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IMPACT OF SILVER AND COPPER NANOPARTICLES ON SEED GERMINATION, GROWTH AND NUTRITIONAL PROFILING OF PAPAYA (*CARICA PAPAYA* L.) SEEDLINGS

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ABSTRACT

A study was laid out to identify the efficacy of metallic silver and copper nanoparticles at various concentrations on seed germination, growth, and plant nutrient properties of red lady papaya seeds at Department of Horticulture, Nagaland University from December 2021 to February 2022. The seeds were treated with metallic silver NPs (< 90 nm) and copper NPs (50 nm) suspension @ 5, 10 and 15 ppm by incubating eight hours at 225 rpm in an orbital shaker. Seeds were sown in a seedbed followed by standard cultural practices under shade net conditions. The seeds treated with AgNPs exerted significantly superior results compared with CuNPs in different plant morphological and biochemical attributes. The highest germination percentage (89.63%) at 25 days after sowing and seed germination index (2.78) were recorded with metallic AgNPs @ 10 ppm. AgNPs clearly demonstrated more accumulation of N (0.77±0.09%), P (0.26±0.03%) and K (0.24±0.01%) content in plant tissue and also significantly improved the seedling vigor index (54.67±14.43) over control (31.58). Priming with nanoparticle suspension enhanced the leaf area, fresh and dry matter content of seedlings and other physiological and biochemical properties of seedlings without any evidence of biotic stress in papaya seedlings.

***Keywords* :** AgNPs, CuNPs, germination, plant growth, papaya and red lady

Introduction

Nanoparticles are atomic or molecular aggregates with at least one dimension between 1 and 100 nm that can drastically modify their physico-chemical properties compared to bulk material (Nel *et al.*, 2006). Nanoparticles are characterized by low weight, small size, and a high surface-to-volume ratio (Khan *et al.*, 2017; Mavani *et al.*, 2013). The application of NPs in agriculture is currently an interesting area for minimizing the use of chemical inputs. Nanoparticles (NPs) of different metal oxides can affect seed germination percentage, improve growth, dry weight, photosynthesis, chlorophyll biosynthesis and plant metabolism (Rezaei *et al.*, 2015). The most common and frequently used of nanoparticles in agriculture sectors are oxides of Zn, Cu, Ti, Ag and Fe. In agriculture, silver nanoparticles play an important role in enhancing seed germination (Shelar and Chavan, 2015; Savithramma *et al.*, 2012), plant growth (Kaveh *et al.*, 2013; Sharma *et al.*, 2012), improving chlorophyll and photosynthetic quantum efficiency (Sharma *et al.* 2012), increasing fertilizer and water use efficiency and antibacterial properties. CuO NPs are used in various industries including agriculture (Rajput *et al.*, 2018) where it can be phytotoxic at higher concentration (Banik and Perez-de-Luque 2017). Papaya is normally propagated by seeds and the seeds are covered by gelatinous sarcotesta that inhibits germination. Quality seedlings are considered as an important criterion for the successful cultivation of papaya from the nursery stage. There is no information available on seed primming with metallic nanoparticles before sowing and its impact on germination, morphological growth and biochemical profile of papaya seedlings. Therefore, keeping in view the above-mentioned research gap, the present study was carried out with metallic CuNPs and AgNPs as new seed priming agents.

Materials and Methods

Experimental details

The experiment was carried out at Department of Horticulture (305.8 m MSL, $25^0$45´ N, 93°53´ E), Nagaland University, Nagaland, India from December 2021 to February 2022. The experiment was laid out in RBD with seven treatments and three replications. The treatments comprised of: control, metallic AgNPs @ 5, 10 and 15 ppm, metallic CuNPs @ 5, 10 and 15 ppm. The Red Lady papaya seeds were surface sterilized with 75% ethanol and rinsed thoroughly with distilled water 3-4 times. Then the seeds were soaked in AgNPs (< 90nm) and CuNPs (50nm) suspension and incubated for 8 hrs in an orbital shaker at 225 rpm. PEG-6000 was used to prevent aggregation of NPs. The primed seeds were washed in non-ionic distilled water 3-4 times and transplanted in seed beds for following observations:

Germination percentage: The germination percentage of seeds was calculated by using the formula as suggested by Hosseini *et al.* (2013). Germination percentage = $\frac{\Sigma n}{N} \times 100$

Where, Σn is the number of seeds germinated until the last day of the experiment and N is the total number of seeds.

Germination Index: The seed germination index was calculated by using the formula as suggested by Roshani *et al.* (2020).

$$\text{Germination Index} = \frac{\text{Number of germinated seeds}}{\text{Days of the first count}} + \frac{\text{Number of germinated seeds}}{\text{Days of the final count}}$$

Mean germination time (days): The mean germination time was calculated according to the formula as suggested by Hosseini *et al.* (2013).

$$\text{Mean germination time} = \frac{\sum n \times d}{N}$$

Where n= number of seed germinated on each day, d= number of days from the beginning of the test and N= total number of seeds germinated at the termination of experiment.

Growth attributing characters: Growth parameters like plant height measured by metric scale at 15 days interval, leaf emergence rate at 7 days interval, stem girth by vernier calliper and fresh and dry weight (at 70 ^{0}C for 48 h in oven) of seedlings by digital weighing balance at 45 days after germination were recorded. Leaf area (cm^2) was taken and traced over the graph paper and counted the number of square blocks enclosed inside the leaf outline.

Seedling vigour index: It was calculated by using the following formula as suggested by Roshani (2020).

Seedling vigor index = Germination % × dry weight (g) of seedlings

Leaf chlorophyll content (mg/g): The amount of chlorophyll present in leaves was estimated before transplanting of seedlings in main field (45^{th} day after germination). 0.2 g of fresh leaf was homogenized using mortar and pestle in 3 mL of 80% acetone with a small amount of quartz sand. The homogenate was filtered through Whatman No.1 filter paper and made up to 25 mL with 80% acetone. The filtrate was ready for chlorophyll estimations. The absorbance of the titration was measured at 645 nm and 663 nm using UV spectrophotometer (Hitachi, Model no.3210). Chlorophyll a, Chlorophyll b and total chlorophyll content were calculated using the following formula (Thimmaiah, 2012).

$$\text{Chl (mg/g)} = (20.2 \times A645 + 8.02 \times A663) \times \frac{\text{Final volume of chlorophyll extract in 80\% acetone}}{\text{Fresh wt. of tissue extracted} \times 1000}$$

Nutrient analysis of leaf tissues: The nutrient content in leaf tissues of 45 days old seedlings were estimated by microjeldahl's method for nitrogen, vanadomolybdate for phosphorus and flame photometric method for potassium adopting standard procedure (Jackson, 1973).

Results and Discussion

Germination percentage

The germination percentage of seeds measured at 15 DAS was quite low ranging from 31.85 to 50.37% that increased steadily with the advancement of sowing time of seeds. The highest germination (89.63%) was noticed by AgNPs @ 10ppm and the lowest (77.03%)

was noticed in unprimed control at 25 DAS, as depicted in Table 1. Early and rapid seed germination has a prerequisite, critical and positive impact as foundation for further plant growth, development and high economic yield. The maximum germination index (2.78) was recorded with AgNPs @ 10ppm followed by CuNPs @ 5 ppm (2.73) which was about 1.67 times higher as compared to untreated control (1.66). Hojjat and Hojjat (2015) also depicted that AgNPs @ 10 µg/L showed the highest value of seed germination index and other seed germination characters of fenugreek seeds while higher concentration caused the slight adverse effect. There was a significant effect in mean germination time (10.05 to 10.65 days) using different nanoparticles. The lowest mean germination times (10.05 days) was recorded by AgNPs @ 10 ppm followed by CuNPs @ 5 ppm (10.10 days) and maximum mean germination time (11.73 days) was recorded in control, as presented in Table 1. It was probably due to enhancement of water absorption by the seeds and increase in the nitrate reductase enzyme (Lei *et al.*, 2008).

Growth parameters

The performance of growth attributes was influenced significantly with different nanoparticle treatments, as presented in Table 2 and Figures 1 and 2. The exponential increment of plant height after seed germination at 15 DAG (6.66 cm), at 30 DAG (14.00 cm) and at 45 DAG (18.87 cm) was recorded in AgNPs @ 10ppm while the minimum was recorded in control at 15 DAG (4.94 cm), at 30 DAG (10.17 cm) and at 45 DAG (15.69 cm) in Figure 1. Both concentration of AgNPs @ 10 and 15 ppm were found to be more effective in increasing the plant height over control. It might be due to the addition of nanoparticles since it increases the α-amylase activity which digests the available carbohydrate into simple sugar and provides energy and nutrition availability easily to faster growing seedlings (Mahakham *et al.* 2016). The maximum leaf emergence rate with advancement of plant growth at 7 DAG (4.87), at 14 DAG (6.73), at 21 DAG (8.80) and at 28 DAG (10.2) were recorded in AgNPs @ 10 ppm over control (Figure 2). The leaf area of plants was significantly influenced by different nanoparticles suspension and was found to vary from 21.43 cm^2 to 40.81 cm^2, as depicted in Table 2. The maximum leaf area (40.81 cm^2) was noticed by AgNPs @ 10ppm followed by AgNPs @ 5ppm (37.03 cm^2) and the minimum leaf area (21.43 cm^2) was recorded in control. The maximum seedling vigor index (78.87) was achieved by AgNPs @ 10 ppm while the minimum was noticed in control (31.58). Low to medium concentration of nanoparticle suspension invariably showed a better impact on seedling vigour index and high concentration caused the negative and slight adverse effect on plant growth characters. AgNPs @ 100 ppm in wheat (Sabir *et al*., 2018) and other different plant (Pallavi *et al.*, 2016) showed a positive and significant influence in seedlings growth. Low to medium concentration (5 to 10 ppm) of AgNPs suspension increased the fresh weight of seedlings among different treatments. The maximum fresh weight (1.75 g) was noticed with AgNPs @ 10 ppm whereas, minimum fresh weight was recorded in control (1.30 g). The result was also confirmed with Salama (2012), who also attributed maximum shoot growth, total biomass production and other photosynthates using AgNPs @ 60 ppm in common bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.). Medium concentration of Ag and CuNPs showed a positive impact in dry weight of papaya seedlings. Seed priming with AgNPs @ 5 and 10 ppm showed more amount of dry weight and almost two folds (0.74 to 0.88 g) over control plant (0.41 g). It might be due to the higher fresh weight of shoots that ultimately led to higher dry matter content of shoots. The girth size of seedlings was found to be more with AgNPs (avg. 0.43 cm) as compared to CuNPs (0.41 cm). The highest stem girth (0.47 cm) was noticed in AgNPs @ 10 ppm while, the minimum seedling girth (0.39 cm) was recorded in control. The results are in conformity with the findings of Acharya *et al*. (2020), who observed maximum growth in stem diameter and longer shoot of watermelon seedlings using AgNPs.

Biochemical parameters

Nanoparticles significantly influenced the chlorophyll content in papaya leaf, as presented in Table 3. The more chlorophyll content in leaves was noticed in AgNPs @ 10 ppm (2.65 mg g^{-1}) and CuNPs @ 15 ppm (2.21 mg g^{-1}) while the minimum was recorded in control (1.68 mg g^{-1}) in Table 3. These results were confirmed by Pandey *et al*. (2014) in Indian mustard leaves and Fatma and Nivien (2015) in tomato. The data pertaining to leaf nutrients (nitrogen, phosphorus and potassium) significantly varied among different treatments are depicted in Table 3. The rapid growth of fully expanded leaves can enhance the rate of nutrient loading that was clearly signified in AgNPs seed primmed plants. Nitrogen content in leaf tissues was quite double in plant treated with AgNPs @ 10 ppm (0.86%) over control (0.40%). The plants treated with CuNPs (0.68 - 0.72%) also significantly improved the leaf nitrogen content. Enhancement of leaf nitrogen and potassium by AgNPs @ 50 ppm was also noticed in oriental lilies by Salachna *et al*. (2019). The maximum leaf phosphorus (0.29%) was recorded in

AgNPs @ 10 ppm while minimum was recorded in control (0.13%). The highest potassium (0.25%) content in leaves was noticed by AgNPs @ 10 ppm, as shown in Table 3.

Conclusion

The findings of this study clearly demonstrated that seed priming with metallic nanoparticles significantly influenced germination, growth and nutritional attributes of papaya seedlings. Among the treatments, AgNPs @ 10 ppm proved to be the most effective, resulting in the highest germination percentage, germination index and reduced mean germination time. It also markedly enhanced seedling vigor, plant height, leaf area, biomass accumulation and stem girth. In addition, AgNPs @ 10 ppm significantly improved biochemical parameters, including chlorophyll content and leaf nutrient status (N, P and K), indicating better physiological efficiency and nutrient uptake. Although CuNPs also showed positive effects at lower concentrations, their performance was comparatively inferior to AgNPs. Higher concentrations of nanoparticles tended to show slight inhibitory effects. Overall, the study suggests that AgNPs @ 10 ppm can be effectively utilized as a seed priming agent to improve early seedling establishment and nutritional quality in papaya.

Table 1: Effect of metallic silver and copper nanoparticles on germination of papaya seeds

Treatment	Germination (%)			Germination index	Mean germination time (days)
	15 DAS	20 DAS	25 DAS		
T_0: Control	$31.85±4.62^d$	$51.85±1.28^e$	$77.03±3.40^d$	$1.66±0.07^c$	$11.73±0.40^a$
T_1: AgNPs @ 5 ppm	$33.33±2.22^d$	$59.26±4.62^d$	$82.96±3.39^c$	$2.11±0.13^b$	$10.65±0.63^b$
T_2: AgNPs @10ppm	$50.37±3.40^a$	$74.81±5.60^a$	$89.63±3.39^a$	$2.73±0.45^a$	$10.05±0.77^b$
T_3: AgNPs @ 15ppm	$45.27±1.68^b$	$66.66±2.22^c$	$88.89±2.22^a$	$2.59±0.16^a$	$10.56±0.93^b$
T_4: CuNPs @ 5 ppm	$50.00±1.00^a$	$71.11±2.23^b$	$85.19±4.63^{bc}$	$2.78±1.00^a$	$10.10±0.45^b$
T_5: CuNPs @10ppm	$46.66±2.22^b$	$72.59±3.40^{ab}$	$85.93±3.40^b$	$1.91±0.11^b$	$10.49±0.43^b$
T_6: CuNPs @ 15ppm	$38.70±2.73^c$	$60.74±3.39^d$	$84.44±5.88^{bc}$	$2.02±0.11^b$	$10.63±0.55^b$
CD (P=0.05)	5.14	6.78	6.52	0.23	1.04

CD at 5 % level of significant, means value with standard deviation (±) bearing different letters a, b, c are significantly different (P< 0.05) on application of Duncan's multiple range test (DMRT)

Table 2: Effect of metallic silver and copper nanoparticles on morphological characters of papaya seedling

Treatment	Leaf area (cm^2)	Seedling vigor index	Fresh wt (g)	Dry wt (g)	Stem girth (cm)
T_0: Control	$21.43±0.91^d$	$31.58±1.12^g$	$1.30±0.04^c$	$0.41±0.026^e$	$0.39±0.05^b$
T_1: AgNPs @ 5 ppm	$37.03±3.82^b$	$61.39±2.17^b$	$1.61±0.10^{ab}$	$0.74±0.04^b$	$0.43±0.02^{ab}$
T_2: AgNPs @10 ppm	$40.81±3.19^a$	$78.87±2.96^a$	$1.75±0.12^a$	$0.88±0.04^a$	$0.47±0.01^a$
T_3: AgNPs @ 15 ppm	$27.72±1.55^c$	$47.11±1.91^e$	$1.44±0.15^{bc}$	$0.53±0.05^{cd}$	$0.40±0.02^b$
T_4: CuNPs @ 5 ppm	$21.64±1.52^d$	$53.67±2.11^c$	$1.54±0.09^b$	$0.63±0.06^c$	$0.41±0.01^b$
T_5: CuNPs @10 ppm	$35.72±2.65^b$	$50.70±2.02^d$	$1.45±0.71^{bc}$	$0.59±0.08^c$	$0.42±0.05^{ab}$
T_6: CuNPs @ 15 ppm	$24.92±0.64^c$	$36.31±1.06^f$	$1.34±0.08^c$	$0.43±0.0.57^{de}$	$0.40±0.03^b$
CD (P=0.05)	3.12	3.47	0.18	0.10	0.04

CD at 5 % level of significant, means value with standard deviation (±) bearing different letters a, b, c, d, f are significantly different (P< 0.05) on application of Duncan's multiple range test (DMRT)

Table 3: Effect of metallic silver and copper nanoparticles on chlorophyll and leaf nutrient status of papaya seedling

Treatments	Chlorophyll (mg g^{-1})	Nitrogen (%)	Phosphorus (%)	Potassium (%)
T_0: Control	$1.68±0.09^c$	$0.40±0.09^c$	$0.13±0.04^b$	$0.21±0.02^a$
T_1: AgNPs @ 5 ppm	$1.95±0.10^{bc}$	$0.69±0.08^b$	$0.23±0.06^a$	$0.23±0.03^a$
T_2: AgNPs @10ppm	$2.65±0.30^a$	$0.86±0.07^a$	$0.29±0.07^a$	$0.25±0.01^a$
T_3: AgNPs @ 15ppm	$2.02±0.09^b$	$0.77±0.15^{ab}$	$0.25±0.08^a$	$0.24±0.09^a$
T_4: CuNPs @ 5 ppm	$2.2±0.093^b$	$0.72±0.14^{ab}$	$0.24±0.08^a$	$0.23±0.02^a$
T_5: CuNPs @10ppm	$2.17±0.02^b$	$0.71±0.04^b$	$0.23±0.05^a$	$0.22±0.02^a$
T_6: CuNPs @ 15ppm	$2.21±0.16^b$	$0.68±0.06^b$	$0.22±0.03^a$	$0.24±0.03^a$
CD (P=0.05)	0.3	0.14	0.07	0.07

CD at 5 % level of significant, means value with standard deviation (±) bearing different letters a, b, c are significantly different (P< 0.05) on application of Duncan's multiple range test (DMRT)

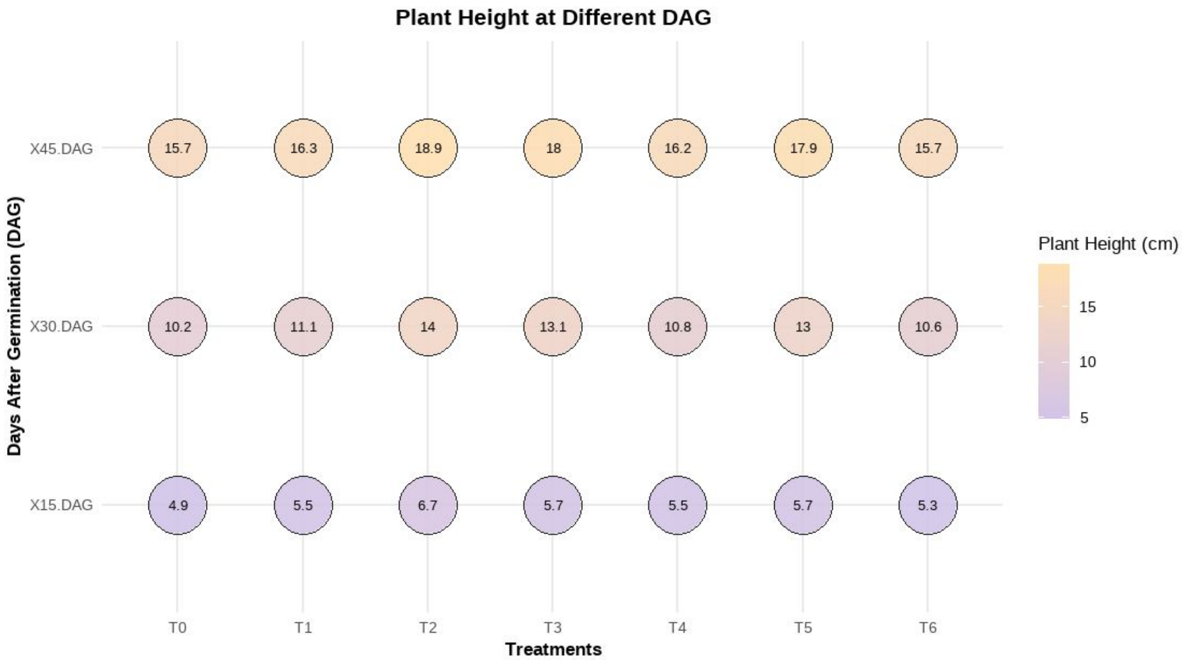

Fig. 1 : Impact of metallic silver and copper nanoparticles on plant height of papaya seedling at different days of germination

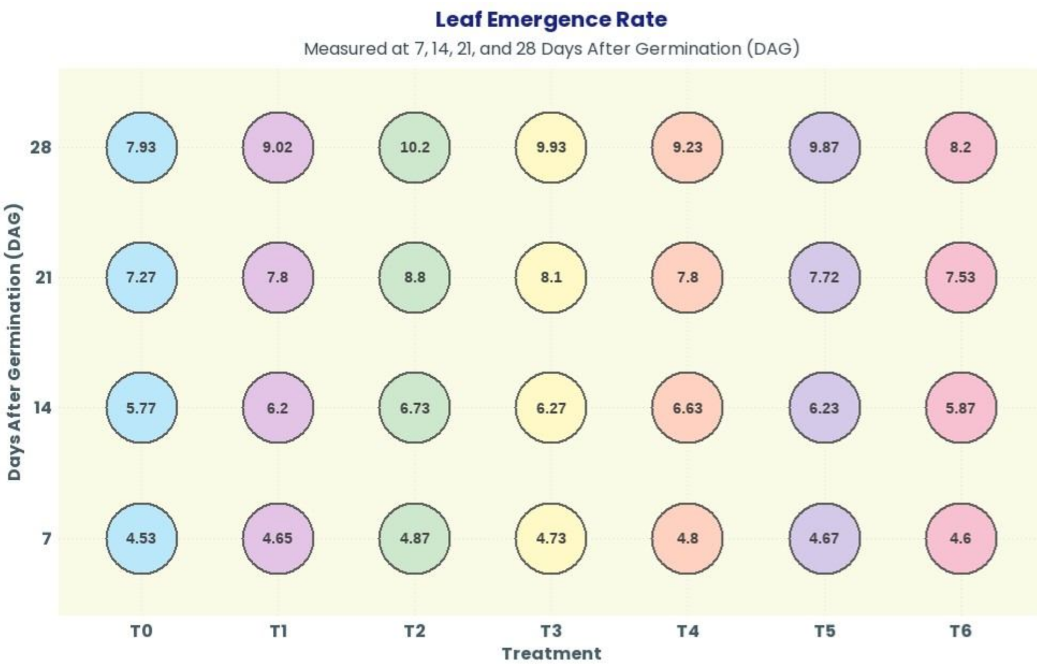

Fig. 2: Effect of metallic silver and copper nanoparticles on leaf emergence rate of papaya seedling at different days of interval

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Declaration of Competing Interest

The authors declare that there is no competing interest

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