSO_4$	7.25	8.25	7.75
T_9 - Hydroprimed (Control)	7.22	7.97	7.59
Mean	7.51	9.06	
	S.Em (±)	C.D (1%)	
Varieties	0.025	0.10	
Treatments	0.053	0.21	
Varieties × Treatments	0.076	0.29	

Table 3: Effect of seed priming with Ag and Cu nanoparticles on root length (cm) of groundnut

Treatments	Root length (cm)		
	V_1 - JL-24	V_2 - Dh-256	Mean
T_1 - Seeds primed with AgNPs at 200 ppm for 1 h	17.17	18.17	17.67
T_2 - Seeds primed with AgNPs at 300 ppm for 1 h	18.25	20.00	19.13
T_3 - Seeds primed with AgNPs at 400 ppm for 1 h	17.22	18.83	18.03
T_4 - Seeds primed with CuNPs at 100 ppm for 1 h	17.22	19.00	18.11
T_5 - Seeds primed with CuNPs at 200 ppm for 1 h	17.00	18.33	17.67

T_6 - Seeds primed with CuNPs at 300 ppm for 1 h	16.58	18.10	17.34
T_7 - Seeds primed with 1mM $AgNO_3$	16.50	18.00	17.25
T_8 - Seeds primed with 1mM $CuSO_4$	16.33	17.98	17.16
T_9 - Hydroprimed (Control)	16.00	17.00	16.50
Mean	16.92	18.38	
	S.Em (±)	C.D (1%)	
Varieties	0.028	0.11	
Treatments	0.059	0.23	
Varieties × Treatments	0.084	0.32	

Table 4: Effect of seed priming with Ag and Cu nanoparticles on seedling dry weight (g) of groundnut

Treatments	**Seedling dry weight(g)**		
	V_1 - JL-24	**V_2 - Dh-256**	**Mean**
T_1 - Seeds primed with AgNPs at 200 ppm for 1 h	3.13	3.12	3.12
T_2 - Seeds primed with AgNPs at 300 ppm for 1 h	3.10	3.12	3.11
T_3 - Seeds primed with AgNPs at 400 ppm for 1 h	4.26	4.40	4.33
T_4 - Seeds primed with CuNPs at 100 ppm for 1 h	3.24	3.25	3.24
T_5 - Seeds primed with CuNPs at 200 ppm for 1 h	3.16	3.08	3.12
T_6 - Seeds primed with CuNPs at 300 ppm for 1 h	3.05	3.08	3.07
T_7 - Seeds primed with 1mM $AgNO_3$	2.98	3.00	2.99
T_8 - Seeds primed with 1mM $CuSO_4$	2.91	2.98	2.95
T_9 - Hydroprimed (Control)	2.57	2.88	2.73
Mean	3.16	3.21	
	S.Em (±)	C.D (1%)	
Varieties	0.014	0.05	
Treatments	0.030	0.11	
Varieties × Treatments	0.042	0.16	

Table 5: Effect of seed nano priming with Ag and Cu nanoparticles on Seedling Vigour Index - I of groundnut

Treatments	**Seedling Vigour Index I**		
	V_1 - JL-24	**V_2 - Dh-256**	**Mean**
T_1 - Seeds primed with AgNPs at 200 ppm for 1 h	2279	2554	2416
T_2 - Seeds primed with AgNPs at 300 ppm for 1 h	2522	2887	2705
T_3 - Seeds primed with AgNPs at 400 ppm for 1 h	2363	2721	2542
T_4 - Seeds primed with CuNPs at 100 ppm for 1 h	2323	2698	2510
T_5 - Seeds primed with CuNPs at 200 ppm for 1 h	2271	2582	2426
T_6 - Seeds primed with CuNPs at 300 ppm for 1 h	2216	2554	2385
T_7 - Seeds primed with 1mM $AgNO_3$	2205	2465	2335
T_8 - Seeds primed with 1mM $CuSO_4$	2178	2431	2304
T_9 - Hydroprimed (Control)	2136	2255	2196
Mean	2277	2572	
	S.Em (±)	C.D (1%)	
Varieties	3.470	13.34	
Treatments	7.361	28.31	
Varieties × Treatments	10.409	40.03	

Table 6: Effect of seed nano priming with Ag and Cu nanoparticles on Seedling Vigour Index - II of groundnut

Treatments	**Seedling Vigour Index II**		
	V_1 - JL-24	**V_2 - Dh-256**	**Mean**
T_1 - Seeds primed with AgNPs at 200 ppm for 1 h	291	292	292
T_2 - Seeds primed with AgNPs at 300 ppm for 1 h	291	295	293
T_3 - Seeds primed with AgNPs at 400 ppm for 1 h	406	424	415
T_4 - Seeds primed with CuNPs at 100 ppm for 1 h	303	308	306
T_5 - Seeds primed with CuNPs at 200 ppm for 1 h	294	290	292
T_6 - Seeds primed with CuNPs at 300 ppm for 1 h	283	289	286
T_7 - Seeds primed with 1mM $AgNO_3$	276	279	278
T_8 - Seeds primed with 1mM $CuSO_4$	269	276	272
T_9 - Hydroprimed (Control)	236	260	248
Mean	294	301	
	S.Em (±)	C.D (1%)	
Varieties	0.976	3.75	
Treatments	2.071	7.96	
Varieties × Treatments	2.929	11.26	

Table 7: Effect of seed nano priming with Ag and Cu nanoparticles on Electrical conductivity (dSm^{-1}) of groundnut

Treatments	Electrical conductivity (dSm^{-1})		
	V_1 - JL-24	V_2 - Dh-256	Mean
T_1 - Seeds primed with AgNPs at 200 ppm for 1 h	0.095	0.085	0.090
T_2 - Seeds primed with AgNPs at 300 ppm for 1 h	0.093	0.055	0.074
T_3 - Seeds primed with AgNPs at 400 ppm for 1 h	0.083	0.045	0.064
T_4 - Seeds primed with CuNPs at 100 ppm for 1 h	0.087	0.054	0.070
T_5 - Seeds primed with CuNPs at 200 ppm for 1 h	0.096	0.093	0.094
T_6 - Seeds primed with CuNPs at 300 ppm for 1 h	0.103	0.105	0.104
T_7 - Seeds primed with 1mM $AgNO_3$	0.109	0.096	0.102
T_8 - Seeds primed with 1mM $CuSO_4$	0.123	0.119	0.121
T_9 - Hydroprimed (Control)	0.198	0.303	0.250
Mean	0.109	0.106	
	S.Em (±)	C.D (1%)	
Varieties	0.001	0.003	
Treatments	0.002	0.007	
Varieties × Treatments	0.003	0.010	

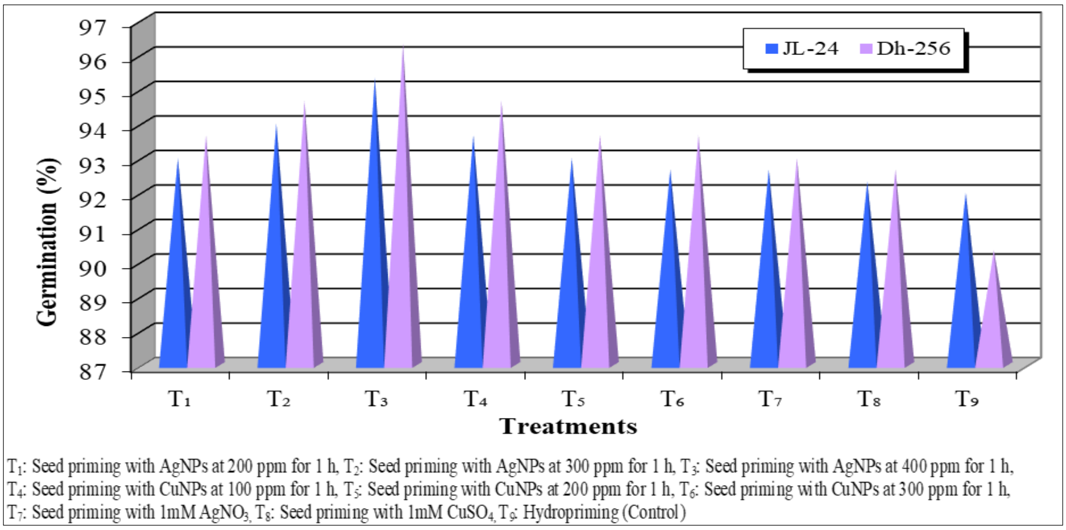

Fig 1: Effect of seed priming with Ag and Cu nanoparticles on germination of groundnut

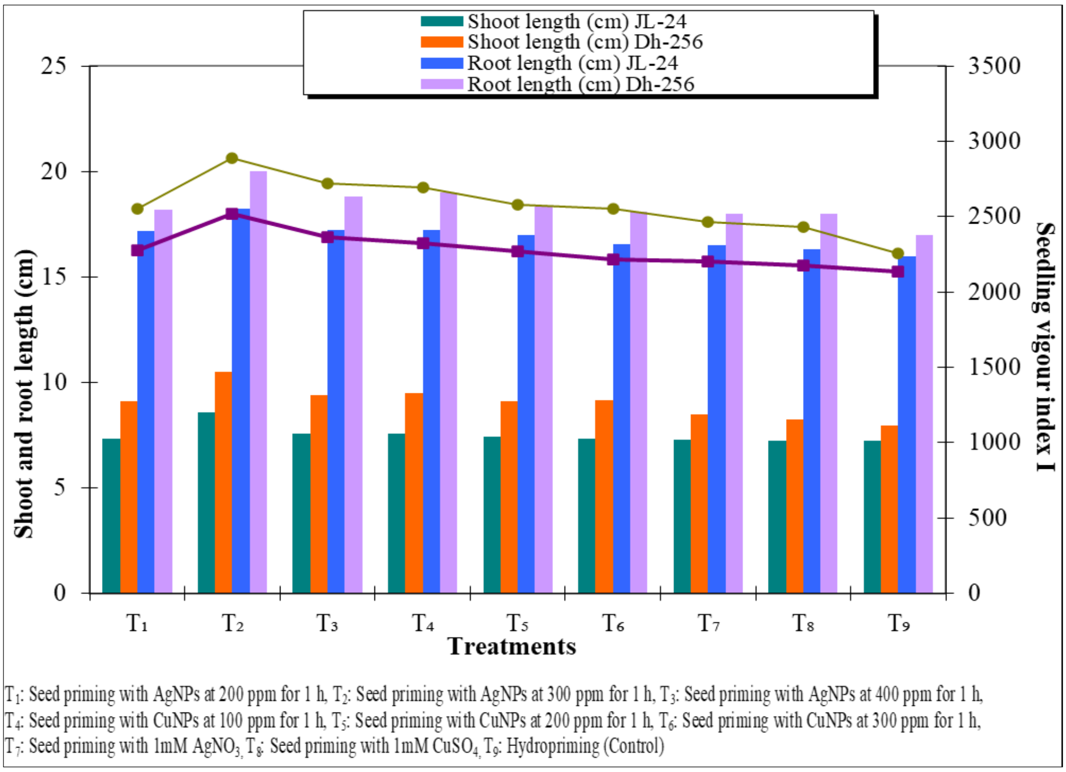

Fig 2: Effect of seed priming with Ag and Cu nanoparticles on shoot length (cm), root length (cm) and SVI-I of groundnut

Conclusion

Green synthesis of AgNPs and CuNPs was successfully carried out through leaf extracts of guava (*Psidium guajava*) as reducing and capping agents. As compared to control significantly higher seed quality parameters were recorded in seeds primed with AgNPs (400 ppm) and AgNPs (300 ppm) under lab conditions. Dh-256 variety recorded higher seed quality parameters and interaction effect was significant compared to JL-24. The outcome of this experiment unequivocally demonstrates that AgNPs can be used as an effective priming agent for enhancing seed quality.

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