Multifaceted Effect of Copper Nanoparticle on the plant of *Artemisia annua* L.

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Abstract

Artemisia annua L., a well-known medicinal plant and the primary natural source of artemisinin, plays a crucial role in pharmaceutical and therapeutic applications. The present study investigates the multifaceted effects of copper nanoparticles (CuNPs) on the growth, biochemical, and phytochemical attributes of *A. annua*. Copper is an essential micronutrient involved in various enzymatic and metabolic processes; however, its nanoparticulate form may exert both stimulatory and toxic effects depending on concentration and exposure duration. The study evaluates the impact of different concentrations 18 µg/ml, 36 µg/ml and 54 µg/m of CuNPs on seed germination, plant height, Internodal length, Days to 50% flowering, chlorophyll content, antioxidant enzyme activity, and secondary metabolite production. Results indicate that lower concentrations of CuNPs enhance germination rate, photosynthetic pigment content, leading to improved growth performance. Conversely, higher concentrations induce oxidative stress, membrane damage, and growth inhibition due to excessive reactive oxygen species generation. Overall, the findings demonstrate a dose-dependent dual role of copper nanoparticles, highlighting their potential as nano-fertilizers or elicitors for enhanced phytochemical production when applied at optimized concentrations. This study provides valuable insights into nanoparticle–plant interactions and supports the sustainable application of nanotechnology in medicinal plant cultivation.

Keywords: *Artemisia annua* L., Nanoparticle, Copper Nanoparticle, Oxidative stress, Genotoxicity

Introduction

Nanotechnology is the new gateway to provide an opportunity for the researchers of plant science and other fields, to build up new tools for integration of NPs into plants that boost existing function and new ones [1]. Nanoparticles (NPs) are wide class of materials that include particulate substances, which have one dimension less than 100 nm at least [2]. In agricultural field, pesticides and herbicides are usually used to get better crop yield and efficiency. But currently, severe debate is going on pessimistic effect of conventional pesticides and herbicides on the environment. Unsystematic usage of pesticides increase pathogen and pest resistance diminishes soil biodiversity, reduce nitrogen fixation; contribute to bioaccumulation of pesticides, pollinator decline and destroy habitat for birds. When NPs are applied with herbicide, little amount of herbicide is required to achieve the weed eradication. NPs owing to their size can enter freely into the cells and can inherent considerable influence on the cellular function. These unique features make them highly attractive for implementation in products for wide application [3]. NPs have unique physiochemical properties and the potential to enhance the plant metabolism [4]. In recent reports, several literature on the ecotoxicity of NPs and nanomaterials as well as the chemistry of both manufactured and natural NPs is reviewed in recent reports [5]. Treatment of AgNPs with 1 or 2.5 mg/L in *Arabidopsis thaliana* plants was found to elevate seedling biomass, whereas treatment with high concentrations was found to decrease seedling biomass [6]. Another study by Zafar *et al.* [7] demonstrated effects of NPs on germination and shoot growth of *Brassica nigra.* In contrast to fine particles, NPs are highly insoluble in water and culture media and show strong genotoxicity in the aqueous environment [8].

Cupric oxide II is an important inorganic compound with the formula CuO. A black solid, it is one of the two stable oxides of copper, the other being Cu_2O or cuprous oxide. Copper (II) oxide belongs to the monoclinic crystal system. The copper atom is coordinated by 4 oxygen atoms in an approximately square planar configuration. [9] reported that copper (Cu) and cadmium sulphide (CdS) NPs provoke stable and heritable phenotypic changes in *Macrotyloma uniflorum* (Lam.) Verdc (Family: Leguminosae).

Aforementioned characteristics of copper in present appraisal CuNPs were used to assess the impact on genetical constitution of *Artemisia* together with morphological and biochemical parameters.

Material and Methodology

Four different accession of *Artemisia annua* L. with high yield potential and seed viability *viz.* EC- 415012, EC-415013, IC-353508 and EC-202429 were obtained from National Bureau of Plant Genetic Resources, Bhowali, Shimla, Uttarakhand. To gauge the seed viability and its acclimatization in the experimental area seeds were germinated *in vivo* and *in vitro* both the conditions. after deep observation accession EC- 415012 was selected for the further experimental study.

Treatment of Nanoparticle

For the nanoparticle treatment, to increase seed permeability healthy seeds of *Artemisia* were presoaked in distilled water for 5 hrs. Copper (II) nanoparticles were selected for the treatment and three different concentration 18 µg/ml, 36 µg/ml and 54 µg/ml were prepared and seeds were immersed for 3 hrs at 25°C. To remove extra solution the seeds were thoroughly washed under running water. After washing treated seeds along with control were sown in pots adopting complete randomized block design (CRBD) to raise the plants. The raised populations were observed until the maturity of the plants for the evaluation of consequences of nanoparticle on the morphology, cytology and biochemical characteristics.

Statistical Analysis

The experiments were performed in three replicates for authenticity of the results. The average data of all parameters was statistically analyzed. The mean, standard error and coefficient of variance (CV) has been calculated at each dose for all treatment. Statistical analysis was performed using the SPSS 16.0 software. A one way analysis of variance (ANOVA) and Duncan‟s multiple range test (DMRT, $P < 0.05$) was subjected for mean separation of studied morphological variables and the graphical illustrations of data was plotted by using Sigma plot 10.0 software.

Result and Discussion

A. Growth line and morphological investigation

The data of germination and survivability of CuNPs treated sets of *Artemisia annua* L. were evaluated and have been tabulated in **Table 1**. Due to copper nanoparticle, treated seeds takes much more time for germination as compared to control sets.The germination and survival percentage in control plants recorded to be 96.63% and 94.41% respectively. In treated sets germination was greatly exaggerated and was calculated to be 94.42% (18µg/ml), 88.34% (36 µg/ml), 79.67% (54 µg/ml) while survivability was noted to be 86.32% (18 µg/ml), 72.61% (36 µg/ml), 61.06% (54 µg/ml), respectively. The morphological traits such as plant height, internodal length, primary number of branches, Days to 50% flowering and leaf area of each treated set was examined **(Graph).** The plant height depreciated from lowest to highest concentration of copper nanoparticle **(Figure 1)**. The chlorophyll and carotenoid content was quantified, which is displayed in **(Graph)**.

Table 1: Effect of on Copper Nanoparticle Germination, survival and pollen fertility (%) of *Artemisia annua* L.

Copper Nanoparticle (µg/ml)	Germination (%)	Survival (%)	Pollen fertility (%)
Control	96.63±2.51[a]	94.41±2.99[a]	95.88±1.94[a]
18	94.42±3.05[a]	86.32±3.44[a]	88.31±2.45[b]
36	88.34±3.73[ab]	72.61±2.43[b]	75.23±1.22[c]
54	79.67±3.86[b]	61.06±2.22[c]	69.76±1.44[c]

*Values followed by the different letters differ at $p < 0.05$ between treatments by DMRT

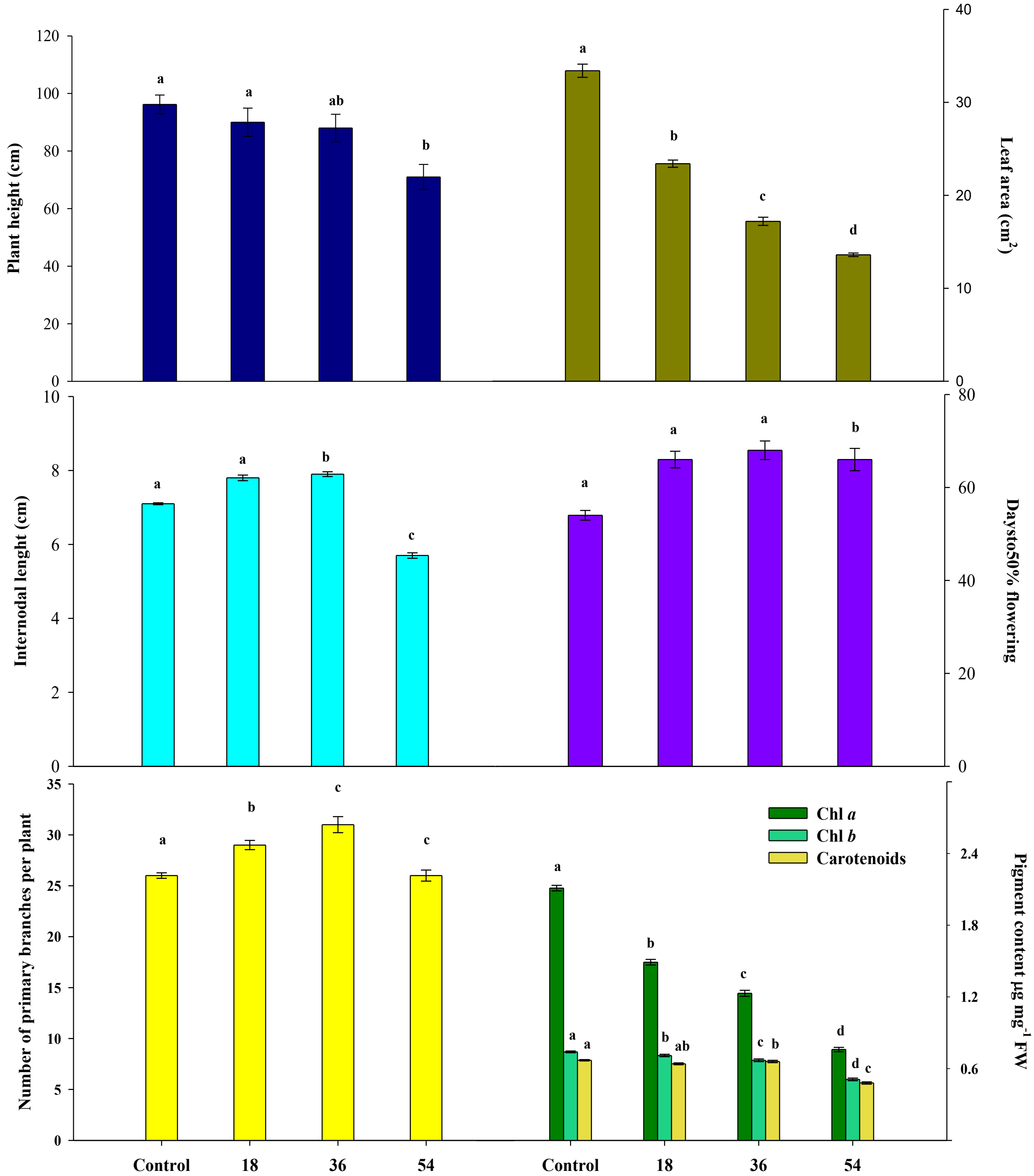
Table 2: Effect of Copper Nanoparticle on the meiotic behaviour of *Artemisia annua* L

Copper Nanoparticle (µg/ml)	No. of PMC's observed	Metaphasic Abnormalities (%)					Anaphasic Abnormalities (%)						Oth.	T.Ab. (%)
		Sc	Pm	St	Sa	Mv	Br	Lg	Un	sti	Asy	Dp		
Control	310	-	-	-	-	-	-	-	-	-	-	-	-	-
18	298	0.34±0.	0.33±0.	0.45±0.	0.44±0.	0.44±0.	0	0	0.11±0.	0.68±0.	0.34±0.	0	0.57±0.	3.69±0.0

		01	19	11	09	09			10	22	14		13	4
36	307	0.43±0. 11	0.33±0. 01	0.77±0. 13	0.65±0. 18	0.54±0. 11	0.65±0. 18	0.75±0. 09	0.43±0. 09	0.86±0. 08	0.43±0. 09	0.44±0. 12	0.54±0. 11	6.830.16 ±
54	339	0.59±0. 02	0.69±0. 10	1.48±0. 06	0.99±0. 14	1.47±0. 15	0.87±0. 14	1.18±0. 05	0.79±0. 20	1.76±0. 10	0.79±0. 11	0.68±0. 07	1.08±0. 07	12.390.0 3±

Abbreviations: **Sc-** Scattering; **Pm**- Precocious movement of chromosomes; **St**- Stickiness; **Sa**- Secondary associations of bivalents; **Mv**- Multivalent; **Br**- Bridge formation;

Lg- Laggard; **Un**- Unorientaion; **Asy**- Asynchronous division; **Dp**- Disturbed polarity; **Oth**- Others; **T.Ab**. –Total abnormalities



Graph- Effect of Copper Nanoparticle on the plant height, interndal length, Leaf area, Days to 50% flowering,No. Of primary branches per plant and chlorophyll content of *Artemisia annua* L.

Figure1 : Showing effect of Copper Nanoparticle on the plant of *Artemisia annua* L.

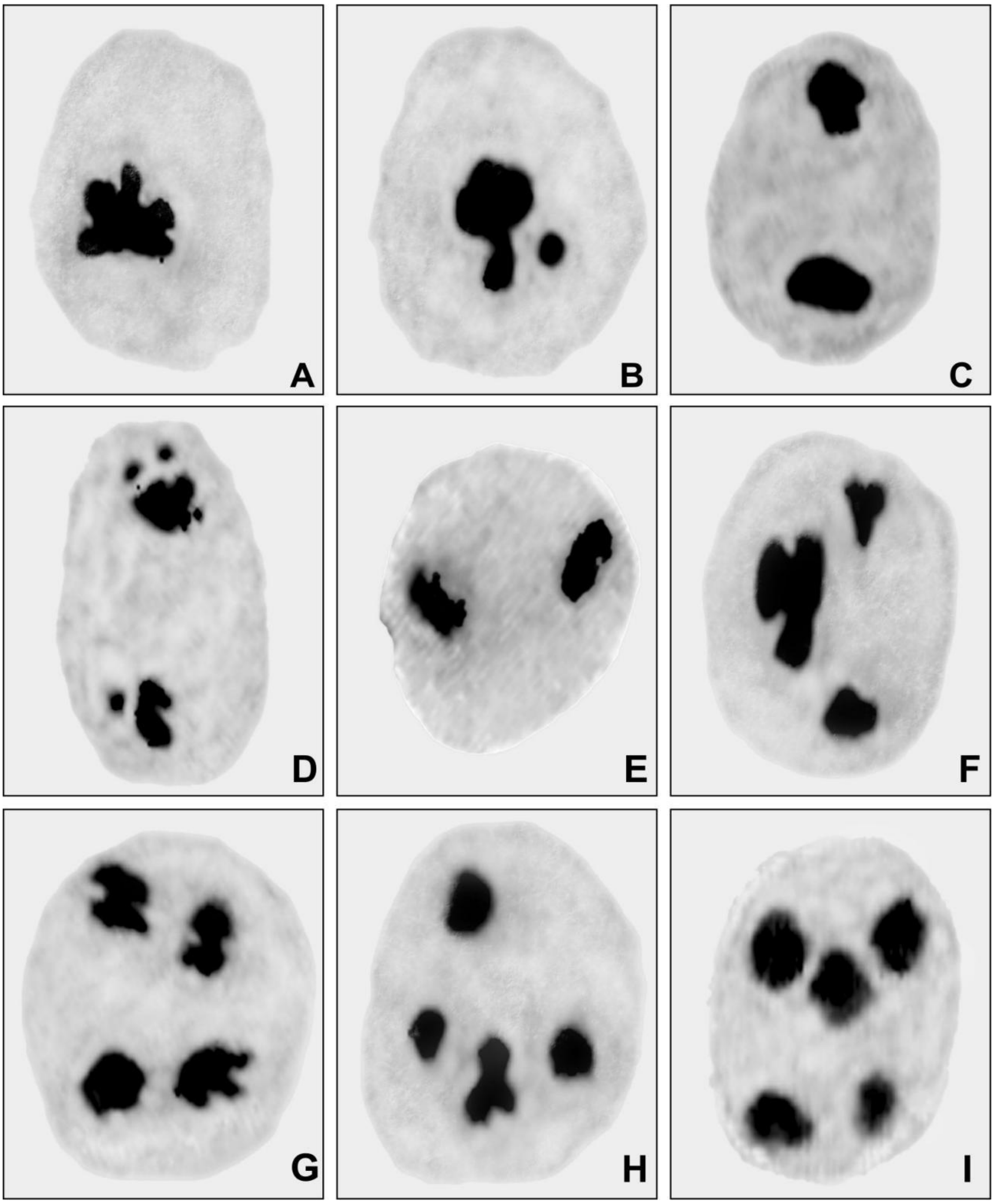


Figure2: Various chromosomal anomalies induced by Copper Nanoparticle treatments –**A)** Stickiness at Metaphase I; **B)** One Precocious chromosome at Metaphase I; **C)** Stickiness at Anaphase I; **D**) Two forward chromosome at Anaphase I with one laggard; **E)** Stickiness at Metaphase II; **F)** Asynchronization at Anaphase II; **G)** Stickiness at Anaphase II; **H)** Disturbed polarity at sticky Anaphase II; **I)** Pentapolarity

B. Meiotic analysis

In control plants a regular meiotic division was evident with 9 bivalent at metaphase I and 9:9 chromsomes at different pole in anaphase I. A varying degree of meiotic anomalies were recognized such as scattering, precocious movement, stickiness (at meta I/II and ana I/II), asynchronization, multivalents, bridges, laggards, unorientation, disturbed polarity, micronuclei formation etc **(Figure 2)**. Most prevalent abnormality examined at metaphase I and anaphase I both was stickiness followed by multivalent formation (**Table 2).** The observation of the present appraisal evidently specified the genotoxicity property of CuNPs on the genomic paradigm of *Artemisia* , which is related with a dose based enhancement in chromosomal aberrations in the treatment sets. A significant increment was observed in the TAB% calculated to be 3.69±0.04 (18 µg/ml) to 12.39±0.0.3 (54 µg/ml) and pollen fertility was also rendered a reduction in comparison to control plants which were accounted as 88.31 % at lower concentration and 69.76% at highest concentration **(Table 1).**

Studies conducted to increase information on genotoxic risks and mitopromotion related to exposure to emerging nanomaterials are of increasing interest for many researchers [10]. Aforementioned results justified that lower concentration of CuONP contribute positive impact on *Artemisia*. Similar results observed in *Elodea densa* (water weed) and stimulate photosynthesis at low concentration (<0.25 mg/L), but the impact scenario completely changes with higher doses and at 1 mg/L concentration a clear suppression in photosynthesis was observed [11]. The root morphology was reported to be negatively affected with Cu and CuO NP, with nearly complete inhibition with a high dose of NP [12,13]. CuO NP influence different stress factors, causes production of ROS that limits the rate of carbon fixation, which leads to photo inhibition [14]. Final production of ROS results in adverse effect on almost all cellular components producing protein modifications, lipid peroxidation, and damage to DNA [15]. In response to Cu naoparticle protective mechanism of plants get activated and different antioxidant compounds were observed to be significantly increased in plants [16,12] . Therefore, the adequate concentration of nanoparticles in the environment may cause a severe impact on agricultural system in future.

At the cellular level, CuO NP increases chromosomal peculiarities which lead to abnormal division. Stickiness was found to be most dominant chromosomal aberrations recorded in the metaphase and anaphase of mitosis. The stickiness presumably is due to intermingling of chromatin fibres, which leads to sub chromatid connections between chromosomes [17,18]. Followed by multivalent formation, which is the results of primary pairing at zygotene and chiasma formation among two or more homologous chromosomes [19]. Therefore studies are needed to better understand the hazardous effects of NPs before utilizing in different sectors.

Conclusion

The present investigation on the multifacet effect of copper nanoparticles (CuNPs) on *Artemisia annua* L. demonstrates that nanoparticle application exerts a concentration-dependent dual impact on plant growth, physiology, and phytochemical accumulation. At optimized low concentrations (18 µg/ml and 36 µg/ml) , CuNPs significantly enhanced seed germination, vegetative growth, chlorophyll content, and biomass production. However, higher concentrations resulted in phytotoxic effects characterized by reduced growth, chlorophyll degradation, membrane damage, and excessive reactive oxygen species generation. Overall, the study highlights the dual nature of copper nanoparticles—beneficial at controlled levels and detrimental at elevated doses. These findings emphasize the importance of dosage optimization for sustainable agricultural and pharmaceutical applications.

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