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Research Article

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Copper Sulfate Nanoparticales In vitro Applications on Verbena bipinnatifida Nutt. Stimulating Growth and Total Phenolic Content Increasments.

Ezzat A. Genady[1], Ebtesam A. Qaid[2] and Ashraf H. Fahmy[3]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar Univ., Nasr City, Cairo, Egypt
[2]Department of Biology, Faculty of Science, Taiz University, Yemen
[3]Agriculture Genetic Engineering Research Institute, Agricultural Research Center, Egypt

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ABSTRACT

Nanotechnology opens a large scope of novel applications in the fields of biotechnology, medicine, nutrition and energy. Since nanoparticles (NPs) have unique physicochemical properties, i.e. high surface area, high reactivity, tunable pore size and particle morphology. Nanoparticles can serve as "magic bullets" containing herbicides, nano-pesticide fertilizers or genes, which target specific cellular organelles in plant and release its contents. Here, we report novel successful in vitro effects of $CuSO_4$-NPs on seeds of Verbena bipinnatifida Nutt. to enhance phenolic contents which are important compounds in pharmaceutical industries especially anti oxidant medicaments.
Keywords: *Copper Sulfate Nanoparticales ($CuSO_4$-NPs), In vitro, Germination, Total Phenolic content, Verbena bipinnatifida Nutt.*

INTRODUCTION

Nanotechnology, a new emerging and fascinating field of science, permits advanced research in many areas and open widely novel applications in the field of biotechnology, agriculture, electronics, energy, medicine and life science(1).Nanotechnology offers an expanding research for reproductive science technology, conversion of agricultural and food wastes to energy, useful byproducts production through enzymatic nanobioprocessing, chemical sensors production, cleaning water, diseases prevention and plant treatment applictions using various nanocides(2,3).The use of modern techniques in the field of nanotechnology is one of the important recent trends for improving active constituents production in the field of tissue culture(4). Researchers from their findings suggested positive effects on plant growth and development. The impact of engineered nanopariticles (ENPs) on plants depends on the composition, concentration, size and physical and chemical properties of ENPs as well as plant species(5).Many researches used nanoparticles like nano silicon(6-8), nano iron (9-11)and nanocopperon plant species(12-14). *Verbena bipinnatifida* Nutt. Family Verbenaceae (VbV) is cultivated in Egypt as an ornamental plant(15) and it is widely distributed as small herbaceous plant with small purple erect flowers (16). The main medicinal uses of verbena species are antioxidant(17), sleep promoting (18)and hepatoprotective activity(19). Several investigations showed that *Verbena bipinnatifida* Nutt. Family Verbenaceae contains phenolic compounds and iridoids (20-22). Here we report on the effect of different concentrations of both CuSO4 and $CuSO_4$-NPs and their effect on growth parameters (seed germination, shoot length, root length and fresh weight) and total phenolic content of *Verbena bipinnatifida* Nutt.

Corresponding Author Dr. Ezzat A. Genady. E-Mail: Genady2004@yahoo.com

MATERIALS AND METHODS

2.1.Preparation of CuSO4-NP suspension

$CuSO_4$-NPs (1g) was dissolved in 50 ml distilled water to prepare different concentrations of CuSO4-NPs (5, 10 and 15 µg L^{-1}). Prior being added to culture media, nanoparticle suspensions were centrifuged and filtered to avoid particles aggregation (23).

2.2. Surface Sterilization:

Seeds of *Verbena bipinnatifida* Nutt. were surface sterilized under aseptic condition in laminar flow hood (NUAIRE™, USA) with 0.1% fungicide for 10 min followed by soaking for 2 min in 70 % (v/v) ethanol, then washed once with sterile D. D. H_2O, then seeds were rinsed with 20 % (v/v) commercial Clorox® (5.25% Sodium hypochlorite) supplemented with few drops of Tween 20 for 20 min, followed by soaking for 2 min in sterile D. D. H_2O, then rinsed five times in sterile D.D. H_2O.

2.3. Culturing of explants

Seeds were cultured onto germination medium (GM) basically contains Murashige and Skoog salts (Murashige and Skoog 1962)(24) supplemented with 30 g sucrose, 8 g agar as a solidifying agent. Seven treatments were conducted as following:

Control(T1); 5 µM L^{-1} $CuSO_4$ (T2); 10 µM L^{-1} $CuSO_4$ (T3); 15 µM L^{-1} $CuSO_4$ (T4); 5 µM L^{-1} $CuSO_4$-NPs (T5); 10 µM L^{-1} $CuSO_4$-NPs (T6); 15 µM L^{-1} $CuSO_4$-NPs (T7)were added to germination medium.

Cultured seeds were then incubated at dark for two days in controlled growth chamber (Shel-Lab, USA) at 25°C and then transferred to a 16-h photoperiod. After 15 d the seedlings were collected and the shoot length, root length and fresh weight were recorded.

2.4. Statistical Analysis

Data obtained were exposed to the proper statistical analysis of complete randomized design as described by Snedecor and Cochran (25) in three replicates. Means obtained were differentiated using Duncan's new multiple range test as described by Duncan (26).

2.5.Determination of total phenolic content:

2.5.1. Extraction of phenolic content.

The method described by the International Organization for Standardization (ISO) 14502-1 was used. Briefly,(0.001 g of each sample was weighed in an extraction tube, and 5 ml of 70% methanol at 70 °C was added. The extract was mixed and heated at 70 °C on a vortex for 10 min. After cooling at room temperature, the extract was centrifuged at 200g for 10 min. The supernatant was decanted in a graduated tube. The extraction step was repeated twice. Both extracts were pooled and evaporated.

2.5.2. Determination of total phenolic content.

The total phenolic content (TPC) was determined by spectrophotometry, using gallic acid as standard, The concentrated extracts were dissolved in the least volume of methanol then completed to 10 ml, 100 µl of this extract was diluted with 8 ml distilled water. To the sample 0.5 ml of 50 % Folin-Ciocalteu reagent was added and left 8 min, and then 1.5 ml of 5% sodium carbonate was added, mixed and allowed to stand for 60 min., protected from light. The absorbance was measured at 725 nm using 95% methanol as a blank. The total phenolic content was measured in terms of gallic acid equivalent.

RESULTS

Response of *Verbena bipinnatifida* Nutt. seedlings grown in MS medium supplemented varied significantly across different concentrations of $CuSO_4$ and $CuSO_4$-NPs (Figure 1 and 2). The verbena seedling treated with the $CuSO_4$-NPs showed the significantly high values in most of the growth parameters tested. Results showed that the $CuSO_4$ and $CuSO_4$-NPs concentrations had significant effect on increment of shoot length over control. At 5 µM of $CuSO_4$-NPs the highest shoot length was recorded which reached about 52 % more than control. A marked increase in root length was observed at different levels of $CuSO_4$-NPswhich reached to 21 % and 20 % at 5 µM and 10 µM , respectively. On the other hand, increasing $CuSO_4$ concentrations above 5 µM caused significant reduction in root length, whereas, highest reduction (75%) in root length was recorded at 15 µM. Moreover, increasing the level of $CuSO_4$ and $CuSO_4$-NPs was accompanied by a significant increasment in fresh weight, whereas15 µM $CuSO_4$-NPs reached to 39% increment as compared with control.

Data recorded in (Table 1) represent the effect of $CuSO_4$-NPs at different concentration on total phenolic content of *Verbena bipinnatifida* Nutt. germinated seeds. It was clearly shown from data that the treatment with$CuSO_4$-NPs at concentrations, 5 µM, 10 µM and 15 µM led to increasments in total phenolic content compared with control and $CuSO_4$ treatments, while the minimum values were recorded at control and 15 µM $CuSO_4$.

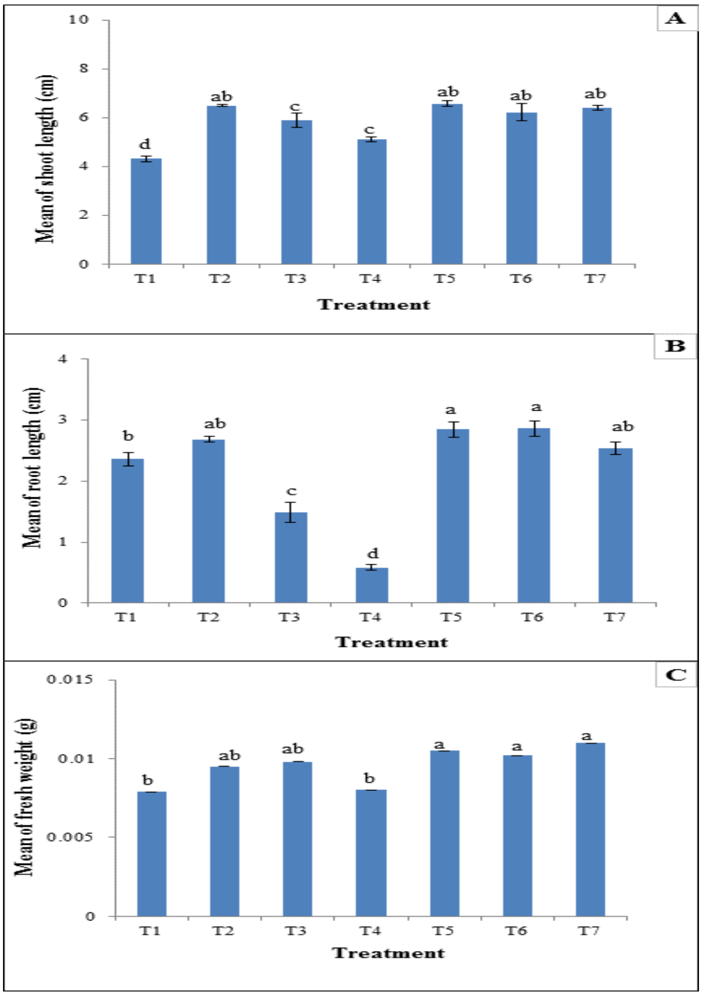

Figure (1): Effect of different concentrations of CuSO4 and CuSO4-NPs addition to control on growth parameters of (VbV): (A) shoot length; (B) root length and (C) fresh weight; Control: T1; 5 µM L^{-1} $CuSO_4$: T2; 10 µM L^{-1} $CuSO_4$: T3; 15 µM L^{-1} $CuSO_4$: T4; 5 µM L^{-1} $CuSO_4$-NPs: T5; 10 µM L^{-1} $CuSO_4$-NPs: T6; 15 µM L^{-1} $CuSO_4$-NPs: T7.

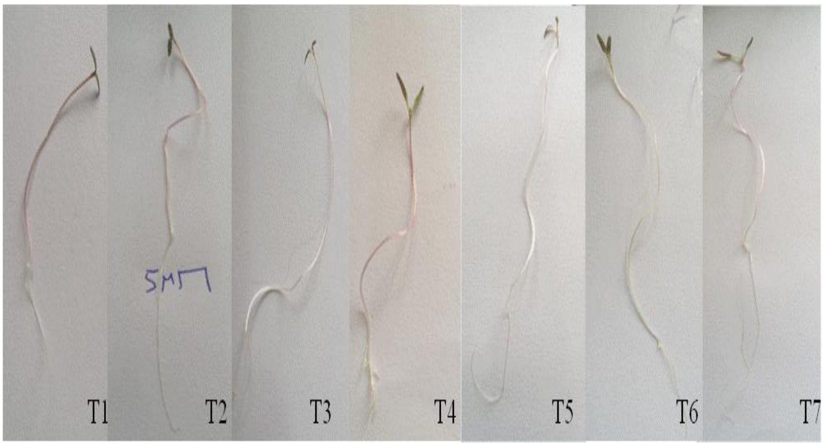

Figure (2): Effect of different concentrations of CuSO4 and CuSO4-NPs addition to control on growth parameters of (VbV): Control: T1; 5 µM L^{-1} $CuSO_4$: T2; 10 µM L^{-1} $CuSO_4$: T3; 15 µM L^{-1} $CuSO_4$: T4; 5 µM L^{-1} $CuSO_4$-NPs: T5; 10 µM L^{-1} $CuSO_4$-NPs: T6; 15 µM L^{-1} $CuSO_4$-NPs: T7.

Table (1): Total phenolic content in *Verbena bipinnatifidaNutt*. with control and different $CuSO_4$ and $CuSO_4$ NPs tested treatments

Sample	Total phenolic content (mg GAE/g DW)
Control	18.2
5 µM L^{-1} $CuSO_4$	28.1
10 µM L^{-1} $CuSO_4$	26.4
15 µM L^{-1} $CuSO_4$	21.2
5 µM L^{-1} $CuSO_4$ NPs	42.7
10 µM L^{-1} $CuSO_4$ NPs	34.3
15 µM L^{-1} $CuSO_4$ NPs	31.4

DISCUSSION

The elements (Co, Cu, Fe, Mn, Mo, Ni and Zn) called micronutrients where they play vital functions in plant cells. Micronutrients are essential for biosynthesis and function of nucleic acids, growth substances, chlorophyll and secondary metabolites, carbohydrates as well as for growth and stress resistance, on the other hand, they are required in a small traces, whereas, turns toxic at higher concentrations. The nanoparticles can be reported with minimal toxicity on test plants if it has no negative effect on root growth at such a high concentration according to the USEPA guidelines.

Properties of nanoparticles, the chemical composition, chemical structure, particle size and surface area play an important role in the mechanism of toxicity of nanoparticles. Toxicity of nanoparticles may be attributed to two different actions (1) a chemical toxicity based on the chemical composition e.g., release of toxic ions and (2) stress or stimuli caused by the surface, size and shape of the particles (27).

Several studies concentrate on the adverse effect of the nanoparticle treatment at higher concentrations. However, here in this research the lower concentration of nanoparticles shows significantly positive response. Our results showed that the lower concentrations of CuSO4 and CuSO4-NPs induced increasing in shoot length, root length and fresh weight of vbv. Verma *et al*.,(2011)(28)mentioned that copper promoted the growth of Vigna radiate seedlings when presented at low level, while at high levels, copper retarded growth by interfering with normal cellular metabolic events. Hafeez*et al*.,(2015)(29) stated that MS medium blended with low concentration of Cu-NPs significantly increased fresh weight of wheat as compared with control plants. Nanoparticles may not have a direct effect on plant growth but may be responsible about the effect via indirect mechanisms. This decrease reported here in root length may be due to reduction in cell division. Similar results has been reported by Verma *et al*., (2011)(28) who found that the growth of root system of mung bean was significantly decreased with increasing level of copper sulfate. Hong et al.,(2015)(30)reported that high level of Cu-NPs reduced root length in hydroponically grown alfalfa by 47.65% compared with control. Copper nanoparticles had shown to be toxic with two crop species i.e. mung bean (Phaseolusradiatus) and wheat (Triticumaestivum) as demonstrated by the reduced seedling growth rate (31).

In brief, the main significant results obtained from determination of TPC by the Folin-Ciocalteu method(32,33) showed that the total phenolic content was increased in *Verbena bipinnatifida* Nutt. by more than two folds surpassing control by using the treatment of 5µM L^{-1} CuSO4 NPs. Moreover, the total phenolic content was also decreased when the concentration of $CuSO_4$ and $CuSO_4$ NPs increased than 5 µM L^{-1}.

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REFERENCES

1. Asra P. and Srimth R. Effect of Nano Silver on Seed Germination and Seedling in Pennisetumglaucum. J. Clust. Sci (2015) 26: 693-701.

2. Carmen IU, Chithra P, Huang Q, Takhistov P, Liu S, Kokini JL Nanotechnology: a new frontier in food science. Food Technol (2003) 57:24–29

3. Nair R, Varghese SH, Nair BG, Maekawa T, Yoshida Y, Kumar DS (2010) Nanoparticulate material delivery to plants. Plant Sci 179:154–163

4. Palaniselvam Kuppusamy, Mashitah M. Yusoff, Gaanty Pragas Maniam, Natanamurugaraj Govindan, Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications – An updated report. Saudi Pharmaceutical Journal (2015) 1-12

5. Ma X, Geiser-Lee J, Deng Y, KolmakovA .Interactions between engineered nanoparticles (ENPs) and plants: phytotoxicity, uptake and accumulation. Sci Total Environ (2010) 408(16):3053–3061

6. Suriyaprabha R, Karunakaran G, Yuvakkumar R, Rajendran V, Kannan N. Silica nanoparticles for increased silica availability in maize (Zeamays.L) seeds under hydroponic conditions. Curr.Nanosci., 2012; 8:902-908.

7. Kalteh M, Alipour ZT, Ashraf S, Aliabadi MM, Falah A. Effect of silica Nanoparticles on Basil (Ocimumbasilicum) Under Salinity Stress. J. Chem. Health Risks, 2014; 4(3): 49 –55.

8. Siddiqui MH, Al-Whaibi MH. Role of nano-SiO_2 in germination of tomato (Lycopersicumesculentumseeds Mill.). Saudi J. Biol. Sci., 2014; 21: 13–17.

9. Afshar RM, Hadi H, Pirzad A. Effect of Nanoiron foliar application on qualitative and quantitative characteristics of cowpea, under end season drought stress. Int. Res. J. Appl. Basic Sci., 2012; 3 (8):1709-1717.

10. Afshar RM, Hadi H, Pirzad A. Effect of nano iron on the yield and yield component of cowpea (Vignaunguiculata) under end season water deficit. Int. J. Agric. Res. Rev., 2013; 3 (1):27-34.

11. Dhoke SK, Mahajan P, Kamble R, Khanna A. Effect of nanoparticles suspension on the growth of mung (Vignaradiata) seedlings by foliar spray method. Nanotechnol. Dev., 2013; 3: 1-5.ol

12. Lee WM, An YJ, Yoon H, Kweon HS. Toxicity and bioavailability of copper nanoparticles to the terrestrial plants mung bean (Phaseolusradiatus) and wheat (Triticumaestivum): plant agar test for water-insoluble nanoparticles. Environ.Toxicol.Chem.,2008;27(9):1915-1921.

13. Dimkpa CO, McLean JE, Latta DE, Manangón E, Britt DW, Johnson WP, Boyanov MI, Anderson AJ. CuO and ZnO nanoparticles: phytotoxicity, metal speciation, and induction of oxidative stress in sand-grown wheat. J. Nanopart. Res., 2012; 14:1125.

14. Sahar M Ouda. Antifungal activity of silver and copper nanoparticles on two plant pathogens, Alternariaalternariaand Botrytis cinerea.Res. J. Microbiol., 2014; 9(1): 34-42.

15. Sharaf, A.M., Sharaf Dictionary, Pharmacognosy, 1956, 938, Faculty ofPharmacy, University of Cairo, Cairo, Egypt.

16. Tăckholm, V., Student's Flora of Egypt, 2nd.,Edition,1974, University of Cairo, Egypt, AngloEgyptian Bookshop.

17. Bilia, A.R.; Giomi, M.; Innocenti, M.; Gallori, S.; Vincieri, F.F. HPLC-DAD-ESI-MS analysis of the constituents of aqueous preparations of verbena and lemon verbena and evaluation of the antioxidant activity. J. Pharm. Biomed. 2008, 46, 463–470.

18. Makino Y.; Kondo, S.; Nishimuna, Y.; Tsukamoto, Y.; Huang, Z.L.; Urade, Y. Hastatoside and verbenalin are sleep-promoting components in Verbena officinalis. Sleep Biol. Rhythms 2009, 7, 211–217.

19. Singh, B.; Saxena, A.K.; Chandan, B.K.; Anand, K.K.; Suri, O.P.; Suri, K.A.; Satti, N.K. Hepatoprotective activity of verbenalin on experimental liver damage in rodents. Fitoterapia1998, 69, 135–140.

20. El-Hela, A.A., Sowinski, P. and KrauzeBaranowski, P. and KrauzeBaranowska, M.,Iridoids and phenylethanoids of Verbenabipinnatifidanutt. ActaPoloniaePharmaceutica-Drug Research,2000,57, No.1, pp 65-68.

21. M.M.Al-Azizi., A.B..Singab, M.M.A.El-Hamouly and E.A.M.Genady, Constituents of Verbena bipinnatifida Nutt., cultivated in Egypt, Al-Azhar Journal of Pharmaceutical Sciences, 2000, 25, 238..

22. Michael HN, Salib JY, IshakMS.,Newmethoxyflavone glycosides from Verbena bipinnatifida Nutt., Pharmazie. ;2001,56(4):348-9.

23. Helaly MN, El-Metwally MA, El-Hoseiny H, Omar SA, El- Sheery NI. Effect of nanoparticles on biological contamination of in vitro cultures and organogenic regeneration of banana.Aust J Crop Sci. 2014;8(4):612-24.

24. Murashige, T. and F. Skoog. A revised medium for rapid growth and bioassays with tobacco tissue cultures.PhysiologiaPlantarum, (1962);15: 473-497.

25. Snedecor, G. W. and W. G. Cochran (1969). Statistical Methods (6th Ed.)Iowa State University Press, Ames,USA.

26. Duncan, D. B . Multiple range and multiple "F-test". Biometrics, (1955); 11: 1-42

27. Brunner T.J., Wick P., Manser P., Spohn P., Grass R.N., Limbach L.K., BruininkA.andStarkW.J.In vitro cytotoxicity of oxide nanoparticles: comparison to asbestos, silica, and the effect of particle solubility.EnvironSciTechnol. 2006 Jul 15;40(14):4374-81.

28. Verma J.P., Singh V. and Yadav J. (2011) Effect of copper sulfate on seed germination, plant growth and peroxidase activity of mung bean (Vigna radiate). International Journal of Botany: 7(2):200-204.

29. Hafeez, A., Razzaq, A., Mahmood, T., Jhanzab, H.M., (2015). Potential of Copper Nanoparticles to Increase Growth and Yield of Wheat, Journal of Nanoscience with Advanced Technology,1(1): 1-6.

30. Hong J., Rico C.M., Zhao L., Adeleye A.S., Keller A.A.Peralta-Videa, J.R., and Gardea-Torresdey J.L. Toxic effects of copper-based nanoparticles or compounds to lettuce (Lactuca sativa) and alfalfa (Medicago sativa)Environ. Sci.:Processes Impacts, 2015, 17, 177–185.

31. Lee W.M., An Y.J., Yoon H. and Kweon, H.S.Toxicity and bioavailability of copper nanoparticles to the terrestrial plants mung bean (Phaseolusradiatus) and wheat (Triticumaestivum): plant agar test for water-insoluble nanoparticles.(2008). Environ. Toxicol.Chem. 27, 1915.

32. McDonald, S., Prenzler, P. D., Antolovich, M., and Robards, K. (Phenolic content and antioxidant activity of olive extracts). Food chemistry, 2001.73(1),73-84.

33. Rohman, A., Riyanto, S., Yuniarti, R.N.S., and Mulatsih, W.(Antioxidant activity, total phenolic, and total flavonoid of extracts and fractions of red fruit (Pandanusconoideus Lam)).International Food Research Journal,2010.17:97-106.