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Copper and Silver Nanoparticle Seed Priming and Foliar Spray Modulate Plant Growth and Thrips Infestation in *Capsicum* spp.

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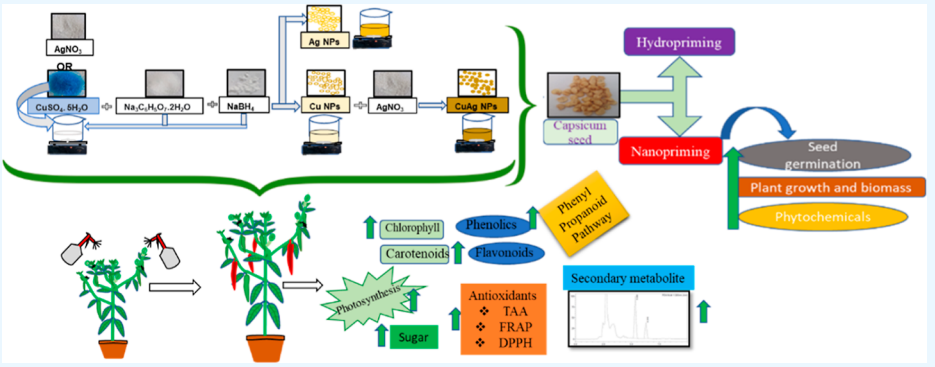

ABSTRACT: Nanoparticles (NPs) have the potential to improve plant health and secondary metabolite production. In the present study, three different NPs, i.e., Ag, Cu, and Cu-Ag NPs were produced in the range from 25 to 86 nm, with zeta potentials ranging from −28.8 to −38.5 mV. The synthesized NPs were used for seed priming and foliar spray on three varieties of *Capsicum annuum. L*, i.e., Arka Sweta (AS), Arka Meghana (AM), and Arka Harita (AH) plants grown under greenhouse conditions. Seed priming at various concentrations of NPs (1, 10, 20 ppm) enhanced the seed germination (96%), seedling vigor index (2494−3112.66), seedling length (6−49%), and biomass (46%) of 45 days old Arka Meghana seedlings. Additionally, all plant tissues accumulated significantly higher amounts of chlorophyll (51−142%), carotenoids (23−94.2%), total phenolic content (73%), and total flavonoid content (57%), compared with the control ($p \leq 0.05$). The foliar spray of NPs (20−100 ppm) has a protective effect on the chili plants against thrips infestation (30−76%). The foliar spray enhanced chlorophyll (15−62%), carotenoids (15−50%), total phenolic content (20−62%), total flavonoid content (64−99%), reducing sugars (15−97%), total antioxidant activity (15−142%), ferric reducing antioxidant power assay (15−109%), DPPH (129−54 mg mL^{-1}), and capsaicinoids (capsaicin and dihydrocapsaicin) (82−128%). This study illustrates that Ag, Cu, and Cu-Ag NPs suppress thrips infestation and proliferation with enhanced plant growth and biochemical activity, which is inversely proportional to the NP size. Chemical NPs play a crucial role in the economic significance of chili plants, offering a promising avenue for developing pesticides to effectively combat thrips infestation. This advancement holds great potential in enhancing the overall agronomic productivity of the chili crops.

1. INTRODUCTION

Pepper (*Capsicum annuum* L.) is an important vegetable in the Capsicum genus (Solanaceae family) with a large growing area and a high nutritional value.[1] Capsicum is well-known for its high concentration of capsaicinoids (oleoresins) and natural red colors (carotenoids). Capsaicinoids are important alkaloids produced via the phenylpropane and branched-chain fatty acid pathways.[2,3] Capsaicin and dihydrocapsaicin, found in the fruit, account for more than 90% of capsaicinoids.[4] The bioactive capsaicinoids have numerous applications in plant health, food, and defense responses.

Nanoparticles' (NPs) small size and high surface area-to-volume ratios distinguish them from bigger particles of the same chemical composition in various ways, including mechanical and biological properties, catalytic activity, thermal conductivity, optical absorption, and melting point.[5] NPs are used in various industries,[6] including pharmaceutics, biomedicine, water purification, wastewater treatment, food processing, packaging, and energy. NPs can be used in agriculture to reduce disease, plant protectants, fertilizer, and slow-release pesticides and act as innovative diagnostic tools.[5] NPs have both stimulatory and inhibitory effects on plant growth, and they rely on the concentration of NPs and plant type.[6,7] Because the production and administration of crops

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with increased germination have gained popularity, scientists have been attempting to encourage plant germination in field situations. Priming a seed before planting is one approach that promotes plant germination in field circumstances. Various priming processes are now commercially employed in various regions of the world. Ag NPs also possess antibacterial and antifungal qualities; Ag NPs are employed as plant growth promoters and for managing plant diseases.[8,9] Low concentrations of Ag NPs enhance crop stress resistance by modulating secondary metabolic pathways to produce more biochemicals, yield, and other components[10,11] and increase plant cytokinin response.[12] Chitosan, the first nanomaterial used in pesticides reduced the chitin synthase gene in *A. gambiae*'s resulting pest management.[13] The formulations of nanobased pesticides such as zinc oxide, copper silver, and silicon dioxide NPs have a broader spectrum, use less water, and reduce environmental pollution.[13] Thabet et al. found that using SiO_2 NPs was superior in preventing the spread of the cowpea aphid, *Aphis craccivora*.[14] The tomato leaf miner, an invasive pest *Tuta absoluta*, could be controlled using dsRNA−NP complexes after specific target dsRNA delivery was successfully mediated by chitosan and star polycation nanomaterials.[15]

Generally, secondary metabolites are triggered in plants in response to abiotic (drought, light, salt) and biotic (pathogens) stress.[16] Low doses of NPs are now explored as safer and more relevant to the environment, but they have yet to garner as much attention as their higher counterparts.[17] The thrips infestation resulted in a decreased nutritional composition of plants while concurrently inducing an elevation in the enzymatic activities of superoxide dismutase, catalase, and peroxidase.[18] Liu et al. reported the reduced enzymatic activities of superoxide dismutase, peroxidase, and catalase observed in damaged leaves of *Phaseolus vulgaris* at elevated carbon dioxide (CO_2). Changing climates may increase thrips damage.[19]

NPs have been illustrated to significantly modulate capsaicin and phytophenol concentrations in *Capsicum* species. Ag NPs increased the capsaicin concentration in *Capsicum frutescens* callus cultures with 2,4-D and kinetin.[20] García-López et al. found that foliar ZnO NPs treatments of 1000 and 2000 mg L^{-1} increased phenols, total flavonoids (soluble + bound), and total capsaicinoids in habanero pepper fruits.[21] Foliar NPs strengthen the crop defenses and resistance to attack from many diseases and pests, as well as crop production and overall quality. Compared to the more conventional soil-root treatment method, the foliar application of NPs offers an exciting new alternative approach.[22]

The effects of Ag, Cu, or Cu−Ag interventions on capsicum have not been thoroughly documented. Ag, Cu, and Cu-Ag NPs are responsible for ROS accumulation and oxidative damage in plant hosts. For better economic prospects and to avoid dangerous chemical sprays, it is imperative to reduce thrips infestations in capsicum. This vegetable crop is commercially significant and crucial to the global market for capsaicin oleoresin. Foliar NPs increase crop yield, quality, defenses, and resilience to pests and diseases.[23] The current study aimed to create a dependable method for synthesizing NPs with potent repellent activity against thrips and increasing the host chili plant growth and distinctive metabolites of fruits. With this rationale, we investigated the physiological and biological impacts of Ag, Cu, and Cu-Ag NPs applied through seed priming and foliar spray to the Capsicum plant at different concentrations.

2. MATERIALS AND METHODS

2.1. Plant Material. Three varieties of pungent chili seeds of *C. annuum*, that is, Arka Sweta (AS), Arka Meghana (AM), and Arka Harita (AH), were obtained from the ICAR-Indian Institute of Horticultural Research (Bangalore, India). Collected seeds were thoroughly washed in running tap water; subsequently, the surface was sterilized with sodium hypochlorite (NaOCl) for 3 min, washed thoroughly with sterile distilled water, and used further.

2.2. Chemicals. Silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$), trisodium citrate ($Na_3C_6H_5O_7$), copper(II) sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$), gallic acid or 3,4,5-trihydroxy benzoic acid, *quercetin* (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one), ascorbic acid, Folin−Ciocalteu reagent, sodium bicarbonate ($NaHCO_3$), aluminum chloride ($AlCl_3$), methanol acetonitrile, acetone, ethanol, ethyl acetate, 3,5-dinitro salicylic acid (DNS), sodium hydroxide, phenol, *potassium sodium tartrate* (*Rochelle salt*), sodium sulfite, glucose, ammonium molybdate, sulfuric acid, sodium phosphate, *potassium phosphate dibasic* anhydrous, potassium dihydrogen phosphate, potassium ferricyanide ($K_3[Fe(CN)_6]$), trichloroacetic acid, ferric chloride ($FeCl_3$), 2,2-diphenylpicrylhydrazyl (DPPH), capsaicin ($C_{18}H_{27}NO_3$), and dihydrocapsaicin ($C_{18}H_{29}NO_3$). All chemicals used were commercially purchased and used without any further treatment (Sigma-Aldrich).

2.3. Synthesis and Characterization of NPs. In a typical synthesis, $AgNO_3$ (50 μL, 0.1 M) NPs were synthesized with $Na_3C_6H_5O_7$ (50 μL, 0.1 M). Twenty mL of Milli-Q water was added in each and kept for continuous magnetic stirring; then, 1 mL of freshly prepared $NaBH_4$ (3.8 mg in 4 mL of H_2O) was added into the stirring solution. After 15 min of the incubation period, the Ag NPs were collected. After the synthesis, NP solution was sonicated for 30 min and stored in a refrigerator (4 °C).[24]

In a typical synthesis, $CuSO_4{\cdot}5H_2O$ (50 μL, 0.1 M) NPs were synthesized with $Na_3C_6H_5O_7$ (50 μL, 0.1 M). Twenty mL of Milli-Q water was added in each and kept for continuous magnetic stirring; then, 1 mL of freshly prepared $NaBH_4$ (3.8 mg in 4 mL of H_2O) was added into the stirring solution. After 15 min of the incubation period, the Cu NPs were collected. After the synthesis, NP solution was sonicated for 30 min and stored in a refrigerator (4 °C).[24]

In a typical synthesis, $CuSO_4{\cdot}5H_2O$ (50 μL, 0.1 M) NPs were synthesized with $Na_3C_6H_5O_7$ (50 μL, 0.1 M). Twenty mL of Milli-Q water was added in each and kept for continuous magnetic stirring; then, 1 mL of freshly prepared $NaBH_4$ (3.8 mg in 4 mL of H_2O) was added into the stirring solution. After 15 min of the incubation period, $AgNO_3$ (50 μL, 0.1 M) was added with continuous magnetic stirring after 15 min, the change in color indicates that the Cu-Ag NPs were formed. After the synthesis, NP solution was sonicated for 30 min and stored in a refrigerator (4 °C).[24]

The ultraviolet−visible spectrum of Ag and Cu ion reduction in colloidal solution was recorded in UV−visible (Thermo Scientific Genesys 150 UV−visible spectrophotometer, Waltham, MA, USA) from 200 to 800 at 2 nm intervals. The zeta analyzer was used to detect the zeta potential and particle distribution of NPs to predict the colloidal stability. The zeta potential and size of the produced NPs were

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determined using a Zetasizer Malvern Nanoseries (Malvern Instrument Zetasizer ZS, Malvern UK). NPs were subjected to Fourier transform infrared spectroscopy studies (Model/Make: IFS 25, Bruker, Germany). Transmission electron microscopy was used to assess the size and form of the produced NPs (TEM, Hitachi Ltd., Japan).[24]

2.4. Storage Stability. The synthesized NPs were stored in a refrigerator (4 °C) and examined after 40 days. The stability of the stored blank and NPs was measured three times, and the average was calculated.[25]

2.5. Effect of NP Priming on Chili Seedlings. The surface sterilized chili seeds were primed with Ag, Cu, and Cu-Ag NPs at 1, 10, and 20 ppm concentrations, and control seedlings (deionized water) for 24 h incubation were used. Since seeds are soaked only for 24 h, no sprouting was observed. In the greenhouse, these seeds were sown in seeding trays containing soil mix comprising four parts compost, 1-part perlite, 1-part vermiculite, and 1-part peat moss. After 45 days, the seedlings were taken out and used to analyze physiological and biochemical parameters. The seedlings were analyzed for physiological parameters (seedling length, seedling vigor index, germination percentage, germination index, germination speed, dead seed). It was an independent experiment with different sets of seedlings. For the seed priming experiment, only the AM chili variety was used.

2.6. Effect of Foliar Spray of NPs on Chili Plant. After 30 days, each seedling is put into a separate pot (capacity 5700 cm^3) holding one plant in the greenhouse at 2500 lx with a photoperiod of 12 h at 29 ± 2 °C, and relative humidity was 75 ± 5% during the light cycle and 85 ± 5% during darkness. At the time of anthesis (45 days after transplantation), treatments with fine foliar spray of Ag, Cu, and CuAg NPs at various concentrations (20, 40, 60, 80, and 100 ppm) and control seedlings (deionized water, $AgNO_3$, $CuSO_4$) were carried out. For foliar spray, all three chili varieties were used. The chili plants were examined at regular intervals for their growth characteristics such as morphological features [color of leaves, plant canopy width, plant height, plant spread, leaf area (cm^2)], the number of primary branches/plants, yield, and fruit yield per plant) IPGRI, and biochemical parameters.[26] The controls for both experiments were treated with deionized water, where 30 mL of water was used for spraying.

2.7. Plant Material and Thrips. Under controlled greenhouse conditions, severely thrips-infested chili plants were maintained throughout the study. Infested leaf material was collected for the study. Under greenhouse conditions, different treatment of NPs was studied for their effectiveness against thrips infestation control. Initially, chili seedlings were raised for 30 days under a sterile zone without thrips infestation, maintaining healthy plants by covering them in perforated polythene bags. After 15 days, plants were infested with thrips with the help of a soft-bristled brush gently, ∼25 to 30 thrips per plant. Throughout the period of growth, it was observed for all the prerequisite growth parameters, thrips activity, and its infestation. Only foliar-sprayed plants were exposed to thrips. All concentrations of NPs were used. Five randomly chosen plants in each replication of each genotype were labeled and used for recording the observations.

2.8. Effect of Foliar NPs on Biochemical Parameters of Chili. The treated chili leaves and fruits were collected for biochemical parameters. Three seedlings were chosen at random from NP sprayed plants, and their physiological and biochemical parameters were measured; the mean values were displayed.

2.8.1. Determination of Chlorophyll and Carotenoids. The leaf samples were extracted with 90% acetone (v/v) by using a mortar and pestle and centrifuged at 7000*g* for 10 min. The clear supernatant was collected, and its absorbance (A) was measured at 661.5, 663, 645, and 450 nm with a double-beam spectrophotometer (Thermo Scientific, Genesys 150 UV−visible Spectrophotometer, Waltham, MA, USA). Chlorophyll *a* (Chl.*a*), chlorophyll *b* (Chl.*b*), and total chlorophyll (Chl.t) concentrations were calculated according to the method described by Lichtenthaler[27] as follows

$$\text{Chl. } a\ (\mu g\ mL^{-1}) = 11.24A_{661.5} - 2.04A_{645}$$

$$\text{Chl. } b\ (\mu g\ mL^{-1}) = 20.13A_{645} - 4.19A_{661.5}$$

$$\text{Chl. t}\ (\mu g\ mL^{-1}) = 7.05A_{661.5} + 18.09A_{645}$$

$$\text{total carotenoids} = \{1000 \times A_{450} - [1{:}\ 9 \times \text{chl}(a) + 63 : 14 \times \text{chl}(b)]\}/214$$

2.8.2. Sample Extraction for Biochemical Assays. Briefly, 1 g of a leaf was extracted with 1 mL (80% ethanol) of solvent using a mortar and pestle, shaken at 100*g* in a gyratory shaker for 30 min, and centrifuged at 10 000*g* for 10 min. The pellet was collected and re-extracted by the same procedure. The two supernatants were pooled and stored in an amber tube to avoid light interference. All extractions and re-extractions were done in triplicate.

2.8.2.1. TPC Determination. The Folin−Ciocalteu technique was used to quantify the total phenolic content (TPC) of the crude extracts. A known amount of extract (0.1 mL) was pipetted into a test tube, which was then filled with distilled water to a volume of 3 mL. The mixture was then incubated for 3 min with 0.5 mL of Folin−Ciocalteu reagent, followed by 2 mL of 20% (w/v) Na_2CO_3 solution. The tube was vortexed and immersed in hot water for exactly 1 min. After the tube contents were cooled, the absorbance at 650 nm was measured. A previously plotted gallic acid standard graph was used to quantify each sample's phenolics. TPC was measured in g gallic acid equivalent (GAE)/100 g weight (FW) of leaf material.[28]

2.8.2.2. TFC Determination. The rude extract (0.1 mL) was diluted to an optimum concentration with 100% ethanol. The diluted sample was then combined with 1 mL of a 2% (w/v) $AlCl_3$ methanolic solution. After 15 min at room temperature, the absorbance of the reaction mixture at 430 nm was measured using a double-beam spectrophotometer. TFC was calculated as g of quercetin equivalent (QE) per 100 g of FW leaf material.[29]

2.8.2.3. Estimation of Sugar Reduction Using the DNS Method. Standard glucose solution (0.1 mg mL^{-1}) was prepared in a series of test tubes in increments of 0.05−0.5 mL, and the volume was adjusted to 1 mL with distilled water. One mL of DNS reagent was added and incubated in a boiling water bath for five min. After incubation, 333 μL of potassium sodium tartarate was added, and absorbance was measured at 510 nm.[30]

2.8.3. Antioxidant Activity. 2.8.3.1. Total Antioxidant Activity. A 0.3 mL extract (2 mg mL^{-1}) was incubated for 90 min at 95 °C with 28 mmol L^{-1} sodium phosphate and 4 mmol L^{-1} ammonium molybdate. The absorbance of the solution was measured at 695 nm using a double-beam

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Table 1. Physicochemical Characteristic Features of NPs

NPs	UV−vis (λ_{max})	zeta potential (mV)	electrophoretic mobility (μm cm/(V s))	zeta avg. (d. nm)	polydispersity index (PdI)	TEM size (nm)
Ag	392.5	−33.8	−1.84	19.42	0.208	25−35
Cu	246.5	−38.5	−3.016	37.8	0.124	66−86
Cu−Ag	406	−28.8	−2.257	23.6	0.182	35−50

Table 2. Effect of NP Seed Priming on Chili Seedling Physiological Parameters after the 45th Day of Germination[a]

NPs	conc. (ppm)	SL (cm)	SW (g)	SVI	GP (%)	GI = $\sum(n_i t_i)$/TNS	GS = $\sum(n_i)/t$	DS
C	0	21.6 ± 1.81	1.056 ± 0.18	1944	90	7.43	2.7	3
Ag	1	25.8 ± 1.3*	1.22 ± 0.13**	2494**	96.66*	6.92***	2.9*	1*
	10	32.2 ± 2.4*	1.44 ± 0.17**	3005.33**	93.33*	6.93***	2.8*	2*
	20	32.2 ± 2.38*	1.42 ± 0.13**	3112.66**	96.66*	6.56***	2.9*	1*
Cu	1	23 ± 0.7 ns	1.01 ± 0.37 ns	2223.33 ns	96.66*	6.73*	2.9*	1*
	10	27.6 ± 1.67 ns	1.2 ± 0.1 ns	2576 ns	93.33*	5.26*	2.8*	2*
	20	28.4 ± 1.94 ns	1.3 ± 0.2 ns	2745.33 ns	96.66*	6.06*	2.9*	1*
Cu−Ag	1	32 ± 1.22**	1.34 ± 0.12**	2986.66**	93.33*	6.7**	2.8*	2*
	10	30.4 ± 2.6**	1.54 ± 0.15**	2938.66**	96.66*	6.8**	2.9*	1*
	20	32 ± 2**	1.54 ± 0.12**	3093.33**	96.66*	6.86**	2.9*	1*

[a]Error represents the standard deviation (n = 3), illustrating the significant difference. Asterisks (*) indicate statistically significant differences among different treatments at the different NPs using paired t-test (***$p \leq 0.001$, **$p \leq 0.01$, *$p \leq 0.05$, and ns—nonsignificant). *C—control, Conc.—concentration, ppm—parts per million, SL—seedling length, SVI—seedling vigor index, GP—germination percentage, GI—germination index, GS—germination rates, and DS—dead seed.

spectrophotometer after it had been cooled to room temperature. The total antioxidant activity (TAA) of the leaf material was reported as g ascorbic acid equivalent (AAE)/100 g FW.[31]

2.8.3.2. Assay for DPPH Free Radical Scavenging Activity. Different dilutions of each extract (0.125−1.25 mg mL^{-1}) were prepared in separate test tubes. Furthermore, 39.4 mg L^{-1} of DPPH in methanolic solution was added to each test tube to the total volume 2 mL. The contents of the test tubes were well mixed and incubated for 15 min in the dark. Using methanol as a blank, the absorbance at 517 nm was measured.[32] The inhibitory or scavenging activity was estimated using the absorbance as DPPH scavenging activity (%) = [(ODcontrol − OD_{sample})/$OD_{control}$] * 100.

OD denotes optical density.

2.8.3.3. Ferric-Reducing Antioxidant Power Assay. The ferric-reducing antioxidant power (FRAP) test was performed per the Oyaizu technique. Each extract was combined with 2.5 mL of 0.2 mol L^{-1} phosphate buffer (pH = 6.6) and 2.5 mL of 1% (w/v) K_3Fe (C.N.)$_6$ and incubated at 50 °C for 30 min. The mixture was then centrifuged at 1000g for 10 min with 2.5 mL of 10% (v/v) TCA. Lastly, 2.5 mL of the upper-layer solution was combined with 2.5 mL of distilled water and 0.1% (w/v) $FeCl_3$. The method was repeated thrice, and the absorbance was measured at 700 nm. The acquired absorbance was translated to g AAE/100 g FW of leaf material.[33]

2.8.4. Extraction and HPLC Analysis of Capsaicinoids. Capsaicinoids extraction was performed according to Sharma et al.[34] High-performance liquid chromatography (HPLC) quantification of capsaicinoids was performed with Shimadzu LC-10A, Japan, instruments equipped with C18 columns. The analysis was performed according to Sharma et al.[34] with a slight modification of acetic acid 0.1%.

Pungency was calculated by the following formula

$$\text{totalScovilleheatunits(SHU)} = [C\ (\text{ppm}) + \text{DHC}\ (\text{ppm})] \times 16.1$$

The conversion to SHU from total capsaicinoids content obtained by the HPLC method in pepper was done by multiplying the coefficient corresponding to the heat value for capsaicin, which is 1.6×10^7.[35]

2.9. Statistical Analysis. The mean, standard error of triplicate tests is provided as the experimental finding for all experiments. GraphPad Prism 8 for the statistical test and an ANOVA. Principal component analysis (PCA) was used to investigate the relationship between morpho-physiological variables and biochemical measurements in the presence of NP treatments (Origin Pro 18). The t-test ($p \leq 0.05$) was utilized to compare treatment means performed using DATAtab software.

3. RESULTS AND DISCUSSION

3.1. Synthesis and Characterization of NPs. The harmonic conversion of the reaction substrate served as a visual indicator for the NP synthesis, as the color transitions of the Ag, Cu, and Cu−Ag NPs to brownish-yellow, light yellow, and yellow, respectively. The NPs are inferred evidence of the reduction of an Ag^+ ion to an Ag^0. The Ag, Cu, and Cu−Ag NPs show distinct peaks at 392, 246, and 406 nm (Table 1), respectively.[23] The synthesized Ag, Cu, and CuAg NPs were characterized[24] and used for further application. See Table 1, S1 Figure A.1−A.4 for the synthesized NP characterization and discussion.

3.2. Effect of NP Treatment via Seed Priming on the Growth of Chili Plants. In the present study, NPs of Ag, Cu, and Cu−Ag recorded varying effects on plant growth and thrips protection in chili plants. At a 20 ppm concentration recorded, significant ($p \leq 0.05$) increases in seed germination 96.66% and seedling vigor index (3112, 2745, and 3093.33) was recorded for Ag, Cu, and Cu-Ag NPs as compared to the control (germination-90%, SVI-1944), respectively (Table 2). Seed treatment with respective NPs promoted seed germination and plant growth under greenhouse conditions compared to the control.

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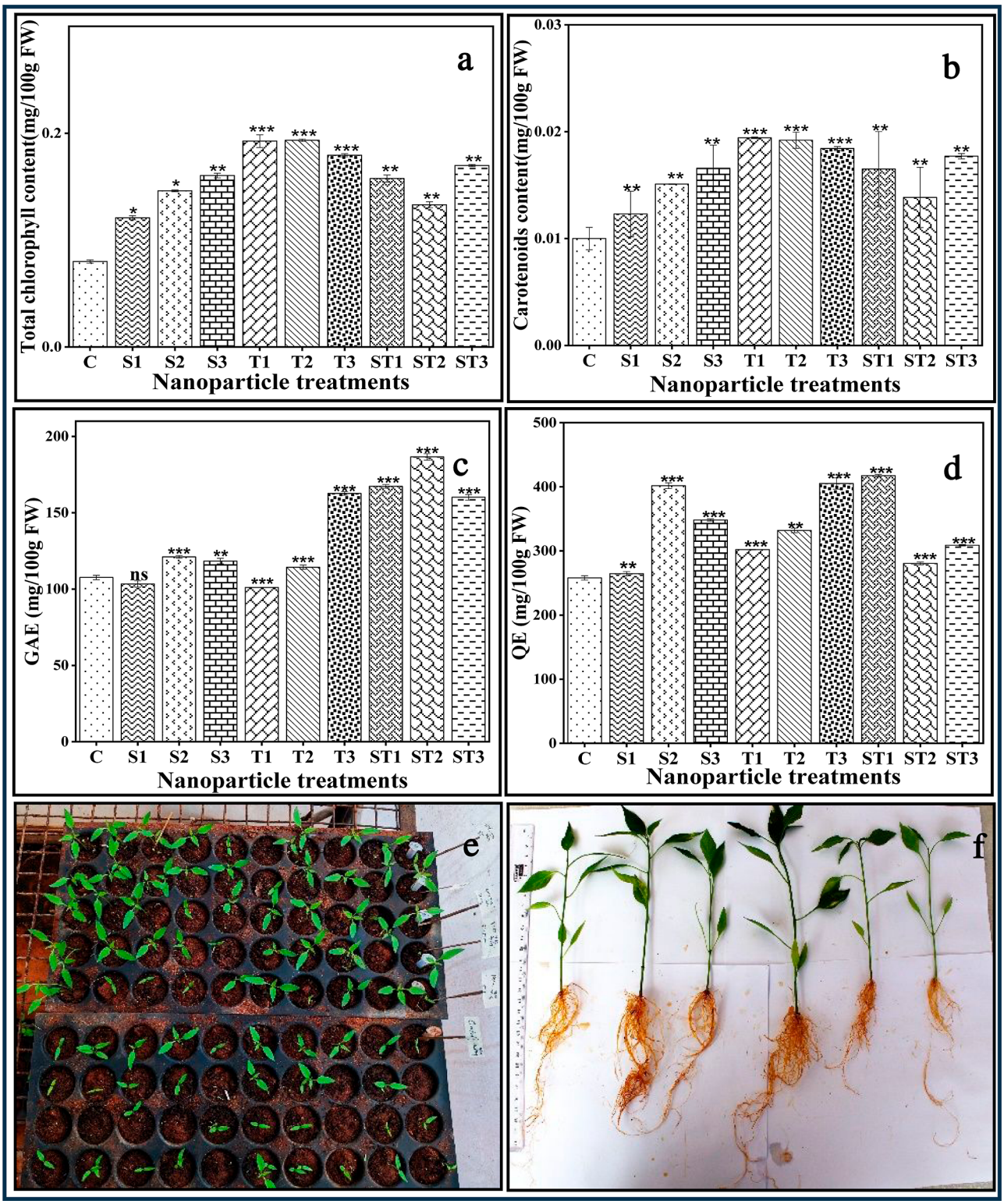

Figure 1. Effect of foliar NP treatment on biochemical contents. (a) Total chlorophyll content, (b) carotenoids content, (c) TPC, (d) TFC, (e) germinated seedlings after 20 days, and (f) treated seedlings after 45 days. *Concentration of NPs - Ag-(S1−S3)-(1−20 ppm), Cu-(T1−T3)-(1−20 ppm), and CuAg-(ST1−ST3)-(1−20 ppm). GAE, gallic acid equivalent. QE, quercetin equivalent. Values ± SD (standard deviation) (n = 3) means three independent replicates. The star (stars) in the figure means a sign for statistical analysis (***$p \leq 0.001$, **$p \leq 0.01$, *$p \leq 0.05$, and ns - non significant). Different letters in the same column indicate significant differences ($p < 0.05$). (Photograph: Courtesy to author).

Among different treatments, the application of NPs significantly ($p \leq 0.05$) improved plant height (32.2 ± 2.38 cm, 28.4 ± 1.94 cm, 32 ± 2 cm for Ag, Cu, and Cu-Ag NPs, respectively) at 20 ppm for AM chili variety (Table 2). The germination results are consistent with earlier research on watermelon, zucchini, and corn crops, which demonstrated that the germination rate was increased when compared to untreated watermelon at various Ag NP concentrations.[36]

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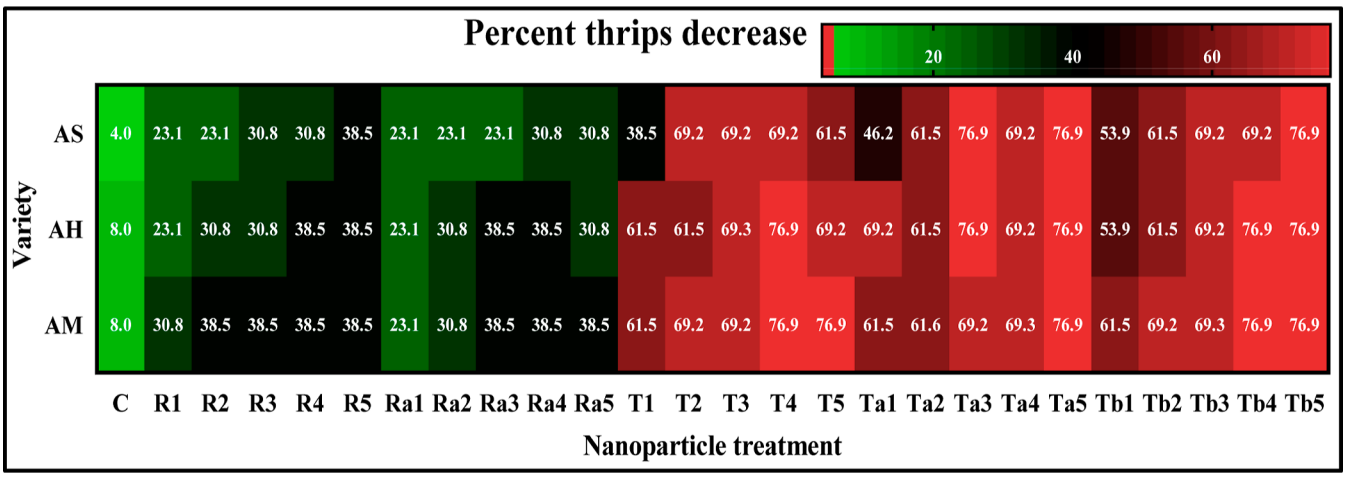

Figure 2. Spearman rank correlation heatmap of supervised list of percent thrips decrease evaluated after foliar application of NP ($AgNO_3$, $CuSO_4$, Ag, Cu, CuAg) with different varieties (AS, AH, AM). The Spearman correlation heatmap was plotted using GraphPad Prism 8. The color legend on the top side of the figure indicates that red corresponds to a value nearly equal to one, whereas bright green is assigned to lower values, which is the correlation r value. *Concentration of NPs - $AgNO_3$ (R1−R5)-(20−100 ppm), $CuSO_4$ (Ra1−Ra5)-(20−100 ppm), Ag-(T1−T5)-(20−100 ppm), Cu-(Ta1−Ta5)-(20−100 ppm), and CuAg-(Tb1−Tb5)-(20−100 ppm). Value ± SD (n = 5). The results are statistically significant using Spearman rank correlation ($p < 0.05$).

Similarly, increased germination was seen when using Ag NPs on *Boswellia ovalifoliolata*[37] and rice.[38] Trichoderma-selenium NP (100 ppm) seed treatment improved all pearl millet growth parameters in greenhouse conditions.[25] Syu et al.[39] investigated the effect of three different morphologies of Ag NPs on the physiological and molecular response of *Arabidopsis* seedlings and found that decahedral Ag NPs had the greatest degree of root growth promotion; however, spherical Ag NPs did not affect root growth promotion but caused the greatest anthocyanin accumulation in *Arabidopsis* seedling.

In comparison to the control plants, the level of chlorophyll in NP-treated chili seedlings increased by 100.4% at 20 ppm in Ag NPs, 141.68% at 10 ppm in Cu NPs, and 112.23% at 20 ppm in Cu-Ag NPs, respectively. Compared to the control, the carotenoids increased by 65.9% at 20 ppm in Ag NPs, 94.2% at 10 ppm in Cu NPs, and 77.1% at 20 ppm in Cu-Ag NPs, respectively. At 10 ppm of Ag NPs, 20 ppm of Cu NPs, and 10 ppm of Cu-Ag NPs, the phenolics were improved by 12.54%, 51.17%, and 73.42% in comparison to the control. At 10, 20, and 1 ppm of Ag, Cu, and Cu-Ag NPs, the TFC was increased by 55.79%, 57.13%, and 61.88%, respectively, compared to the control (Figure 1).

Chlorophyll has a positive relationship with a photosynthetic rate and total photosynthate production.[40] Increased chlorophyll content in Ag NPs' primed seedlings can be ascribed to increased water and nutrient absorption, which leads to improved plant growth and physiological development. The enhanced chlorophyll content seen in the Ag NP-treated seedlings in this study might aid in the buildup of soluble proteins for plant metabolic activities, allowing for great physiological performance. Increased chlorophyll levels may hasten the rate of photosynthetic CO_2 fixation, resulting in more soluble sugars and larger plant biomass. Ag NPs enhanced cellular electron exchange efficiency, photosynthetic quantum efficiency, and chlorophyll concentration in *Brassica juncea* seedlings compared to control *Brassica juncea* seedlings.[41] CuO NPs' effects on lettuce (*Lactuca sativa* L.) seedling development were assessed, and exposure to concentrations ranging from 0.2 to 300 g mL^{-1} improved seed germination and antioxidant activity.[42] Various concentrations of copper NPs, Uddin et al. 2022 observed that in chili plant growth, its physiochemical characteristics, and its function in nutrient delivery to the plants were all enhanced.[43]

3.3. Effect of NP Foliar Spray on Thrips Infestation. All treated plants clearly show a decrease in thrips at all of the NP concentrations, which can clearly be distinguished from the control to all treated plants. The maximum number of thrips decreases within these three varieties with NP treatment. The impact of synthesized NPs on thrips suppression differed significantly among the Ag, Cu, and Cu-Ag NPs. Ag NPs at concentrations above 40 ppm, and Cu and Cu-Ag NPs at concentrations above 60 ppm, significantly ($p \leq 0.05$) suppressed sporulation by about 70−85% compared to the effects of $AgNO_3$, $CuSO_4$, and the control group. Maximum pest control is indicated by the optimal concentration value of 60 ppm. Foliar spraying with NPs had no visible phytotoxic effects and had no effect on any of the plant growth parameters. All NPs have improved thrips defense capabilities in a concentration-dependent manner. The effectiveness of the combined thrips protection was further enhanced when more NPs were used as a spray treatment (Figure 2).

Physicochemical barriers, chemical signaling leading to the synthesis of defense-related chemicals like phytohormones, secondary metabolites, or antimicrobial peptides, induced systemic resistance, and the antimicrobial characteristic features of NPs may all influence or gate the influence of NPs on the host chili plant in all three varieties. In many chili-growing regions, thrips, mites, and borer complexes are the most damaging insect pests significantly reducing chili production. It has been reported that these inorganic NPs can be used as nanopesticides and nanofungicides to prevent plant diseases and pests.[44] While providing shielding protection in a dosage-dependent way also, it is important to note that the effect of NPs is quite complicated and is influenced by a wide variety of factors, including dosage,[45] plant species,[46] rhizosphere and foliage microorganisms,[47] particle size, shape, and charge of NPs.[23] Foliar NPs have been found to enhance the crops' defense mechanisms and improve their resistance against various diseases and pests. Additionally, applying foliar NPs has been shown to impact crop yield and

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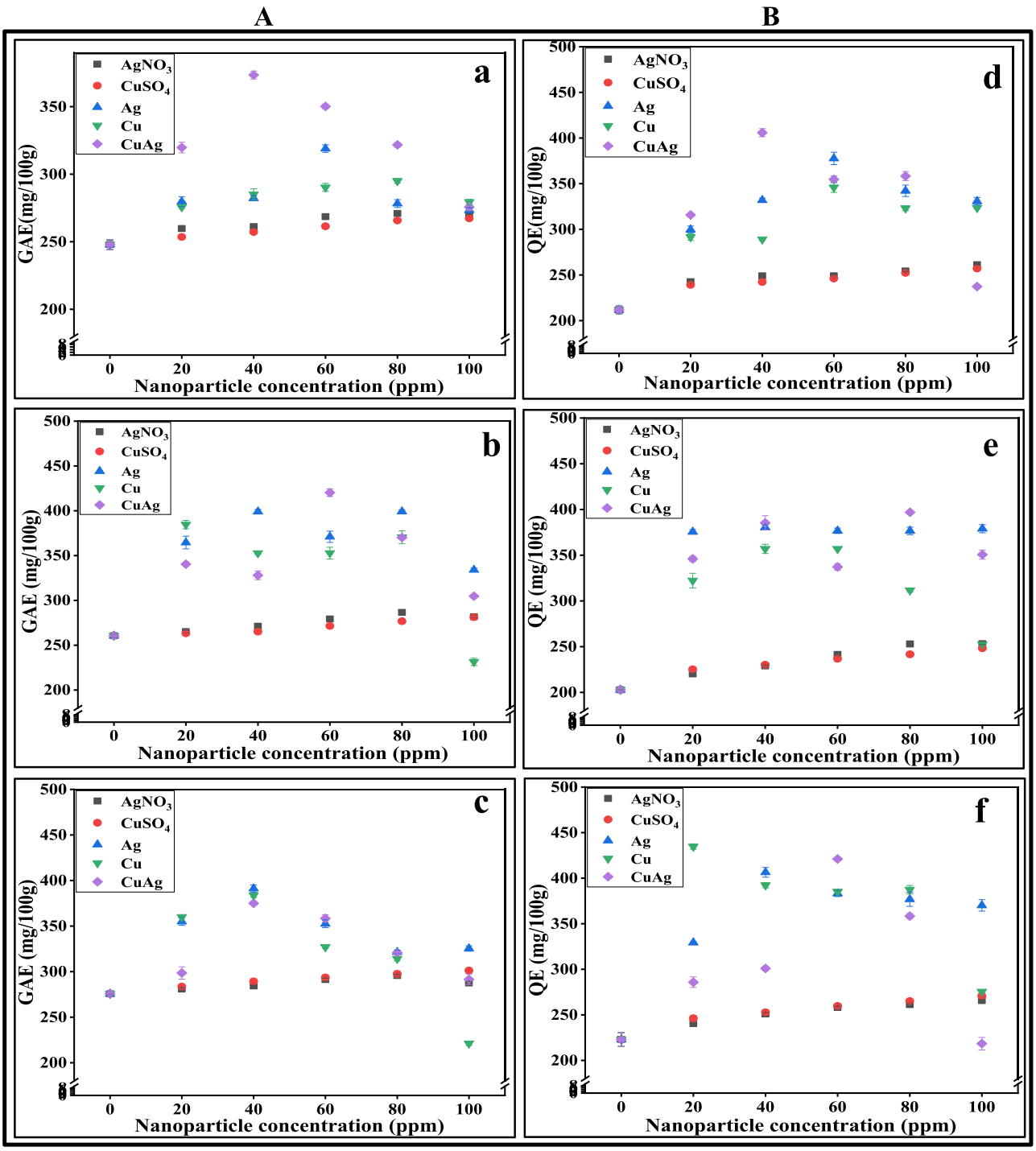

Figure 3. Effect of foliar application of NP on *Capsicum* spp. (A) TPC and (B) TFC. (a,d) AS variety; (b,e) AH variety; and (c,f) AM variety. Values ± SD (standard deviation) (n = 5) are the means of three independent replicates. The results are statistically significant ($p < 0.05$) (t-test).

overall quality positively. In contrast to the conventional soil-root treatment method, the foliar application of NPs presents a promising alternative approach.[23] Certain biologically produced NPs, such as Ag NPs produced by soil microorganisms, have significant antibacterial action. Ag NPs are frequently used as fungicides.[48] Inorganic metal-based NPs, such as Cu–Zn bimetallic NPs, possess antibacterial properties and are commonly employed as insecticides and antibacterial agents.[49] They are also effective against plant illnesses caused by fungus and mold,[50] as well as pests and diseases.[51]

NPs may boost the plant's immune response, preparing it for a more effective defense against pests and diseases. In our study, the curtailing of thrips infestation under NP application is further supported by a recent report[52,53] on the efficacy of NP formulations as antifeedants and plant disease control[54] in various plants.

3.4. Impact of Foliar NPs on Chili Plant Growth Parameters. The plant treated with Ag NPs at 60 ppm, Cu at 80 ppm, and Cu-Ag NPs at 80 ppm concentrations showed an increase in height, measuring 79 cm in the AS chili variety, respectively. Similarly, the NP-treated plants in the AH chili

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variety showed an increase in height of 90 cm in Ag NPs at 100 ppm, Cu NPs at 60 ppm with 94 cm, and Cu−Ag at 60 ppm of 94 cm concentrations, respectively. Subsequently, the treated plants with Ag, Cu, and Cu−Ag at concentrations of 40, 80, and 100 ppm showed an increase in height, measuring AM-94, 91, and 93 cm, respectively. These measurements were compared to the control group, which had heights of AS-55 cm, AH-60 cm, and AM-59 cm, respectively. Number of fruits (AS-44, 51, and 48 for Ag, Cu, CuAg NPs at 60, 80, 100 ppm; AH-52, 60, and 63 for Ag, Cu, Cu-Ag NPs at 60 ppm for all; AM-55, 51, and 55 for Ag, Cu, and Cu−Ag at 80, 20, and 80 ppm, respectively, as compared to the control (AS-42, AH-45, and AM-49), number of flowers, fruits pods, the diameter of fruit, plant spread, fruit color (AS-light green, AH-green, and AM-dark green), leaf thickness (AS-thick, AH-moderate thick, and very thick), leaf curling(up, down and both), leaf area, and fruit length as compared to the control. Results suggest that foliar NP treatment progressively improves the chili plant growth and productivity progressively.

Factors like dose, plant species in which, due to variations in their physiology, genetic makeup, or defense mechanisms, different plant species respond to NPs in different ways, and rhizosphere microorganisms like mycorrhizal fungi and rhizobacteria interact with NPs and modify their effects on plant defense. These bacteria can boost or reduce NPs' plant health benefits.[55] The zinc oxide (ZnO) NPs at a concentration of 100 mg L^{-1} significantly increased the leaf area, shoot and root dry weight, and chili plants fruit yield compared to the control group.[21] A subsequent study illustrated that the application of Ag NPs to chili plant leaves, at a concentration of 50 mg L^{-1}, resulted in increased photosynthetic activity, growth, and yield, when the plants were subjected to drought-stress conditions.[56]

3.5. Influence of NP Foliar Treatment on Phytochemical Constituents of the Chili Plant. Applying NPs improves the chili crop efficiency by regulating the biochemical and physiological characteristics. The current study found that treating the chili plants with foliar NPs significantly increased the net photosynthetic rate and related attributes. Additionally, it also enhanced the levels of carotenoids in the plants. The Ag NP application resulted in an increase in chlorophyll contents by 32.89%, 53.33%, and 14.92% in the AS-(2.02 ± 0.03 mg 100 g^{-1}), AH-(2.53 ± 0.05 mg 100 g^{-1}), and AM-(2.08 ± 0.00 mg 100 g^{-1}) at 20, 40, and 80 ppm, respectively, as compared to the control. In Cu NP application resulted in an increase in chlorophyll contents by 15.79%, 52.12%, and 23.76% in AS-(1.76 ± 0.01 mg 100 g^{-1}), AH-(2.51 ± 0.03 mg 100 g^{-1}), and AM-(2.24 ± 0.01 mg 100 g^{-1}) at 60, 20, and 20 ppm in foliar NP treatment. Whereas, in Cu-Ag NP foliar treatment at 60, 80, and 20, chlorophyll content increased by 27.63%, 61.82%, and 11.05% in AS-(1.94 ± 0.01 mg 100 g^{-1}), AH-(2.67 ± 0.01 mg 100 g^{-1}), and AM-(2.01 ± 0.00 mg 100 g^{-1}), respectively, as compared to the control (S1 Figure A.5A).

The carotenoid contents increased by 50%, 14.75%, and 50% in AS-(0.06 ± 0.00 mg 100 g^{-1}) at 100 ppm, AH-(0.07 ± 0.00 mg 100 g^{-1}) at 80 ppm, and AM-(0.06 ± 0.00 mg 100 g^{-1}) at 80 ppm concentrations, respectively, upon foliar application of Ag NP treatments as compared to the control. Similarly, Cu NPs resulted in greater carotenoid contents of 25%, 14.75%, and 50% in AS-(0.05 ± 0.01 mg 100 g^{-1}) at 60 ppm, AH-(0.07 ± 0.0005 mg 100 g^{-1}) at 60 ppm, and AM-(0.06 ± 0.0002 mg 100 g^{-1}) at 40 ppm concentration of foliar NP treatment as compared to the control, respectively.

The Cu-Ag NP application increased carotenoid contents of 32.50%, 31.55%, and 25% in the AS-(0.053 ± 0.001 mg 100 g^{-1}), AH-(0.08 ± 0.00001 mg 100 g^{-1}), and AM-(0.05 ± 0.0001 mg 100 g^{-1}) at 80, 20, and 60 ppm, respectively, as compared to the control (S1 Figure A.5B).

The TPC showed 28.66%, 42.30%, and 41.93% improvement for Ag NP foliar treatment in AS-(318.96 ± 1.62 mg 100 g^{-1}), AH-(399.13 ± 3.45 mg 100 g^{-1}), and AM-(391.2 ± 2.74 mg 100 g^{-1}) at 60, 80, 40 ppm, respectively, compared to the control. The TPC showed 18.99%, 47.59%, and 39.23% improvement for Cu NP foliar treatment in AS-(295.0 ± 2.91 mg 100 g^{-1}), AH-(384.58 ± 4.71 mg 100 g^{-1}), and AM-(383.75 ± 3.92 mg 100 g^{-1}) at 80, 20, and 40 ppm, respectively, compared to the control. The TPC showed 50.63%, 61.29%, and 36.05% improvement for Cu-Ag NP foliar treatment in AS-(373.43 ± 2.94 mg 100 g^{-1}) at 40 ppm, AH-(420.23 ± 4.2 mg 100 g^{-1}) at 60 ppm, and AM-(375.0 ± 1.71 mg 100 g^{-1}) at 40 ppm concentration, respectively, compared to the control (Figure 3A).

All three NPs augmented the TFC in the leaves of all three chili varieties. The response is concentration-dependent and also varies with the chili variety. Compared to the control, a surge of 78.36%, 87.52%, and 82.29% in AS-(377.69 ± 6.74 mg 100 g^{-1}), AH-(380.46 ± 2.63 mg 100 g^{-1}), and AM-(406.46 ± 5.35 mg 100 g^{-1}) at 60, 40, and 40 ppm, respectively, was seen as compared to the control for Ag NPs. Cu NP treatment increased TPC by 63.37%, 75.97%, and 94.97% in AS-(345.95 ± 5.52 mg 100 g^{-1}) in AH-(356.91 ± 0.47 mg 100 g^{-1}), and AM-(434.72 ± 3.00 mg 100 g^{-1}) at 60, 60, and 20 ppm, respectively, as compared to the control. In Cu-Ag NP foliar treatment, TFC shows an increase by 99%, 95.69%, and 88.86% in AS-(405.85 ± 4.38 mg 100 g^{-1}), AH-(396.91 ± 1.64 mg 100 g^{-1}) and AM-(421.1 ± 1.85 mg 100 g^{-1}) at 40, 80, and 60 ppm, respectively, as compared to the control (Figure 3B).

The Ag NP treatments had a significant effect on reducing sugar content and it is observed that the reduced sugar content is increased by 71.21%, 96.80%, and 15.14% in AS-(453.56 ± 0 mg 100 g^{-1}), AH-(385.18 ± 0.38 mg 100 g^{-1}), and AM-(305.02 ± 2.29 mg 100 g^{-1}) at 60, 80, 40 ppm, respectively, for Ag NPs. Similarly, Cu NP foliar treatment supported an elevation in the reducing sugar contents by 22.24%, 55.10%, and 15.10% in AS-(323.83 ± 1.11 mg 100 g^{-1}), AH-(303.56 ± 1.91 mg 100 g^{-1}), and AM-(304.91 ± 3.82 mg 100 g^{-1}) at 40,80, 100 ppm, respectively, as compared to the control. In Cu-Ag NP foliar treatment, the increase in reducing sugars by 24.89%, 45.16%, and 29.79% in AS-(329.51 ± 1.87 mg 100 g^{-1}), AH-(284.1 ± 1.91 mg 100 g^{-1}), and AM-(343.83 ± 0.76 mg 100 g^{-1}) at 20, 100, and 80 ppm, respectively, was observed as compared to the control (S1 Figure A.6A).

The biochemical composition mainly attributed to the chlorophyll, carotenoids, TPC, and TFC, and reducing sugar content estimation in all three varieties of the plant leaf materials was found to be significantly enhanced in all of the NP treatments. The metal NPs can increase the efficiency of chemical energy production in photosynthetic systems.[57,58] These findings are consistent with those of An et al.,[59] who revealed that NPs boosted the levels of ascorbate and chlorophyll in the Asparagus leaves. Plants often absorb foliar NPs through stomata, fissures or water holes, ion channels, protein transporters, endocytosis, stigma, wounds, and trichomes.[60] Stomata permeation, epidermal adsorption, and internalization are the primary mechanisms responsible for the metal absorption by foliage. NPs can also enter plants through

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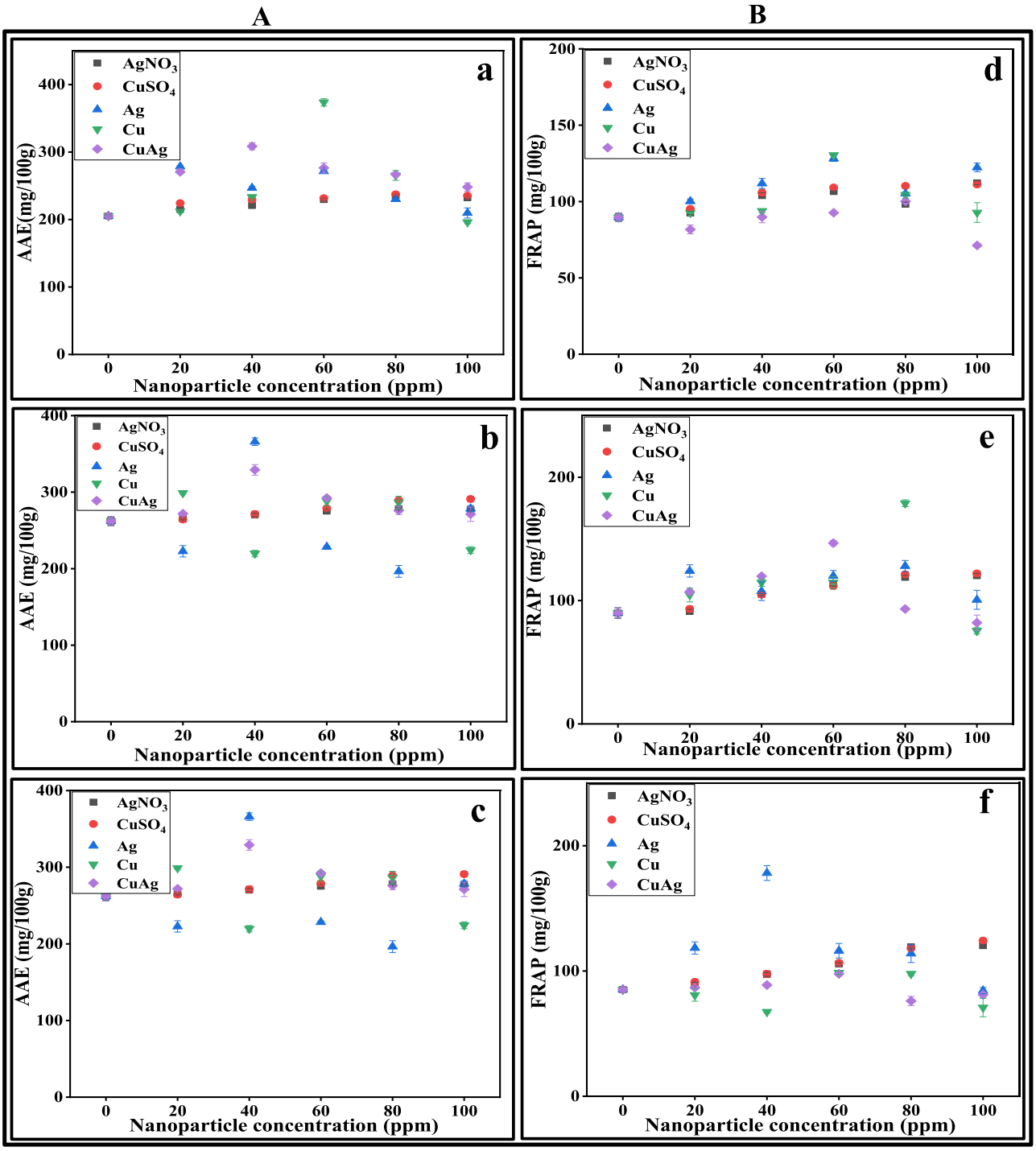

Figure 4. Effect of foliar NP on antioxidant activity in chili plant leaves. (A) TAA and (B) FRAP. (a,d) AS variety; (b,e) AH variety; and (c,f) AM variety. Values ± SD (standard deviation) (n = 5) are the means of three independent replicates. The results are statistically significant ($p < 0.05$) (t-test).

damaged root sites, lateral root growth zones, and root tissue.[61] Under greenhouse conditions, the foliar spray appliances of NPs, the host plant show a significant variation in the plant phytochemical constituents during the growth period with significant thrips—repellent activity (77%), which is regularly monitored throughout the cultivation period. Leads of using foliar applications of NPs include a reduction in the production of reactive oxygen in vivo[62] improved seed germination,[63] the increased shelf life of agricultural produce, improved absorption and assimilation of foliar fertilizer,[21] and controlled release of NPs at targeted locations.[64]

Sadak[17] reveals that foliar application of Ag NPs to fenugreek plants boosted all growth parameters (shot length, number of leaves/plants, and shoot dry weight) compared to untreated plants.[12] Salama et al.[65] reported similar results of Ag NPs that stimulated the development of common bean and corn plants. Silver NP foliar spray significantly affected treated wheat seedlings at all doses, as evidenced by increased biomass, carbohydrate, and protein stimulation.[66] The induced growth

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increases, and Ag NP involvement in inhibiting ethylene signaling in the fenugreek plant depends on the size and form of the NPs. This is consistent with the findings of Latif et al.,[67] who found that Ag NPs greatly improve photosynthesis and are strongly associated with nitrogen metabolism. Foliar application of Cu NPs increased vitamin content, fruit firmness, and antioxidant enzyme activity, hence increasing tomato fruit quality and freshness.[68] It also increased the levels of phenols, carotenoids, total sugar, indole, and amino acids in maize and coriander (*Coriander sativum*).[69]

3.6. Antioxidant Assay. *3.6.1. Total Antioxidant Activity.* The antioxidant activity observed for Ag NPs is 35.65%, 71.26, and 39.65% increase in AS-(278.4 ± 1.16 mg 100 g^{-1}) at 20 ppm, AH-(273.17 ± 2.29 mg 100 g^{-1}) at 20 ppm, and AM-(365.94 ± 4.93 mg 100 g^{-1}) at 40 ppm concentrations, respectively, as compared to the control. TAA in Cu NP treatment enhances by 82.07%, 143.01%, and 14.06% in AS-(373.68 ± 5.43 mg 100 g^{-1}), AH-(387.62 ± 1.37 mg 100 g^{-1}), and AM-(298.9 ± 1.68 mg 100 g^{-1}) at 60, 80, and 20 ppm, respectively. In Cu−Ag treatment, antioxidant activity increased by 50.28%, 144.59%, and 25.59% in AS-(308.44 ± 5.13 mg 100 g^{-1}) at 40 ppm, AH-(390.14 ± 3.54 mg 100 g^{-1}) at 60 ppm, and AM-(329.1 ± 6.84 mg 100 g^{-1}) at 40 ppm concentrations, respectively, as compared to the control (Figure 4A).

3.6.2. DPPH Scavenging Activity. DPPH is a popular, fast, and repeatable approach for determining antioxidant activity in plant and leaf extracts. The antioxidant action is mostly attributed to phenolic and flavonoid chemicals, and the current findings also relate to their content. The DPPH scavenging activity assay shows the inhibitory concentration (IC_{50}) value in Ag NP treatment of AS-87.27 ± 5.18 mg mL^{-1} at 20 ppm, AH-72.11 ± 0.72 mg mL^{-1} at 60 ppm, and AM-99.87 ± 0.53 mg mL^{-1} at 60 ppm.

In Cu NP foliar treatment, DPPH IC_{50} value was observed in AS-76.67 ± 0.06 mg mL^{-1} at 40 ppm, AH-82.28 ± 0.20 mg mL^{-1} at 60 ppm, and AM-85.95 ± 1.18 mg mL^{-1} at 60 ppm concentrations. Furthermore, the IC_{50} value of Cu-Ag NP foliar treatment shows AS-78.43 ± 0.78 mg mL^{-1} at 40 ppm, AH-54.16 ± 0.66 mg mL^{-1} at 60 ppm, and AM-78.07 ± 0.72 mg mL^{-1} at 60 ppm concentrations (S1 Figure A.6B).

3.6.3. Ferric-Reducing Antioxidant Power Assay. The ability of different extracts of different foliar NP-treated samples varies to reduce the ferric ions. The FRAP capacity improved by 42.93%, 42.42%, and 109.25% in AS-(128.15 ± 1.86 mg 100 g^{-1}), AH-(128.04 ± 4.53 mg 100 g^{-1}), and AM-(178.2 ± 5.87 mg 100 g^{-1}) at 60, 80, and 40 ppm concentrations, having the highest FRAP capacity as compared to the control in Ag NPs, respectively. The most prevalent iron in plant systems is ferrous iron, and its redox potential is significant in cellular oxidative stress, leading to various physiological dysfunctions. Foliar treatment demonstrated strong chelating efficacy by collecting ferrous ions before ferrozine. In Cu NPs, the foliar treatment enhances by 45.45%, 99%, and 15.69% in AS-(130.41 ± 0.45 mg 100 g^{-1}), AH-(178.9 ± 2.79 mg 100 g^{-1}), and AM-(98.52 ± 1.17 mg 100 g^{-1}) at 60, 80, and 60 ppm concentrations, having the highest FRAP capacity when compared to the other treatments, respectively. In Cu-Ag NP foliar treatment, an increase of 11.69%, 63.11%, and 14.73% in AS-(100.14 ± 1.33 mg 100 g^{-1}), AH-(146.64 ± 2.14 mg 100 g^{-1}), and AM-(97.7 ± 1.33 mg 100 g^{-1}) at 80, 60, and 60 ppm, having the highest FRAP capacity when compared to the other treatments, respectively (Figure 4B).

NPs have a high surface area-to-volume ratio, allowing them to interact more efficiently with plant cells and tissues. They can enter the plant through the stomata on the leaves and go to other portions of the plant. NPs can harm plants in a variety of ways, together with inducing oxidative stress and producing excessive reactive oxygen species, promoting mechanical damage such as stomata blockage caused by particle physical properties; NP dissolution and release of toxic metal ions; damaging DNA, protein, and other biomacromolecules; and interfering with normal cell metabolism.[61] These factors considered, the NPs at applied concentrations did not adversely affect plants. ROS is a metabolic byproduct of aerobic metabolism that can be harmful to plants.[70] NPs cause the buildup of ROS, causing lipid and protein damage. The failure to balance the synthesis and clearance of reactive oxygen causes oxidative stress, which destroys genetic material, inhibits key enzymes, and triggers lipid peroxidation.[71] Most particles are sufficiently soluble to release the component metal ions; foliar Ag NPs may release silver ions and produce hazardous reactions in plants (e.g., disrupt electron transport, cause inactivation of respiratory-related enzymes, membrane permeability, gene mutation, and cytolysis).[72] Copper NPs might act in the formation of OH radicals. Phytotoxicity in leaves impacts macromolecular metabolic pathways, causing genotoxicity. Plants can be harmed by NPs that undergo chemical changes such as redox and valence alteration.[73]

3.7. Total Capsaicinoids. In this study, we observed that the AM variety is the most pungent among the three varieties. The total capsaicinoids content was observed as 314.28 ± 3.36 mg 100 g^{-1} at 60 in Ag, 276.97 ± 2.6 mg 100 g^{-1} at 80 ppm in Cu, and 346.89 ± 3.74 mg 100 g^{-1} at 60 ppm for Cu-Ag NP treatments, respectively, as compared to the control (152.02 ± 2.95 mg 100 g^{-1}) in the fruits of treated plants (Figure 5). These values can be observed as pungency SHU as of 50,599.31 at 60 ppm for Ag, 44,592.13 at 80 ppm for Cu, and 55,849.51 at 60 ppm for Cu-Ag NPs, respectively, as compared to the control of 24,474.95 SHU.

Capsaicinoids are prominent secondary metabolites in chili, enhanced in response to the NP, which is consistent with

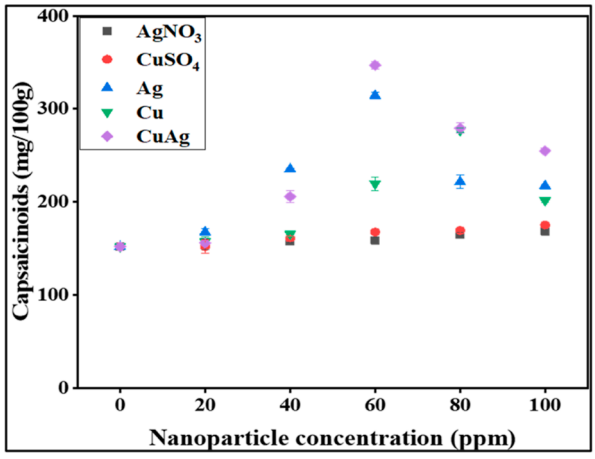

Figure 5. Capsaicinoids analysis of the chili plant treated with different foliar concentrations of NPs on the AM variety. Values ± SD (standard deviation) (n = 5) are the means of three independent replicates. The results are statistically significant (p < 0.05) (t-test).

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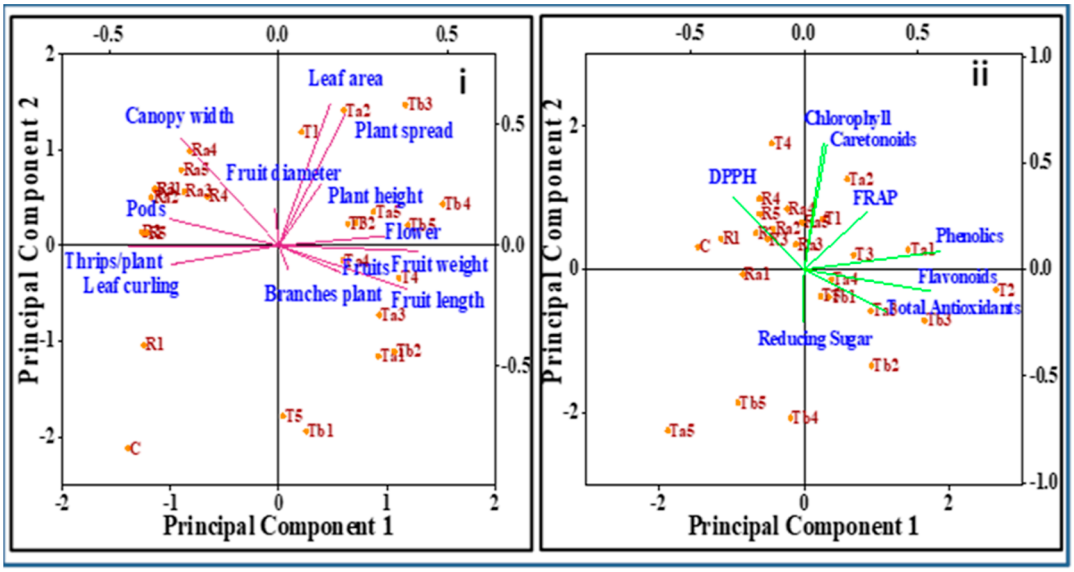

Figure 6. PCA plot of Arka Meghana against the foliar treatment of NPs. (i) Physiological parameter. The studied parameters were thrips, fruit weight, number of fruits, number of flowers, leaf area, pods, plant height, fruit length, fruit diameter, plant spread, canopy width, branches/plant, and leaf curling; (ii) Biochemical parameter. Total chlorophyll, carotenoids, TPC—total phenolic content, TFC—total flavonoid content, TAA—total antioxidant activity, FRAP—Ferric-reducing antioxidant power, DPPH—DPPH$^{\bullet}$ scavenging activity, reducing sugars, *Concentration of NPs - $AgNO_3$ (R1−R5)-(20−100 ppm), $CuSO_4$ (Ra1−Ra5)-(20−100 ppm), Ag-(T1−T5)-(20−100 ppm), Cu-(Ta1−Ta5)-(20−100 ppm), and CuAg-(Tb1−Tb5)-(20−100 ppm). Values ± SD (n = 5) (standard deviation) are means of three independent replicates; Means not significantly different according to one-way ANOVA at $P \leq 0.05$ levels.

previous study findings that secondary metabolites are crucial compounds that assist plants in responding to abiotic (drought, light, salt) and biotic (pathogens) stress.[16] Silver ions in silver nitrate are well explored for their growth-promoting and secondary metabolite-enhanced production in various plants cultured in vitro and ex vitro,[74−77] wherein silver nitrate was tested in milligram or microgram concentrations. Recently, silver-based NP applications to achieve improved plant growth have gained significance. However, to some extent, the lack of understanding of plant responses to NP-induced stress in terms of secondary metabolism is a barrier to the use of NPs in agricultural activities. The augmentation of pungency metabolites in chili through abiotic elicitors was known.[78−80] However, augmentation of pungency metabolites using NPs could be more effective in terms of quality. The reaction of plants to NPs for the enhancement of phytoalexins was reported earlier. Phytoalexins are a diverse set of antimicrobial chemicals that plant produce de novo in response to pathogens at the site of infection and are thought to be biological indicators of disease resistance.[81]

3.8. Principal Component Analysis. *3.8.1. Evaluate the NP−Physiological Interactions of Arka Meghana Using PCA.* PCA was used to determine the association between morpho-physiological parameters and NP treatments. The results of PCA revealed five major principal components (PCs) (eigenvalue 1) (S1 Figure A.8 (iii)), which accounted for 77.07% of the overall variance of physiological characteristics following NP therapy. The PC scores were distinguished across treatments based on their positive and negative values across PC1 and PC2. PC1 and PC2 accounted for 49.09% of the data variability in the morpho-physiological features of Arka Meghana. PC1 had 34.75% data variability, distinguishing the NP treatment from the other two treatments (control and raw chemical control) regarding positive and negative PC ratings. PC1 distinguished the treatment from the others due to larger positive coefficients of flower, fruit weight, and fruit length. PCA2 showed 14.34% data variability, and this PC distinguished the control treatment from the other treatment based on plant spread, leaf area, and canopy width. PC3 accounted for 11.12% of the total variance and was impacted mostly by the number of fruits, pods, and fruit diameter. PC4 accounted for 9% of the overall variance and was mostly affected by branches/plants. PC5 accounted for 7.86% of the overall variance and was impacted mostly by plant height, leaf curling, and thrips/plant. A biplot was produced utilizing the two top PCs to aid in selecting treatment concentrations for the many measured physiological parameters. The biplot of physiological activity under NP indicated that the number of fruits, flowers, fruit weight, fruit length, and branches/plants were positively associated with NPs with various concentrations of T1, T2, T3, T4, T5, Ta1, Ta2, Ta3, Ta4, Ta5, Tb1, Tb2, Tb3, Tb4, Tb5, Ta2, Ta5, and Tb3, Tb4, Tb5. Although the biplot allowed us to discover the NP concentration-physiological connection, selecting the optimum NP based on a single physiological measure under the evaluated NP settings was problematic. As a result, we employed T3, Ta3, and Tb3 concentrations to pick out the best growth.

3.8.2. Evaluate the NP−Biochemical Interactions of Arka Meghana Using PCA. PCA was used to determine the association between biochemical parameters and NP treatments. The results of PCA revealed three major PCs (eigenvalue 1) (S1 Figure A.8 (iii)), accounting for 71.84% of the total variance of biochemical characteristics after NP therapy. The PC scores were distinguished across treatments based on their positive and negative values across PC1 and PC2. PC1 and PC2 accounted for 50.45% of the data variability in the biochemical features of Arka Meghana. PC1 had a data variability of 27.04% and distinguished the NP treatment from the other two treatments (control and raw chemical control) in terms of positive and negative PC ratings.

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PC1 distinguished itself from the other treatments by having greater positive coefficients of TPC and TFC. PCA2 had a data variability of 23.41%, and this PC distinguished the control treatment from the other treatment by accounting for chlorophyll, carotenoids, and DPPH activity. PC3 accounted for 21.39% of the overall variance and was impacted mostly by TAA, FRAP, and reducing sugars. A biplot was produced utilizing the two top PCs to aid in selecting treatment concentrations on the many measured biochemical parameters. TPC and TFC were favorably related with NPs with varied concentrations of T2, T3, Ta1, Tb2, and Tb3, whereas chlorophyll, carotenoids, and DPPH were positively associated with Ta2, T1, T4, R1, Ra1, Ra2, Ra3, R2, and R1. TAA, FRAP, and RS were shown to be related to T2, Tb4, R3, R4, Ra5, and Ra5. Although the biplot helped us to find the NP concentration-biochemicals link, selecting the optimum NP based on a single biochemical parameter under the investigated NP settings was problematic. As a result, we chose the biochemicals with the greatest concentrations of T2, T3, Ta2, and Tb3 (Figure 6 (ii)).

The results of our work illustrated that a foliar spray of Ag, Cu, and a combined form of Cu-Ag NPs is more effective and efficient in exhibiting thrips-repelling activity in chili host plants. The lowest number of thrips infestation was observed in plants subjected to a foliar spray of Cu-Ag NPs at an 80 ppm concentration in treated seedlings of chili plants. Our study examined the effects of chili plant growth parameters on seed priming, foliar spray, chlorophyll, and carotenoid levels, as well as plant health and stress response. These pigment concentrations may indicate light harvesting, energy transmission, or oxidative stress protection. Further research may reveal whether pigment content increases due to photosynthetic performance or stress reactions.[57,58] Defense mechanisms were examined via phenolics and flavonoids as they help plants fight pests and diseases. These substances are antioxidants, antibacterials, and antifeedants. TPC and TFC increases may indicate improved defense mechanisms, making plants more resistant to pests and diseases.[69] Crop productivity was investigated in our study by discussing the biochemical composition changes and keen observation of fruit under NP treatment. Evaluating the relationship between observed changes and crop productivity would illuminate the practical effects of NPs in agriculture.

Furthermore, an efficient upregulation of plant growth and phytochemicals in host plants supports the evident avenue of Ag and Cu NPs as a potent pesticide formulation on a large scale with a wide mass scale at a proficient level.

4. CONCLUSIONS

In the present work, we demonstrated the two-step synthesis of silver NPs by using $NaBH_4$ and trisodium citrate as reducing and stabilizing agents, respectively. Ag, Cu, and Cu-Ag NPs were synthesized within a size range of 25−86 nm. All the synthesized NPs had shown significant seedling growth parameters along with enhanced phytochemical constituents with prevalent thrips protection in a dose-dependent manner under greenhouse conditions. The nanometer size of NPs and long-term stability show synergistic pesticide potential against thrips on chili spp. In contrast, the Cu-Ag NPs boosted chili morphological traits, particularly plant height, spread, biochemical synthesis, antioxidant potential, and phytochemical expression. The sustainable treatment of foliar NPs on chili strongly correlates to increment in the industrial important phytochemical and thrips resistance. Phytochemicals are widely used in the medical, cosmetic, and pharmaceutical industries. Thus, the present study provides meaningful insights into the sustainable, cost-effective, and promising use of NPs in the valorization of chili plants.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c06961.

NP characterization and discussion, biochemical assays, and PCA analysis and discussion (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

TPC, total phenolic content; TFC, total flavonoid content; RS, reducing sugars; TAA, total antioxidant activity; FRAP, ferric-reducing antioxidant power; DPPH, 2,2-diphenyl-1-picryl-hydrazine-hydrate; Ag, silver; Cu, copper; CuAg, copper silver; NPs, nanoparticles; AS, Arka Sweta; AH, Arka Harita; AM, Arka Meghana; ROS, reactive oxygen species; SHU, Scoville heat unit; ppm, parts per million

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