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Physiological Effect of ZnO Nanoparticles on Peanut (*Arachis hypogaea* L.)

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Abstract: *This study was carried out to determine the physiological effects of ZnO nanoparticles, which have been used for different purposes in recent years, on peanuts (Arachis hypogaea), which are ecologically and economically important agricultural plants. In the study, the effects of different ZnO concentrations (0, 5, 25, 50, 100 and 250 mg/L) on germination, growth and development and some physiological parameters of peanut were determined. Final germination rates at 100 and 250 mg/L ZnO concentrations were significantly reduced compared to control. Adverse effects of high concentrations applied on the growth and development of seedlings were determined. The protein content of the seedling tissues decreased significantly at high concentrations. While the total carbohydrate content increased at low concentrations compared to the control, it was determined that it decreased at high concentrations. The changes in the amount of phenolic compounds and non-protein sulfidriyl groups showed that high concentrations of ZnO cause stress. In addition, it has been clearly shown that increases in the amount of hydrogen peroxide, which are reactive oxygen species, and malondialdehyde, which is an oxidative stress indicator, cause oxidative stress in the peanut tissues.*

Keywords— Nano-ZnO, germination, physiological effect, stress

INTRODUCTION

Among different types of nanomaterials, metal and metal oxide nanoparticles differ in their small size and large surface area, and various physical and chemical properties such as differences in surface charges, shapes, conductivity, melting and freezing points (Dizaj et al., 2014; Falcaro et al., 2016). Due to their unique properties, many metal, metal oxide and alloy nanoparticles are produced and used in many fields (Karlsson et al., 2008). Metal nanoparticles such as titanium dioxide, copper, zinc, aluminum and silver nanoparticles are used as additives in industrial products. For example, ZnO is a multipurpose inorganic material and ZnO nanoparticles are one of the most widely used materials in various industrial fields (Baek et al., 2012). Thus, considering that the concentration of the products in the environment will gradually increase with the use of nanotechnological products, great attention has been drawn to environmental pollution and ecological effects in recent years (Nel et al., 2006; Batley et al., 2013). For this reason, it is thought that plants, like other living things, will be directly affected by these nanotechnological substances (Navarro et al., 2012). Many studies have been conducted in the literature on the effects of nanometals on plants in recent years (Dimkpa et al., 2012; Dimkpa et al., 2015; Singh and Kumar, 2020).

Peanut (*Arachis hypogaea*) is a legume belonging to the Fabaceae family, which is widely grown in tropical or subtropical climates, accounting for 12% of the world's oilseed production (Schilling, 1996). Since peanut is a hot climate plant, it is produced in the irrigable coastal plains of the Mediterranean and Aegean regions in Turkey, where the Mediterranean climate is dominant. Since peanuts form their fruits under the ground, it is successfully grown in the relatively light sandy-loam soils of these regions. Although Turkey's share in world production is very low, the yield per hectare is higher than the world average (Kadiroğlu, 2008).

This study was carried out to determine the physiological effect of zinc oxide, one of the nanoparticles used for different purposes in recent years, on the growth and development of peanut, which is an ecological and economically important agricultural plant.

MATERIALS AND METHODS

Plant material and treatment

Peanut (*A. hypogaea* L.) seeds were sterilized with 5% sodium hypochlorite for fifteen minutes before starting the study. These seeds were then rinsed three times with distilled water to remove the sodium hypochlorite from their surface. These seeds were studied in sterile plastic containers with 10 seeds in each container in four replications. The seeds were allowed to germinate and develop under the influence of 0, 5, 25, 50, 100 and 250 mg/L ZnO NPs (particle size <50 nm). ZnO NPs concentrations were prepared by dilution of the stock suspensions with deionized water. The solutions were stirred for two minutes using small magnetic bars prior to use to avoid aggregation. Applications were carried out with ten seeds in each container. Germination and growth stage were carried out in a controlled climate cabinet (Snijders Scientific, The Netherlands). The seedlings continued to grow in a climate chamber at a light level of 120 μE $m^{-2}.s^{-1}$ and a temperature of 23±1 °C in a 16/8 h light/dark regime. If the seedlings needed, these seeds were watered with their own solutions. Peanut seedlings grown under the influence of nanoparticles were harvested on the 10th day of application.

Physiological analyses

For protein analysis, fresh plant samples were homogenized in 0.1 M phosphorus buffer (pH 7.4). The protein contents of the samples were determined by Lowry et al. (1951). Bovine serum albumin was used as a standard to determine the amount of protein. Total carbohydrate

determination was determined according to Plummer (1998) using anthron solution. The prepared samples were kept in a boiling water bath for 10 minutes and then shock-cooled and read in a UV/VIS spectrophotometer at 620 nm. Glucose was used as a standard to determine the amount of total carbohydrate. Determination of total phenolic compounds of plant samples was done according to Ratkevicius et al. (2003). Samples to be homogenized were centrifuged for 10 minutes. Then, 50 µl of the supernatant was taken and the final volume was 1 ml, 3% sodium carbonate and 0.3 N Folin-Ciocalteau were added and left at room temperature for 2 hours. These samples were then read in the spectrophotometer at 765 nm. The homogenized fresh plant organs were centrifuged for 15 minutes. 500 µl of supernatant was taken and 2.5 ml of phosphate buffer (pH 7.4) was added. Finally, 0.5 ml of 5,5'-dithio-bis(2-nitrobenzoic acid) was added and vortexed. After 20 minutes of incubation, the prepared samples were read in the spectrophotometer at 412 nm (Ellman, 1959). Reduced glutathione was used as a standard for the determination of non-protein sulfhydryl groups. For malondialdehyde (MDA) contents of peanut seedlings, samples were homogenized in 10% TCA. These samples were centrifuged for 15 minutes. Then, 2 ml of supernatant was taken, 2 ml of thiobarbutiric acid was added, and it was kept in a water bath at 95°C for 30 minutes. Following this process, the samples were shock-cooled in an ice-water environment. Samples were read at 532, 600 and 450 nm in a UV/VIS spectrophotometer to determine the amount of MDA (Zhou, 2001). The H2O2 content of the organs of peanut seedlings was determined according to Sergiev et al. (1997). After the weighed seedling organs were homogenized, 0.5 ml was taken from the samples. 0.5 ml of phosphorus buffer and 1 ml of 1 M KI were added to it. This mixture was read in a UV/VIS spectrophotometer at 390 nm.

Data analyses

Tolerance index (TI) was calculated as follows:

$$TI = \frac{\textit{Length of seedlings under ZnO effect}}{\textit{Length of seedlings in control}} \; x\,100 \quad -100$$

Vigor index was calculated as follows (Abdul-Baki and Anderson, 1973):

Vigor index= Final germination (%) X Total seedling length (cm)

All analyzes in this study were performed in four replications. SPSS program (11.0) was used for statistical analysis of the obtained data. For this purpose, the least significant difference test (LSD) was used to compare the data.

RESULTS AND DISCUSSION

Since seed germination and early seedling development constitute the most important stage of a plant's development, determining the effects of stress factors at these stages makes it more meaningful. Seed germination experiments are simple and one of the parameters used in toxicity experiments. Final germination rates and statistical evaluation of peanut seeds under the influence of different ZnO concentrations are given in Fig. 1a. While there was no statistically significant change in germination rates under the effect of 5, 25 and 50 mg/L concentrations of ZnO ($p>0.05$), decreases were found significant at 100 and 250 mg/L concentrations ($p<0.05$). There have been some studies on the effects of ZnO NPs on seed germination (Burman et al., 2013; Marslin et al., 2017). The fact that similar results were reported in the germination of *Brassica nigra* seeds of ZnO nanoparticles (Rehman et al., 2020), supports our study findings.

Root and shoot lengths were measured to determine seedling growth under the influence of ZnO NPs (Fig. 1b). Accordingly, while root growth decreased insignificantly at 5 mg/L concentration ($p>0.05$), it showed statistically significant decreases at 25-250 mg/L concentrations ($p<0.05$). On the other hand, shoot growth under the effect of ZnO showed insignificant change at 5-50 mg/L concentration ($p>0.05$), on the contrary, it was found to decrease significantly at 100 and 250 mg/L concentrations ($p<0.05$). There were decreases in vigor indices due to the increase in applied concentrations (Fig. 1c). Similarly, it was determined that the tolerance indices of the seedlings under the influence of ZnO were also decreased (Fig. 1d). In previous studies, it has been reported that the effects of nanometals on the growth and development of plants vary according to plant species, concentration, application environment and conditions (Bondarenko et al., 2013; Zafar et al., 2017; Singh and Kumar, 2019; Rehman et al., 2020). Zn NPs are most heavily used in agriculture (Awasthi et al., 2017; Agarwal et al., 2017). It has been reported that the use of ZnO-NP nano-fertilizer may cause toxic effects on plants (Xiang et al., 2015). In addition, some studies have reported that ZnO NPs can inhibit the plant root growth (Wang et al., 2013).

Although protein contents of roots did not show insignificant changes under the effect of 5 and 25 mg/L ZnO in peanut seedlings ($p>0.05$), significant decreases were observed at 50, 100 and 250 mg/L concentrations ($p<0.05$) (Fig. 2a) . On the other hand, significant reductions in protein content of shoots were determined only at 100 and 250 mg/L ZnO concentrations ($p<0.05$). Decreases in the amount of protein in plants have been reported at high ZnO concentrations (Rahmani et al., 2016). Determined decrease in protein content may be attributed to decline in the synthesis of some proteins and/or induced activities of proteolytic enzymes (Khudsar et al., 2001; Panda and Choudhury, 2005).

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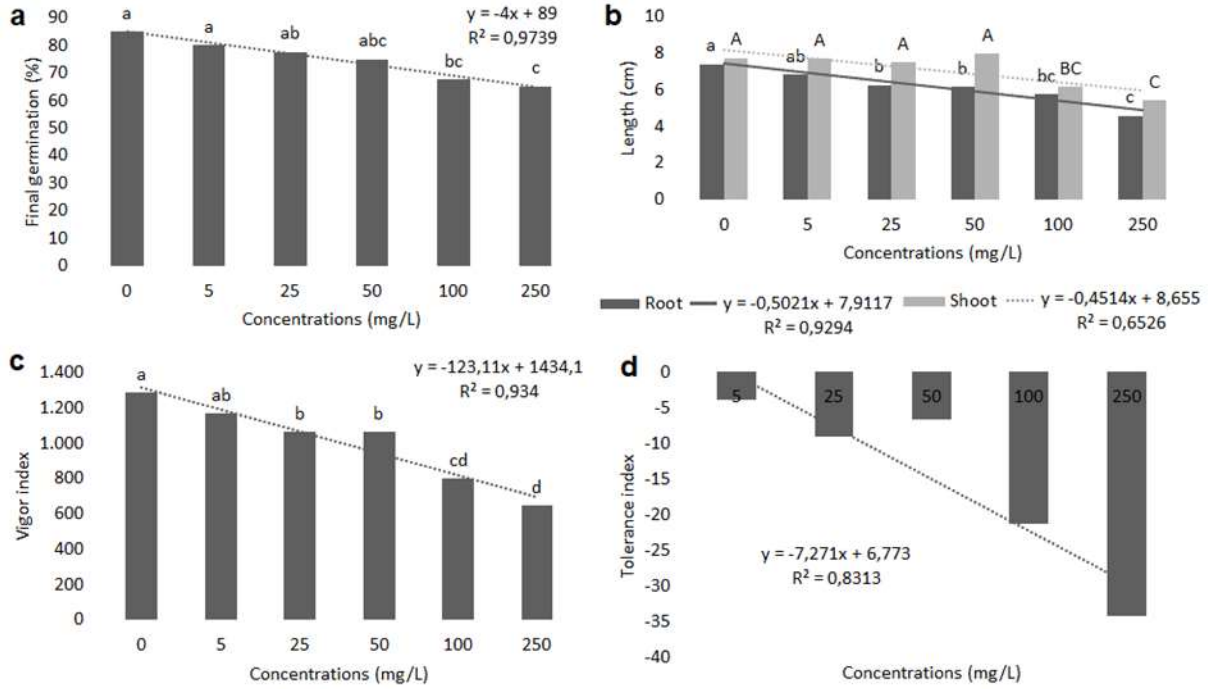

Fig. 1. *Final germination rates (a), root and shoot lengths (b), vigor index (c) and tolerance index (d) of peanut seedling after ZnO NPs applications and their statistical evaluations. Means with different letters are significantly different from one another according to LSD test (p<0.05).*

Total carbohydrate content increased up to 50 mg/L concentration under the effect of ZnO. It was determined that it decreased at 100 and 250 mg/L concentrations (Fig. 2b). Similar findings were also observed in shoots. It has been reported that ZnO applied to *Brassica napus* causes an increase in the amount of sugar (Rahmani et al., 2016). Heavy metal induced alterations in the osmatic balance of plants have been documented (Glenn et al., 1997). Our findings are in line with previous work reporting an increase in sugar levels under abiotic stresses (Gill et al., 2001).

There were changes in the contents of phenolic compounds of roots and shoots (Fig. 2c). Although insignificant increases were found in roots and shoots at low concentrations ($p>0.05$), significant increases were found in the levels of phenolic compounds, especially at high concentrations applied ($p<0.05$). Baskar et al. (2018) reported that total phenolic contents were higher in all NiO, CuO and ZnO nanoparticle concentrations applied to eggplants than control eggplants. In addition, they found that all applied nanoparticle concentrations increased up to 500 mg/L and decreased total phenolic contents at 1000 mg/L concentrations. While the amount of non-protein sulfhydryl groups increased at 5 mg/L ZnO concentration in seedling roots ($p<0.05$), other concentrations decreased insignificantly compared to control ($p>0.05$) ($p>0.05$) (Fig. 2d). In the shoots, these changes were insignificant compared to the control ($p>0.05$). It has been reported that nanoparticles cause oxidative stress in plants as a result of their interaction with cellular components resulting in the formation of reactive oxygen species (ROS) (Krishnaraj et al., 2012; Rico et al., 2013). Phenolic compounds and non-protein sulfhydryl groups as defense molecules in alleviating oxidative stress caused by excess ROS (Corral-Diaz et al., 2014).

It has been determined that the levels of MDA and hydrogen peroxide, which are indicators of oxidative stress, increase with increasing ZnO concentrations. Hydrogen peroxide contents of peanut seedling organs showed insignificant changes at 5 and 25 mg/L ZnO concentrations ($p>0.05$). On the contrary, it increased significantly ($p<0.05$) at concentrations of 50, 100 and 250 mg/L (Fig. 2e). The MDA content of roots and shoots increased statistically insignificantly up to 50 mg/L concentration ($p>0.05$), whereas it increased significantly at 100 and 250 mg/L concentrations ($p<0.05$) (Fig. 2f). Rao and Shekhawat (2014) reported that MDA formation in root, shoot and leaf tissues of *B. juncea* exposed to ZnO increased in a dose-dependent manner. In conclusion, besides the increase in hydrogen peroxide amounts, a dose-dependent increase in MDA contents showed that ZnO NPs could also induce oxidative stress in peanut seedlings.

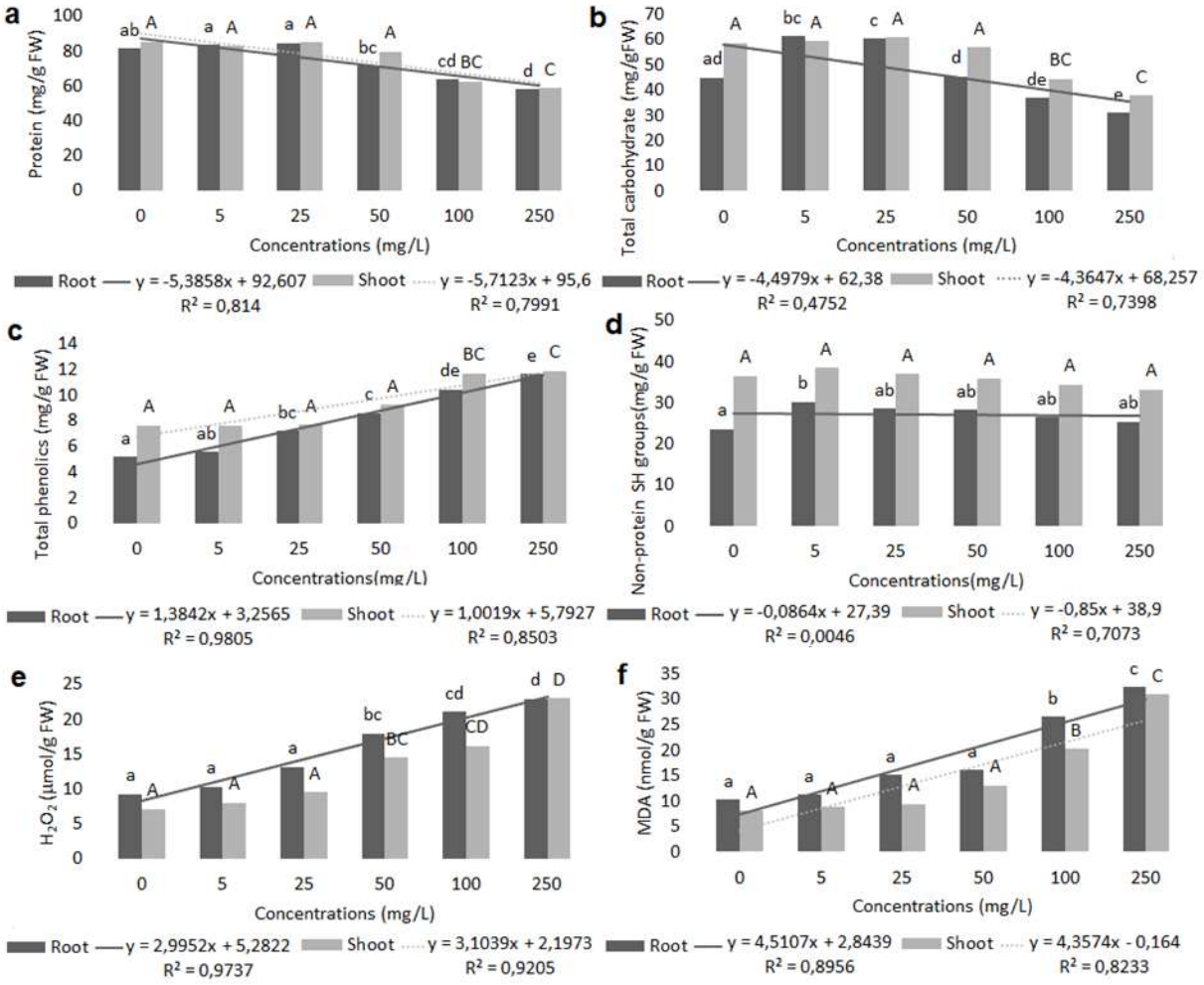

Fig. 2. *The contents of protein (a), total carbonhydrate (b), total phenolics (c), non-protein sulphydryl groups (d), hydrogen peroxide (e) and MDA (f) in peanut seedling after ZnO NPs applications and and their statistical evaluations. Means with different letters are significantly different from one another according to LSD test (p<0.05).*

As a result, the effects of different ZnO concentrations on germination, growth and development and some biochemical events of peanut seeds were determined in this study carried out under controlled conditions. Especially at high concentrations, negative effects on the growth and development of seedlings were determined. In addition, it is understood from the changes in the amounts of phenolic compounds and non-protein sulfidyryl groups, as well as the increase in the amounts of hydrogen peroxide and MDA, that the seedlings are stressed at high concentrations of ZnO.

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REFERENCES

Abdul-Baki, A.A. & Anderson, J. D. (1973). Vigor determination in soybean seed by multiple criteria 1. Crop science, 13(6), 630-633.

Agarwal, H., Kumar, S.V. & Rajesh Kumar, S. (2017). A review on green synthesis of zinc oxide nanoparticles—an eco-friendly approach. Resour. Effic. Technol. 3, 406–413.

Awasthi, A., Bansal, S, Jangir, L.K., Awasthi, G., Awasthi, K.K. & Awasthi, K. (2017). Effect of ZnO nanoparticles on germination of Triticum aestivum seeds. Macromol Symp 376(1700043), 1–5

Baek, M., Chung, H. E., Yu, J., Lee, J. A., Kim, T. H., Oh, J. M., Lee, W.J., Paek S.M., Lee J.K., Jeong J. & Choi, S.J. (2012). Pharmacokinetics, tissue distribution, and excretion of zinc oxide nanoparticles. International journal of nanomedicine, 7, 3081.

Baskar, V., Safia Nayeem S., Kuppuraj, S.P., Preethy, S., Muthu, T. & Ramalingam, S. (2018). Assessment of the effects of metal oxide nanoparticles on the growth, physiology and metabolic responses in in vitro grown eggplant (*Solanum melongena*). 3 Biotech 8, 362.

Batley, G. E., Kirby, J. K. & Mclaughlin, M. J. (2013). Fate and Risks of Nanomaterials in Aquatic and Terrestrial Environments. Accounts of Chemical Research 46, 854–62.

Bondarenko, O., Juganson, K., Ivask, A., Kasemets, K., Mortimer. M. & Kahru, A. (2013). Toxicity of Ag, CuO and ZnO nanoparticles to selected environmentally relevant test organisms and mammalian cells in vitro: a critical review. Arch Toxicol. 87(7), 1181-200.

Burman, U., Saini, M. & Kumar, P. (2013). Effect of zinc oxide nanoparticles on growth and antioxidant system of chickpea seedlings. Toxicol Environ Chem 95, 605–612.

Corral-Diaz, B., Peralta-Videa, J.R., Alvarez-Parrilla, E., Rodrigo-García, J., Morales, M.I., Osuna-Avila, P., Niu, G., Hernandez-Viezcas, J.A. & Gardea-Torresdey, J.L. (2014). Cerium oxide nanoparticles alter the antioxidant capacity but do not impact tuber ionome in *Raphanus sativus* (L). Plant Physiol. Biochem. 84, 277–85.

Dimkpa, C.O., McLean, J.E., Britt, D.W. & Anderson, A.J. (2015). Nano-CuO and interaction with nano-ZnO or soil bacterium provide evidence for the interference of nanoparticles in metal nutrition of plants. Ecotoxicology 24, 119–129.

Dimkpa, C.O., McLean, J.E., Latta, D.E., Manangon, E., Britt, D.W, Johnson, W.P., Boyanov, M.I. & Anderson, A.J. (2012). CuO and ZnO nanoparticles: phytotoxicity, metal speciation, and induction of oxidative stress in sand-grown wheat. J. Nanoparticle Res. 14, 1125.

Dizaj, S. M., Lotfipour, F., Barzegar-Jalali, M., Zarrintan, M. H. & Adibkia, K. (2014). Antimicrobial activity of the metals and metal oxide nanoparticles. Materials Science and Engineering C 44, 278-284.

Ellman, G.L. (1959). Tissue sulphydryl groups. Archives Biochemistry and Biophysics 82, 70-77.

Falcaro, P., Ricco, R., Yazdi, A., Imaz, I., Furukawa, S., Maspoch, D. & Doonan, C. J. (2016). Application of metal and metal oxide nanoparticles MOFs. Coordination Chemistry Reviews 307, 237-254.

Gill, P.K., Dev Sharma, A., Singh, P. & Singh Bhullar, S. (2001) Effect of various abiotic stresses on the growth, soluble sugars and water relations of sorghum seedling grown in light and darkness. Bulgarian Journal of Plant Physiology, 27, 72–78.

Glenn, E.P., Brown, J.J. & Khan, M. J. (1997). Mechanisms of salt tolerance in higher plants. In Basra A. S. & Basra R. K. (Eds.), Mechanisms of environmental stress resistance in plants (pp. 83–110). The Netherlands: Harwood Academic Publishers.

Kadiroglu, A. (2008). Yerfıstığı Yetiştiriciliği, Batı Akdeniz Tarımsal Arastırma Enstitüsü Müdürülüğü Yay., 53, Antalya.

Karlsson, H.L., Cronholm, P., Gustafsson, J. & Möller, L. (2008). Copper oxide nanoparticles are highly toxic: a comparison between metal oxide nanoparticles and carbon nanotubes. Chemical Research in Toxicology 21(9), 1726-1732.

Khudsar, T., Mahmooduzzafar, M. & Iqbal, M. (2001). Cadmiuminduced change in leaf epidermes, photosynthetic rate and pigment concentrations in *Cajanus cajan*. Biologia Plantarum, 44, 59–64.

Krishnaraj, C., Jagan, E.G., Ramachandran, R., Abirami S.M., Mohan, N. & Kalaichelvan. P.T. (2012). Effect of biologically synthesized silver nanoparticles on *Bacopa monnieri* (Linn.) Wettst. plant growth metabolism. Process Biochem 47(4), 651–658.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. The Journal Of Biological Chemistry, 193, 165-175.

Marslin, G., Sheeba, C.J. & Franklin, G. (2017). Nanoparticles alter secondary metabolism in plants via ROS burst. Front Plant Sci 8:1–8.

Navarro, D. A., Bisson, M. A., Aga, D.S. (2012). Investigating Uptake of waterdispersible Cdse/Zns quantum dot nanoparticles by *Arabidopsis thaliana* Plants. Journal of Hazardous Materials 211–212, 427–35.

Nel, A, Xia T, Madler L & Li N. (2006). Toxic potential of materials at the nanolevel. Science 311, 622–627.

Panda, S.K., Choudhury, S. (2005) Changes in nitrate reductase (NR) activity and oxidative stress in moss *Polytrichum commune* subjected to chromium, copper and zinc phytotoxicity. Brazilian Journal of Plant Physiology, 17, 191–197.

Plummer D.T. (1998). An introduction to Practical Biochemistry. Third edition. Tata McGraw-Hill Publishing Company Ltd., New Delhi.

Rahmani, F., Peymani, A., Daneshvand, E. & Biparva, P. (2016) Impact of zinc oxide and copper oxide nano-particles on physiological and molecular processes in *Brassica napus* L. Ind. J. Plant Physiol. 21(2):122–128.

Rao, S. & Shekhawat, G.S. (2014). Toxicity of ZnO engineered nanoparticles and evaluation of their effect on growth, metabolism and tissue specific accumulation in *Brassica juncea*. J. Environ. Chem. Eng. 2(1):105–114.

Ratkevicius, N., Correa, J. A. & Moenne, A.(2003). Copper accumulation, synthesis of ascorbate and activation of ascorbate peroxidase in *Enteromorpha compressa* (L.) Grev. (Chlorophyta) from heavy metal-enriched environments in northern Chile. Plant, Cell and Environment 26, 1599–1608.

Rehman, R.U., Khan, B., Aziz, T., Gul, F.Z. & Nasreen, S., Zia, M. (2020). Postponement growth and antioxidative response of *Brassica nigra* on CuO and ZnO nanoparticles exposure under soil conditions. IET Nanobiotechnology, 14(5):423-427.

Rico C.M., Morales M.I., McCreary R., Castillo-Michel H., Barrios A.C., Hong J., Tafoya A., Lee W.Y., Varela-Ramirez A., Peralta-Videa J.R. & Gardea-Torresdey J.L.

(2013). Effect of cerium oxide nanoparticles on rice: a study involving the antioxidant defense system and in vivo fluorescence imaging. Environ Sci. Technol. 47(11):5635–5642.

Sergiev, L., Alexieva, E. & Karanov, E. (1997). Effect of spermine, atrazine and combination between them on some endogenous protective systems and markers in plants. Comptes Rendus de l'Académie Bulgare des Sciences 51, 121-124.

Singh, D. & Kumar, A. (2020). Binary mixture of nanoparticles in sewage sludge: Impact on spinach growth. Chemosphere 254, 126794.

Wang P, Menzies NW, Lombi E, McKenna BA, Johannessen B, Glover CJ, Kappen P, Kopittke PM (2013). Fate of ZnO nanoparticles in soils and cowpea (*Vigna unguiculata*). Environ. Sci. Technol. 47, 1–25.

Xiang, L., Zhao, H.M., Li, Y.W., Huang, X.P., Wu, X.L., Zhai, T., Yuan, Y., Cai, Q.Y., Mo, C.H. (2015). Effects of the size and morphology of zinc oxide nanoparticles on the germination of Chinese cabbage seeds. Environ. Sci. Pollut. Res. 22, 10452–10462.

Zafar, H., Ali, A. & Zia, M. (2017). CuO nanoparticles inhibited root growth from *Brassica nigra* seedlings but induced root from stem and leaf explants. Environ Geochem Health. 42(1), 147-158.

Zhou, Q. (2001). The measurement of malondialdehyde in plants. In: Zhou Q. (ed.): Methods in Plant Physiology. China Agricultural Press, Beijing: pp. 173–174.