Effects of Silver and Copper Oxide Nanoparticles on the Germination Indices of *Brassica juncea* Seeds

Ahmad Faraz, Mohammad Faizan and Shamsul Hayat*

Plant Physiology Section, Department of Botany, Aligarh Muslim University, Aligarh, 202002, India
***Corresponding author: hayat_68@yahoo.co.in**

Abstract

Diverse application of nanoparticles (NPs) made it possible that they can be used in agriculture as nanofertilizers which increase the plant growth and yields. This paper investigates the *in vitro* stuides of two nanoparticles, silver (Ag) and copper oxide (CuO) nanoparticles on the germination of mustard seeds. Different concentration (0, 2, 4, 8 or 16 mg/L) of both nanoparticles was used in this experiment. CuO NPs shows the better result than AgNPs on germination and maximum increase was found at 4 mg/L of CuO NPs which was about 19% by CuO NPs at 4 mg/L as compared to control, whereas, vigour index was 267% and germination index was 29% higher than the control. At the same concentration (4 mg/L), AgNPs increased the germination rate by 8%, vigour index by 104% and germination index by 9% over the control.

Keywords: Agriculture, copper, mustard, nanoparticles, silver

Introduction

Nanotechnology is one of the most promising field of science which have potential application in many sectors. According to definition it is the branch of science which deals with the manipulation of matters at atomic, molecular or macromolecular level where properties significantly differ to those at larger scale. Particles have at least one dimeson and size between 1-100 nm are called nanoparticles (NPs). Importance of NPs is due to their unique physicochemical properties, *i.e*., high surface area, high reactivity, size and (Siddqui *et al*., 2015). Due to rapid progress and widespread use, it is now possible to synthesize and produce engineered nanoparticles (ENPs) with several types, dimensions, and morphologies (Gui *et al*., 2015).

A number of studies showed the response of nanoparticles on germination, seedling growth, physiology of plants but the result on seed germination is not yet conclusive due to variation in result (Hao *et al*., 2016). It was reported that TiO_2 NPs improve the seed germination of fennel (Liu and Lal, 2015). Similar result also found in the case of zinc oxide nanoparticles (ZnO NPs), for cucumber (Rosa *et al*., 2013). Increased germination of corn, barley, and soybean seeds in response to multiwalled carbon nanotubes (MWCNTs) was also found (Lahiani *et al*., 2013).

AgNPs is one of the most used nanoparticles due to their antimicrobial properties. Evidence suggests that AgNPs affects the plants growth and physiological activities (Vishwakarma *et al*., 2017; Sable *et al*., 2018). It was found that percent germination was accelerated by AgNPs in pearl millet (Parveen and Rao 2015). Cu-based NPs have various uses (Wang *et al*., 2012; Zuverza-Mena *et al*., 2015; Xiong *et al*., 2017).

In this study, we have investigated the impact of AgNPs and CuO NPs on the germination parameters of mustard seeds.

Materials and Methods

Source of mustard seed

Mustard Seed (*Brassica juncea* cv. RGN-48) purchased from Indian Agricultural Research Institute PUSA, New Delhi. Healthy and uniform size seeds were selected for the study. These seeds were surface sterilized with 0.01% mercuric chloride solution for five minutes, followed by rinsing with double distilled water (DDW), at least thrice, to remove even the traces of adhered mercuric chloride to the seed surface before treatments and transferred into the

petri dishes. The petri dishes were sealed with paraffin tape and placed inside the incubator at temperature of 22 ± 2 ^{o}C. Germination was asses in the form of percent germination, germination index (GI) and vigour index (VI)

Preparation of AgNPs and CuO NPs

AgNPs and CuO NPs purchased directly from the Sigma-Aldrich, New Delhi. Various of concentration (0, 2, 4, 8 and 16 mg/L) of both AgNPs and CuO NPs was prepared in DDW.

Seed germination rate

Germination rate was recorded every day from 2 to 7 days. The number of germinated seeds was noted daily for 7 days. Seeds were considered as germinated when their radicle showed at least 2-3 mm in length.

Vigor index (VI)

Seedling vigor index was calculated by the following formula as suggested by Abdul Baki and Anderson (1972).
Vigor index = % germination X seedling length (root + shoot) cm

Germination index (GI)

Germination index was calculated by the formula given by Tao and Zheng, (1990). Germination index (GI) = % germination ÷ germination days

Results

Seed germination rate

Seed germination rate was significantly improved by all the treatments. Out of the two nanoparticles, the response of CuO NPs was significantly higher than that of AgNPs, irrespective of the concentration. CuO NPs (4 mg/L) proved best that increased the seed germination rate by 19%, over the control, whereas, at the same level AgNPs had 8% more seeds to germinate **(Figure 1).**

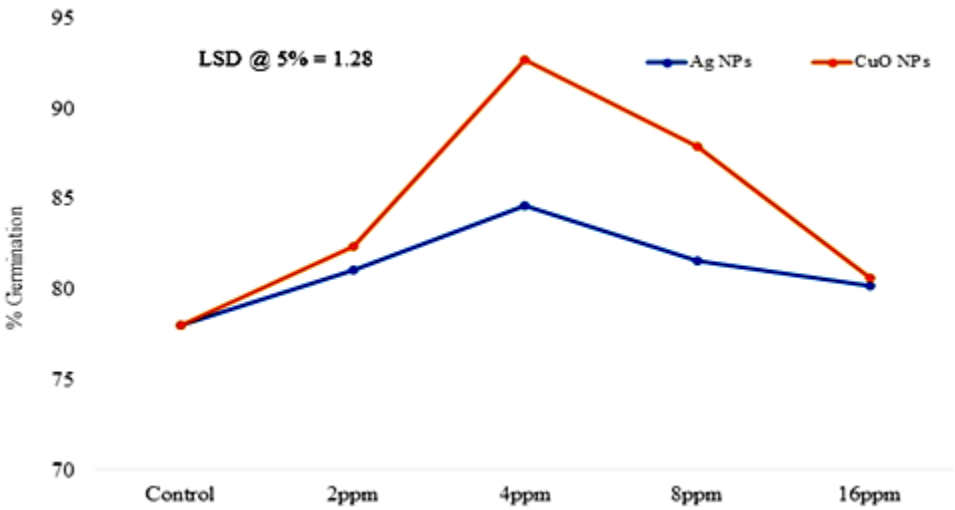

Figure 1: Effect of AgNPs and CuO NPs on percent germination of mustard seeds at 7th days. Vertical bars show standard errors (±SE)

Vigour index

The results presented in **figure 2** indicate that vigor index increased significantly irrespective of the concentration and the nanoparticle used. CuO NPs produced more pronounced result as compared to Ag NPs. The 4 mg/L of CuO NPs proved best that enhanced the vigor index by 267%, over the control. Moreover, at the same concentration AgNPs improved it by 104% more than the control.

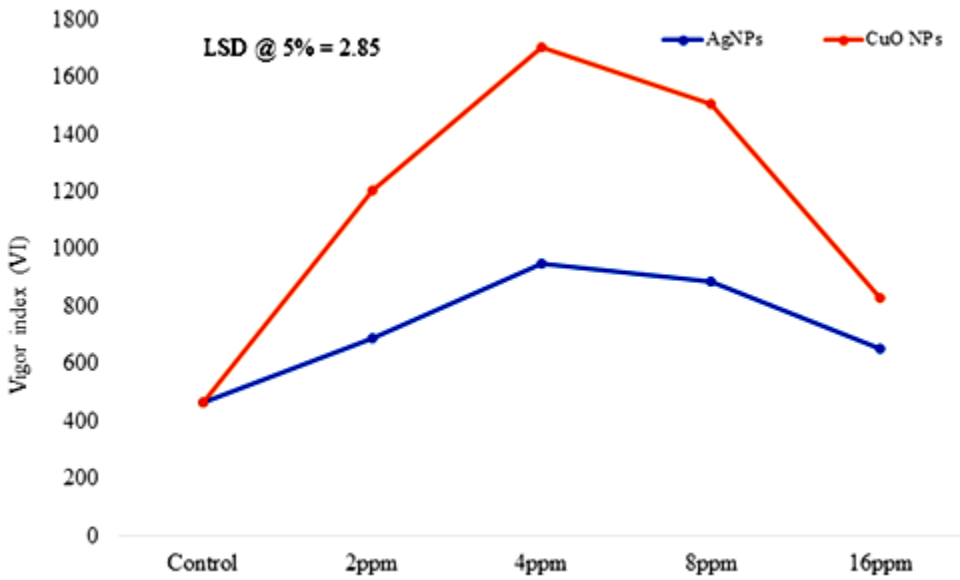

Figure 2: Effect of AgNPs and CuO NPs on vigour index of mustard seeds at 7th after treatments days. Vertical bars show standard errors (±SE)

Germination index

It is deduced from the **figure 3** that germination index was positively affected by AgNPs and CuO NPs, at all their concentrations but latter improved it to a significant level. CuO NPs (4 mg/L) exhibited maximum increase of 29%, over the control.

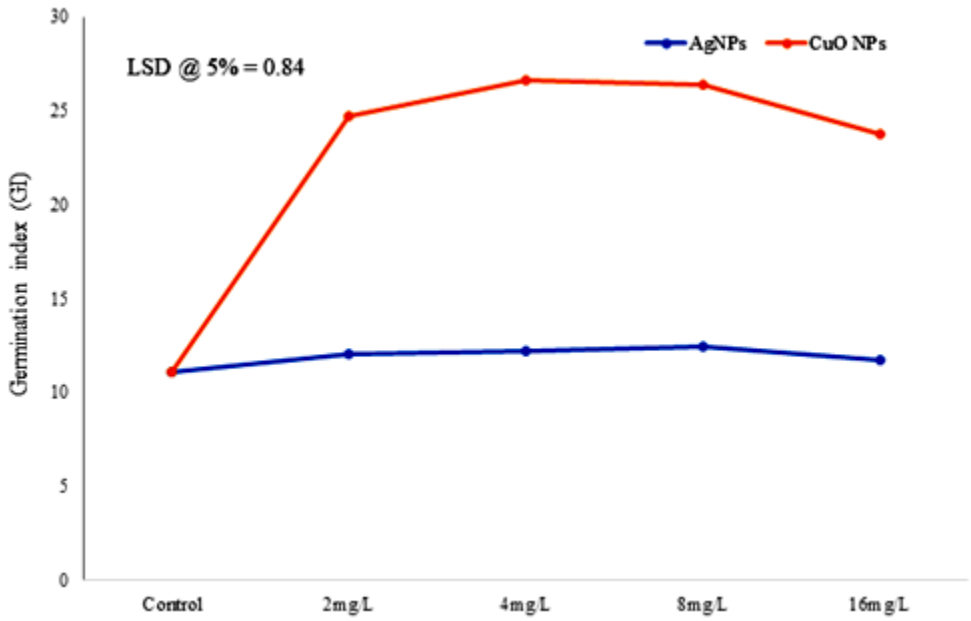

Figure 3: Effect of AgNPs and CuO NPs on germination index of mustard seeds at 7th days after the treatment Vertical bars show standard errors (±SE)

Discussion

Seed germination initiate from the imbibition of water which activates the physiological processes that's where plant's life start (Wierzbicka and Obidzińska, 1998). The present study was implicit to inspect the effects of different concentration of AgNPs and CuO NPs on germination of mustard seed. All the concentrations of both the NPs increased the rate of germination **(Figure 1, 2 & 3),** where 4 mg/L of CuO NPs proved best which excelled over that of AgNPs. Our result is well supported by Raju *et al*., (2016) who find that Fe NPs significantly increase the germination of green gram seeds by enhancing the water uptake. Similarly, improved seed germination by AgNPs (Krishnaraj *et al.*, 2012; Sharma *et al.*, 2012; Yin *et al*., 2012 Almutairi and Alharbi, 2015), ZnO NPs (Afrayeem and Chaurasia, 2017), CNTs (Khodakovskaya *et al.*, 2009; Ghodake *et al*., 2010), CuO NPs and SiNPs (Shah and Belozerova, 2009; Siddiqui and Alwhaibi, 2013; Suriyaprabha *et al*., 2014), TiO2 NPs (Zheng *et al*., 2005) and AuNPs (Gopinath *et al*., 2014), biogenic NPs (Bhati-Kushwaha *et al*., 2013) with varied crops has already been reported. Moreover, SiO2 and MoNPs activated the germination in rice seeds (Adhikari *et al.*, 2013). Soybean seeds exposed to ZnO NPs also overcome the impact of drought stress with improved germination (Sedghi *et al.*, 2013). The prominence in the effect of CuO NPs over AgNPs on seed germination of mustard seeds **(Figure 5)** is possibly because of its impact on the generation of ethylene which favoured seed germination (Taiz and Zeiger, 2002). Ag is also the inhibitor of ethylene action so that nanoform may also exert the same effects but this study still needed effective mechanism. However, seed germination response to NPs is considered as poor indicator (Rico *et al*., 2011) but needs a thorough investigation to understand the actual mechanism by testing various crops.

Conclusion

It can be concluded from the present study that NPs have ability to improve the seed germination at lower concentration. Both the NPs i.e. AgNPs and CuO NPs positively influence the seed germination process of mustard seed. Out of the two tested NPs response of CuO NPs was more pronounced than AgNPs.

Acknowledgements

The authors are thankful to Chairman, Department of Botany, Aligarh Muslim University, India, for providing the necessary facilities for conducting this experimental work.

References

Adhikari, T., Kundu, S., Biswas, A. K., Tarafdar, J. C., and Rao, A. S. 2012. Effect of copper oxide nano particle on seed germination of selected crops. ***Journal of Agricultural Science and Technology.* 2:** 815–823.

Afrayeem, S. M., and Chaurasia, A. K. 2017. Effect of zinc oxide nanoparticles on seed germination and seed vigour in chilli (*Capsicum annuum* L.). ***Journal of Pharmacognosy and Phytochemistry***. **6(5):** 1564–1566.

Almutairi, Z. M., and Alharbi, A. 2015. Effect of Silver Nanoparticles on Seed Germination of Crop Plants. ***Journal of Advances in Agriculture***. **4(1):** 280–285.

Bandyopadhyay, S., Plascencia-Villa, G., Mukherjee, A., Rico, C. M., Jose-Yacaman, M., Peralta-Videa, J. R., and Gardea-Torresdey, J. L. 2015. Comparative phytotoxicity of ZnO NPs, bulk ZnO, and ionic zinc onto the alfalfa plants symbiotically associated with *Sinorhizobium meliloti* in soil. ***Science of the Total Environment*. *515***: 60–69.

Bhati-Kushwaha, H., Kaur, A., and Malik, C. P. 2013. The synthesis and role of biogenic nanoparticles in overcoming chilling stress. ***Indian Journal of Plant Sciences***. **2(4):** 54–62.

Ghodake, G., Seo, Y. D., Park, D., and Lee, D. S. 2010. Phytotoxicity of carbon nanotubes assessed by *Brassica juncea* and *Phaseolus mungo*. ***Journal ofNanoelectronics and Optoelectronics***. **5(2)**: 157–160.

Gopinath, K., Gowri, S., Karthika, V., and Arumugam, A. 2014. Green synthesis of gold nanoparticles from fruit extract of *Terminalia arjuna*, for the enhanced seed germination activity of *Gloriosa superba*. ***Journal of Nanostructure in Chemistry***. **4(3)**: 115.

Gui, X., Deng, Y., Rui, Y., Gao, B., Luo, W., Chen, S., and Xing, B. 2015. Response difference of transgenic and conventional rice (*Oryza sativa*) to nanoparticles (γFe_2O_3). ***Environmental Science and Pollution Research.* 22(22):** 17716-17723.

Khodakovskaya, M., Dervishi, E., Mahmood, M., Xu, Y., Li, Z., Watanabe, F., and Biris, A. S. 2009. Carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth. ***ACS Nano***. **3(10)**: 3221-3227

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

Lahiani, M. H., Dervishi, E., Chen, J., Nima, Z., Gaume, A., Biris, A. S., and Khodakovskaya, M. V. 2013. Impact of carbon nanotube exposure to seeds of valuable crops. ***ACS Applied Materials and Interfaces*. 5(16):** 7965–7973.

Liu, R., and Lal, R. 2015. Potentials of engineered nanoparticles as fertilizers for increasing agronomic productions. **Science of Total Environment. 514:** 131–139.

Parveen, A., and Rao, S. 2015. Effect of nanosilver on seed germination and seedling growth in *Pennisetum glaucum*. **Journal of Cluster Science. 26(3):** 693-701.

Raju, D. Mehta, U. J., and Beedu, S. R. 2016. Biogenic green synthesis of monodispersed gum kondagogu (*Cochlospermum gossypium*) iron nanocomposite material and its application in germination and growth of mung bean (*Vigna radiata*) as a plant model, ***IET Nanobiotechnology*. 10**: 141–146.

Rosa D. G., Lopez-Moreno M. L., de Haro D., Botez C. E., Peralta-Videa J. R., and Gardea-Torresdey J. L. 2013. Effects of ZnO nanoparticles in alfalfa, tomato, and cucumber at the germination stage: root development and X-ray absorption spectroscopy studies. **Pure and Applied Chemistry. 85**: 2161.

Sable, S. V., Ranade, S., & Joshi, S. (2018). Role of AgNPs in the enhancement of seed germination and its effect on plumule and radicle length of Pennisetum glaucum. ***IET nanobiotechnology*. 12(7)**: 922-926.

Sedghi, M., Mitra, H., and Toluie, S. G. 2013. Effect of nano zinc oxide on the germination parameters of soybean seeds under drought stress. ***Annals of West University of Timisoara: Series of Biology*. 16(2):** 73–78.

Shah, V., and Belozerova, I. 2009. Influence of metal nanoparticles on the soil microbial community and germination of lettuce seeds. ***Water, Air, and SoilPollution*. 197(1–4):** 143–148.

Sharma, P., Bhatt, D., Zaidi, M. G. H., Saradhi, P. P., Khanna, P. K., and Arora, S. 2012. Silver nanoparticle-mediated enhancement in growth and antioxidant status of *Brassica juncea*. ***Applied Biochemistry and Biotechnology*. 167(8):** 2225–2233.

Siddiqui, M. H., Al-Whaibi, M. H., Firoz, M., and Al-Khaishany, M. Y. 2015 Role of Nanoparticles in Plants. **Springer International Publishing**. 19–35.

Siddiqui, M. H., and Al-whaibi, M. H. 2014. Role of nano-SiO2 in the germination of tomato (*Lycopersicum esculentum*). ***Saudi Journal of Biological Sciences***. **21(1):** 13– 17.

Srivastava, G., Das, C. K., Das, A., Singh, S. K., Roy, M., Kim, H., Sethy, N., Kumar, A., Sharma, R. K., Philip, D., and Das, M. 2014. Seed treatment with iron pyrite (FeS_2) nanoparticles increases the production of spinach. **RSC Advances. 4:** 58495–58504.

Suriyaprabha, R., Karunakaran, G., Yuvakkumar, R., Rajendran, V., and Kannan, N. 2014. Foliar application of silica nanoparticles on the phytochemical responses of maize (*Zea mays* L.) and its toxicological behavior. ***Synthesis and Reactivityin Inorganic, Metal-Organic and Nano-Metal Chemistry***. *44* (8), 1128–1131.

Taiz, L., and Zeiger, E. 2002. ***Plant Physiology***. Sinauer Associates. 3rd edn Sunderland, Massachusetts.

Vishwakarma, K., Upadhyay, N., Singh, J., Liu, S., Singh, V. P., Prasad, S. M., and Sharma, S. 2017. Differential Phytotoxic Impact of Plant Mediated Silver Nanoparticles (AgNPs) and Silver Nitrate ($AgNO_3$) on *Brassica* sp. ***Frontiers in plant science***. **8**: 1501.

Wang, Z., Xie, X., Zhao, J., Liu, X., Feng, W., White, J. C., and Xing, B. 2012. Xylem-and phloem-based transport of CuO nanoparticles in maize (*Zea mays* L.). ***Environmental science & technology*. 46 (8):** 4434-4441.

Wierzbicka, M. S., and Obidzińska, J. 1998. The effect of lead on seed imbibitions and germination in different plant species. ***Plant Science***. **137(2):** 155–171.

Xiong, T., Dumat, C., Dappe, V., Vezin, H., Schreck, E., Shahid, M., and Sobanska, S. 2017. Copper oxide nanoparticle foliar uptake, phytotoxicity, and consequences for sustainable urban agriculture. ***Environmental Science & Technology*. 51(9):** 5242-5251.

Yin, L., Colman, B. P., McGill, B. M., Wright, J. P., and Bernhardt, E. S. 2012. Effects of Silver Nanoparticle Exposure on Germination and Early Growth of Eleven Wetland Plants. ***PLoS ONE***. **7(10):** 47674.

Zheng, L., Hong, F., Lu, S., and Liu, C.2005. Effect of nano-TiO2 on strength of naturally aged seeds and growth of spinach. ***Biological Trace Element Research*. 104(1):** 83-91.

Zuverza-Mena, N., Medina-Velo, I. A., Barrios, A. C., Tan, W., Peralta-Videa, J. R., and Gardea-Torresdey, J. L. 2015. Copper nanoparticles/compounds impact agronomic and physiological parameters in cilantro (*Coriandrum sativum*). ***Environmental Science: Processes & Impacts*. 17(10):** 1783-1793.