An Acad Bras Cienc (2021) 93(3): e20190739 DOI 10.1590/0001-3765202120190739
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

ECOSYSTEMS

Effects of copper oxide nanoparticles on germination of *Sesbania virgata* (FABACEAE) plants

ELIENE S. DOS SANTOS, DANIELA E. GRACIANO, WILLIAM F. FALCO, ANDERSON R. LIMA CAIRES & EDUARDO J. DE ARRUDA

Abstract: Nanotechnology is a field that, over the years, has been growing in several research areas, such as medicine, agriculture and cosmetics, among others. As a result, there is a continuous increase in the production, use and disposal of these materials in the environment. The behaviour and (bio) activity of these materials in the atmosphere, water and soil are not fully studied. Therefore, it is necessary to carry out an analysis of the risks of contamination, as well as the possible effects and impacts of nanoparticles (NPs) on the ecosystem. In an attempt to investigate these effects on plants, the present study aimed to investigate the impact of copper oxide nanoparticles (CuO NPs) on the seed germination process of *Sesbania virgata*. For this, the *Sesbania virgata* seeds were subjected to different concentration of CuO NPs (0, 100, 200, 300 and 400 mgL^{1}) and their germination and development were monitored by optical analysis (thermography and chlorophyll *a* fluorescence). The results show that the CuO NPs induced a reduction on the maximum emission of chlorophyll *a*, which was concentration-dependent. The data also showed that CuO NPs promoted an increase in the energy dissipated by non-photochemical pathways and the surface temperature of the seeds. Additionally, our findings revealed that CuO NPs caused a root growth inhibition. In summary, the present study demonstrates, for the first time, that CuO NPs can negatively affect the physiological status and development of the *S. virgata* plant, by altering the efficiency of the functioning of photosystem II in its initial developmental stage, depending on the concentration of CuO NPs.

Key words: root growth, oxidative stress, metals, chlorophyll fluorescence, nanomaterials.

INTRODUCTION

Nanotechnology is revolutionary, applicable in various sectors and has the potential to benefit human health and the environment; however, it is necessary to consider the risks and impact of the production and disposal of nanoparticles (NPs) on the environment and organisms (Carniel 2013). The most accepted definition for the term *nanomaterials* (NMs) includes natural or man-made particles with at least one dimension less than 100 nm; NPs are widely accepted as materials with at least two dimensions less than 100 nm (Klaine et al. 2008). The possible interactions with ecosystems and organisms occur when commercial products are released into the environment, for example silver nanoparticles (Ag NPs) used in the textile industry or titanium dioxide nanoparticles (TiO_2 NPs) in cosmetics. Thus, the studies with NPs carried out to date should be regarded as a hypothesis because, on the level of exposure that occurs today, these results would not be observable naturally (Brayner et al. 2013). However, an increasing concentration of NPs in

the environment (bioaccumulation) should be expected over the time, as mostly engineered NPs are not biodegradable.

A previous study reported that the chlorophyll *a* fluorescence of Soybean (*Glycine max* L. Merr.) plants was impacted when subjected to gold nanoparticles (Au NPs) during the seed germination stage. Au NPs induced a suppression of the chlorophyll *a* fluorescence, and that was dependent on the size and concentration of NPs. This phenomenon was mainly attributed to the effect of the transfer of electrons (e^-) through photoinduction of chlorophyll molecules (Chl) contacting Au NPs, resulting in a decrease in the chlorophyll fluorescence signal. The smallest Au NPs (5 nm) induced the highest fluorescence suppression (Falco et al. 2011). In another study, leaves of fava bean (*Vicia faba* L.) had their chlorophyll fluorescence impacted when subjected to Ag NPs. In addition, the interaction between Chl and Ag NPs was dependent on the size and concentration of the metal NPs (Queiroz et al. 2016). Therefore, the particle size and distribution are of the great importance for the adverse effects of NPs on plants. On the nanometric scale, the effective area available is considerably increased, changing the nature of the interactions between the molecular forces of the material. This affects the impacts that these processes or nanotechnological products can cause to the environment, human health and society in general.

The impacts caused by the release of pollutant loads with NPs and/or NMs and intervention scenarios is an area of interest for researchers, mainly those investigating plants, which are an important part of the ecosystem; plants may be able to remove, immobilise or even reduce the availability of nanomaterials (Andrade et al. 2007). In addition to the relevance of plant-NP interactions as trophic relationships, they have potential uses as bioindicators of environmental contamination, through the evaluation of sensitivity responses depending on the type, size and/or concentration of NPs.

Bioindicators or environmental indicators are important tools used to correlate a given anthropic factor (human-made changes) or a natural factor with a potential impact. This may include an important assessment of ecological integrity, as in the condition of an area or organism. These studies can be performed by comparing individuals, the structure and function of a biological community between an impacted area and reference areas (Andrade 2010). For example, *Pistia stratiotes* and *Eichhornia crassipes* are indicator species of polluted aquatic environments because they develop in places with high concentrations of organic matter, micronutrients and macronutrients. These species are used as indicators of pollution levels, because they have a high growth rate and an excellent ability to absorb and concentrate metallic pollutants in aquatic systems; moreover, they do not show symptoms of poisoning at high pollutant concentrations (Bettinelli et al. 2000). Species such as *Utricularia breviscapa* (utricularia), *Nymphaea elegans* (water lilies) and *Nymphoides indica* (nymph) are also bioindicators of environmental pollution (Pompêo 2008). Research conducted with the plant *Cicer arietinum* L. (chickpea) exposed to different concentrations of copper (II) oxide NPs (CuO NPs) showed that the NPs interfered in the growth of the plant, causing a delay in the growth of roots and stems (Nair & Chung 2015a). Another study showed that the growth of rice (*Oryza sativa* CV. *Swarna*) was affected at three different levels of stress caused by copper II oxide (CuO NPs) (0.5 mM, 1.0 mM and 1.5 mM CuO NPs, < 50 nm particle size). Based on these results, it was concluded that the rate of germination of the plant was significantly reduced. In addition, it was evident

that, in leaves, oxidative eruptions occurred (Shaw & Hossain 2013).

Another study examined the effects of CuO NPs on the germination of seedlings of green peas (*Pisum sativum* L.) at different concentrations (0, 50, 100, 200, 400 and 500 mgL^{-1}). The experimental results demonstrated that there was a significant reduction in plant growth (shoot and root length), increased generation of reactive oxygen species (ROS) and lipid peroxidation, and oxidative degradation of lipids induced by NPs (Nair & Chung 2015b, Nair & Chung 2017).

In Van et al. (2016), strains of transgenic cotton (Bt-29317) and conventional cotton (Jihe 321) were subjected to different concentrations (0, 10, 200 and 1000 mgL^{-1}) of CuO NPs. They found that CuO NPs significantly inhibited the growth and development of transgenic and conventional cotton, impacting the height, root length, number of roots and biomass production when subjected to CuO NP concentrations over 10 mgL^{-1}. They also demonstrated that the CuO NPs mainly aggregated in the root outer epidermis. Nevertheless, CuO NPs aggregates were found in the leaf epidermis, and also reached leaf cells by endocytosis.

Metal-oxide nanoparticles (NPs) such as copper oxide (CuO NPs) offer promising perspectives for the development of novel agro-chemical formulations of pesticides and fertilisers. (Simonin et al. 2018) showed that CuO NPs can have detrimental effects on microbial activity in soils with different physicochemical properties that had been previously exposed to various agricultural practices. The experiments showed that CuO NPs had detrimental effects on soil microbial activities, but most effects occurred at the highest concentration tested (100 $mgkg^{-1}$). Similar to previous studies, they observed that the negative effects of CuO NPs increase over time, indicating that short-term studies (hours, days) may underestimate the risks posed by these contaminants. The presence of plants influences the microbial response to CuO NPs exposure but does not mitigate or compensate for the effects.

Mosa et al. (2018) investigated toxicity on the physiological, phenotypical, biochemical and genomic levels. It was found that Cu NPs in the size range of 10-30 nm (50, 100 and 200 mgL^{-1} of Cu NP powders) were toxic to *Crocus sativus*. Cu NPs showed a decrease in the total biomass of the treated *C. sativus* plants. The analysis demonstrated that Cu NPs accumulated in *C. sativus* plant tissues, with higher accumulation levels in root tissues. The genotoxic effect of Cu NP induced genomic DNA modifications in *C. sativus*. Additionally, Cu NP led to a significant decrease in chlorophyll a and b contents, an increase in H_2O_2 and MDA contents, as well as an increase in electrolyte leakage which induced damage to the cucumber root plasma membrane. They demonstrated that Cu NP induced oxidative stress in *C. sativus* and Cu-Zn SOD gene expression was induced under Cu NP treatment.

Sesbania virgata (Cav.) Pers. belongs to the family Leguminosae (Fabaceae), the subfamily Faboideae (Papilionoideae) (Shuguang et al. 2009). The species is popularly known as Sesbania, bean, sarazinho and mother-jose. The genus *Sesbania* is characterised by species tolerant of poor soils with a high content of heavy metals and a high level of adaptability when subjected to biotic and abiotic stresses (Yang et al. 2003). *S. virgata* is a fast-growing pioneer species, a perennial weed that reproduces by seeds, with a shrubby habit that can achieve 2 to 4 m tall. It is a native species with quick growth and hardiness, showing its potential for planting in degraded areas or in recovery, as well as desertified areas and for the restoration of riparian forests (Delarmelina et al. 2014, Araújo

et al. 2004). In addition to bringing physical benefits to the soil with its highly branched root system, it prevents erosion and serves to support reforestation and the maintenance of areas in recovery (Zanandrea et al. 2009). It produces a large number of seeds with high viability, which are scattered within indehiscent fruits that can float in the water for the dispersal of the species (Pott & Pott 1994). The seeds of the species undergo rapid germination and are homogeneous, an unusual feature among the seeds of native plants. They present dormant endosperm seeds that accumulate tegmental galactomannan as a polysaccharide in the cell wall. The germination process of these seeds occurs on the second or third day after soaking (Buckeridge & Dietrich 1996, Tonini et al. 2007).

In this context, the present study aimed to detect changes in the functioning of the photosynthetic apparatus of *Sesbania virgata* (Cav.) Pers. induced by CuO NPs. For this, non-destructive optical methodologies were applied for investigating the phytotoxicity effects of NPs on *Sesbania virgata* (Cav.) Pers. seedlings when subjected to different concentrations of NPs.

METHODOLOGY

Copper oxide nanoparticles (CuO NPs)

Copper (II) oxide nanoparticles (CuO NPs were acquired from Aldrich (USA) in powder - cod. 544868 - 5g - Copper(II) oxide - nanopowder < 50 nm particle size (TEM) (www.sigma-aldrich.com)). The specification of the product does not indicate the purity of the nanoparticles. Transmission Electron Microscopy (TEM) - Morgagni 268D 100 kV (FEI) was used to determine the morphology and mean diameter of the nanoparticles.

Prior to the TEM image collection, the CuO NPs were sonicated for 15 min in isopropyl alcohol solution. Then, samples were prepared by placing a drop of isopropyl alcohol nanoparticle solution on a holey carbon grid and dried in vacuum during 3 h before the microscopy analysis. The nanoparticle size distribution was estimated from the measurement of about 100 particles. The analyzed material was extremely agglomerated, which made it difficult to count a larger number of nanoparticles. The morphology and diameter of the CuO NPs were determined by means of ImageJ software.

Dynamic Light Scattering (DLS) technique was used to determine the hydrodynamic size of the CuO NPs, as well as to calculate their mean hydrodynamic size distribution, Polydispersity Index (PDI), and Zeta Potential (ZP). The analysis was performed using the Zetasizer Nano ZSP equipment (Malvern instruments), using the following parameters: laser wavelength of 633 nm (He-Ne), scattering angle of 173º, temperature of 25°C and average refractive index of 1.390. The samples were prepared with ultrapure water and DMSO at concentration of 1% (v/v) and then placed in a cuvette. For each sample, three measurements were performed and the mean value was recorded.

The CuO NPs (~50 nm), in powder, were analyzed by scanning electron microscopy (SEM) with a dispersive x-ray energy spectroscopy system (Energy Dispersive X-ray Spectroscopy - EDS). The equipment is a bench system, brand Phenom-World, model Phenom Pro X. The system has a nominal resolution ≥ 14 nm, magnification of 80 - 130000x, elementary detection range that varies from Carbon (C) to Amerício (Am), source electron CeB6, color navigation camera with 20 to 135x zoom and variable voltage acceleration from 5 - 15 kV. The equipment has an EDS detector of the SDD type (Silicon Drift Detector) with integrated software for determining the elemental composition, simultaneously identifying the different elements of the sample.

The measurements were carried out using carbon tape for conductivity and deposition of the samples.

Seed selected in this study

We used the seed of *S. virgata* collected in the Pantanal-MS, Passo da Lontra 184, Corumbá / MS in October 2015. The processing of the seeds was manual. The seeds were obtained from plants, in a number exceeding 20 individuals for the maintenance of genetic diversity. The tested seed lot was kept in a dark seed dispersal box under refrigeration. Prior to germination, the seeds underwent a mechanical scarification process by sanding, which consisted of submitting the seeds to friction, wear and tear with sandpaper, to damage the skin. This is one of the most efficient processes to enhance hydration, permeation and germination in this species (Camargos et al. 2008).

For the bioassays, the CuO NPs were dispersed in aqueous solutions using ultra-pure water and agitated in an ultrasonic bath for 30 minutes. The seed was scarified by sanding, to facilitate the hydration of the reserve, and then soaked in CuO NPs dispersion at concentrations of 0 (control group), 100, 200, 300 and 400 mgL^{-1}. Each group was kept submerged in 5 mL of the solution in a beaker for 12 h in an orbital agitator (TECNAL) with a 5 rpm rotation adjustment to facilitate soaking the seeds and for better absorption/internalization of the NPs.

Evaluation of germination

After immersion, the seeds were placed in Petri dishes with moistened double filter paper and kept in a germination chamber with controlled temperature, humidity and photoperiod, providing ideal conditions for the germination and growth of the species. The seeds were maintained at 25°C with a 12 / 12 h dark / light photoperiod and 65 / 70% humidity with an actinic intensity of approximately 300 $\mu molm^{-2}s^{-1}$. For each concentration of NPs used, 12 seeds were evaluated. *In vivo* analyzes were performed for 6 days. The first 24 h were immersed and, from the second day on, thermal image measurements were performed, the kinetics of the chlorophyll fluorescence image and the stationary fluorescence were evaluated in 5 days and after 5 days, the root size of each seed was measured. After the collected data, the average for each seed lot was applied and the standard deviation for concentration was applied. The data presented will be relative to the means of each group (0, 100, 200, 300 and 400 mgL^{-1}). These procedures were applied to the study of the proposed material, copper oxide nanoparticles (CuO NPs).

OPTICAL TECHNIQUES USED FOR DETECTING STRESS IN PLANTS

Infrared thermography

Thermal images obtained through a thermographic Testo® camera with an infrared detector with a 2.3-megapixel resolution, in which the temperature scale is indicated by false-colour gradients. The equipment has a temperature range between -20 to 350°C. An image was collected for each Petri dish containing 12 seeds for each concentration tested. From the Testo® software, the average temperature of each seed was obtained from the analysis of the temperature in different parts of the 12 seeds. These data were subsequently submitted to analysis of variance; the comparisons between means were performed using Student's t-test with 95% reliability. These measures were carried out after a period of adaptation to the dark for 30 minutes, to avoid any temperature changes influenced by light irradiation and with a control temperature at 25°C.

Kinetics of chlorophyll a fluorescence imaging

To conduct the study on the influence of NPs on the photosynthetic apparatus of plants *in vivo*, the kinetic fluorescence imaging technique was applied, using a closed FluorCam FC-800 C Mark Photon Systems instrument. Composed basically of panels of LEDs used as an excitation source with actinic light intensity above 2500 µmol (protons) m^{-2} s^{-1} and a CCD camera collecting plant fluorescence in the region between 400 to 1000 nm, this apparatus provides images with a resolution of 512 x 512 pixels at a rate of 50 frames per second. The system is close, allowing the adjustment of the dark seed, before it was expose to radiation. Before the measurements, the seeds were adapted to the dark for 30 minutes to ensure that all reaction centres were open, allowing for a more effective measure of fluorescence. For the kinetic fluorescence imaging technique, white light was used as an excitation source and a filter was used to select only the emission of chlorophyll a, in the region of 680 nm; the measurements were performed on one side of the seed. The parameters observed in the analysis of chlorophyll fluorescence were Fm' (maximums fluorescence of dark-adapted measurement) and NPQ´ (non-photochemical quenching).

Stationary fluorescence

Fluorescent analyses of *in vivo* seeds were performed using a portable spectrophotometer consisting of two lasers, operating at 405 and 532 nm, a monochromator SB 2000 (FL-OceanOptics), a Y-type optical fibre and a laptop, to obtain the spectra. The samples were excited at 405 nm and the spectra were obtained from 450 nm to 800 nm. The analyses were carried out directly on the top of each seed and were performed on all days of the germination process (five days).

Root size

After 5 days of germination, the root length was determined with the aid of a calliper (Digimess®), to an accuracy of 0.05 mm.

RESULTS AND DISCUSSION

CuO NPs Characterization

The EDS results show the characteristic copper peaks of greater relative intensity: at 0.929 keV, referring to the Lα1.2 transition; 0.949 keV for the Lβ1 transition; 8.047 keV referring to the Kα1 transition and 8.905 keV referring to the Kβ1.3 transition. From the data it is also possible to verify the existence of a characteristic oxygen peak at 0.525 keV of the Kα1.2 transitions, confirming that the samples are composed of copper (II) oxide (CuO).

Although the manufacturer reported that the CuO NPs (powder) were composed of particles with a diameter smaller than 50 nm, the results revealed that the CuO NPs had a large diameter size with different populations (in the range of 28-70 nm) with an average diameter of 48.26 ± 8.0 nm. In addition, the TEM results also demonstrated that CuO NPs had predominantly regular spherical shapes (see Figure 1).

The DLS results revealed that CuO NPs were highly polydispersed, with average hydrodynamic diameter, PDI and ZP of 410.9 ± 147.4 nm, 0.7 ± 0.1 and –14.1 ± 2.7 mV, respectively. These results demonstrate that a higher hydrodynamic diameter ($D_{hidrodynamic}$; 410.9 ± 147.4 nm) was obtained when compared to the diameter determined by TEM, an expected result because the DLS measures the total diameter of the NPs together with molecules and ions (layers) adsorbed on the nanoparticles of surface when they are placed in a solution (Kass et al. 2017). The data also show that CuO NPs are not monodispersed, as the obtained PDI was 0.7

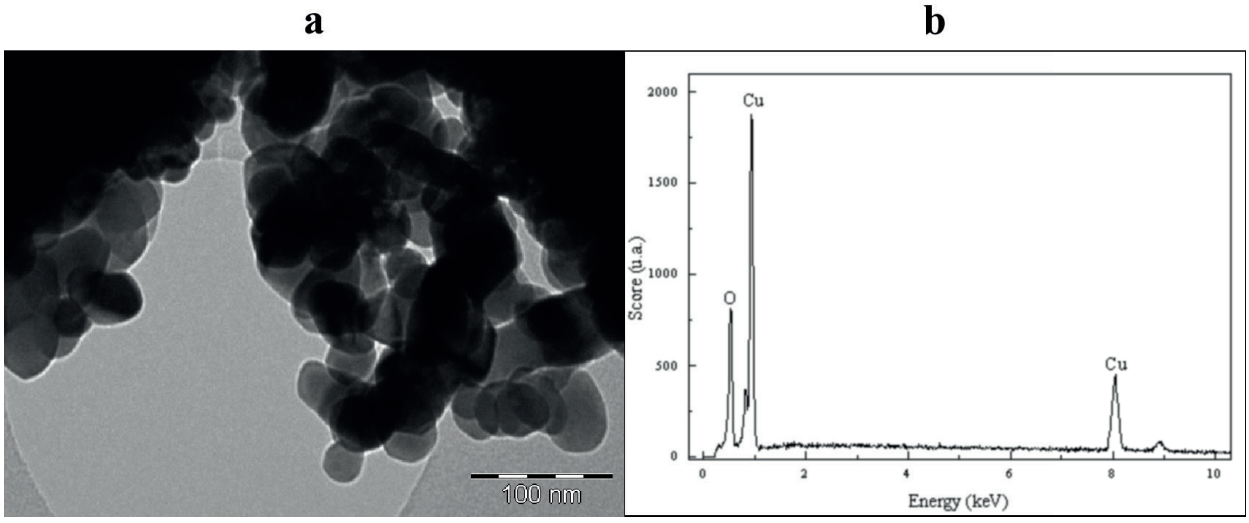

Figure 1. (a) Transmission electron microscopy micrography and (b) EDS spectrum of CuO NPs.

± 0.1, being classified as highly polydispersed. A sample is defined as highly monodispersed when PDI ≤ 0.1, almost monodispersed for values between 0.1 and 0.7, and highly polydispersed for values > 0.7 (Stetefeld et al. 2016). In addition, the results demonstrate that CuO NPs have a ZP value of -14.1 ± 25 mV, indicating that CuO NPs dispersed in aqueous solution are relatively stable. Guidelines classifying NP dispersions with ZP values of ± 0–10 mV, ± 10–20 mV, ± 20–30 mV and > ± 30 mV as highly unstable, relatively stable, moderately stable and highly stable, respectively. (Bhattacharjee 2016)

CuO NPs Effects on *Sesbania virgata* Seeds

Infrared thermography

The thermal image analyses performed showed a significant increase in temperature, especially in seeds subjected to CuO NPs in the first days of germination, but the seeds recovered, as is common in living organisms, possibly due to homeostasis, when compared with the control (0 mgL^{-1}). The average temperature of twelve points for each seed and the average of the 12 seeds was analysed for each concentration.

Figure 2 shows that the temperature of the seeds was significantly changed after subjected to the CuO NPs (t-test, $p < 0.05$; 95% degree of confidence). However, due to the temperature behaviour of the germination and hydrolysis processes, there was a slight difference in temperature as a function of time, and this was clearly not linear, presenting oscillatory behaviour between 72 and 96 h. This may be because the germination process can be affected by several internal and external (environmental) factors at the same time, which act alone or jointly, which when act as trigger internal signals at the molecular level, which can induce the activation or inactivation of various compounds and metabolic reactions (Kerbauy 2008, SEO et al. 2009). More studies on the temperature of seeds during germination are needed, because this is still lacking bibliographic references.

Fluorescence kinetics of chlorophyll a

A kinetic fluorescence image analysis of the top seeds of *S. virgata* was performed after 120 h of soaking, at concentrations of 0, 100, 200, 300 and 400 mgL^{-1}. The parameters presented are Fm' and

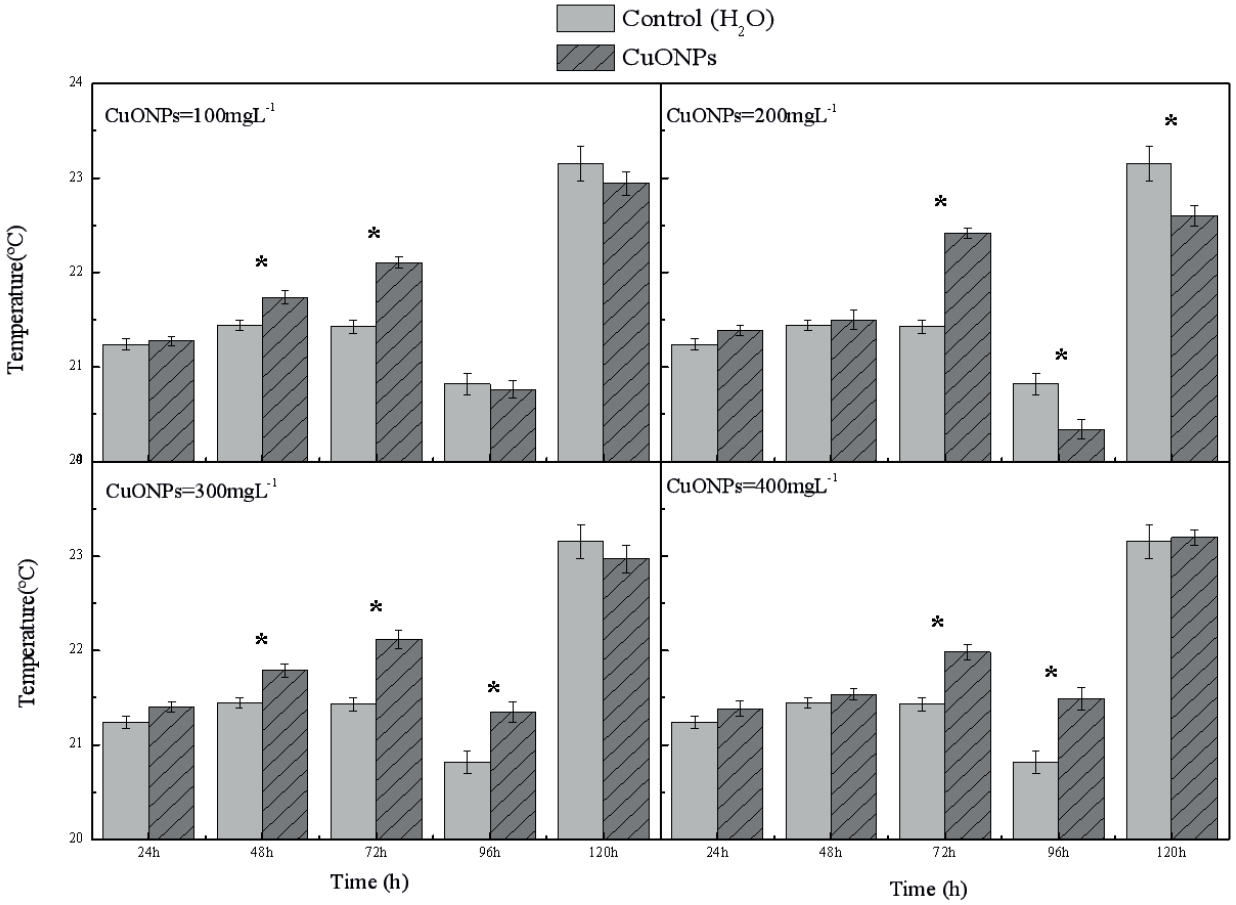

Figure 2. Mean temperature of 12 seeds of *Sesbania virgata,* as a function of time at all concentrations of CuO NPs (< 50 nm) compared with the control (0 mgL^{-1}). * Significant difference (t-test, $p < 0.05$, $n = 12$).

NPQ', measures obtained in the saturating pulse, when the seeds were already adapted (Figure 3).

Figures 4a and 4b illustrate a comparison, in relation to time, of seeds subjected to the presence of CuO NPs and control seeds, as Fm' (maximum fluorescence in the light-adapted state) and NPQ' (indicative of excess radiant energy dissipation in the form of heat in PSII). The analysis of NPQ' i.e the last saturated light pulse, when the seeds were already adapted to darkness, shows that CuO NPs induced changes in this parameter at all concentrations, evaluated relative to the control, causing a significant increase at 48 and 72 h when cotyledons are being formed. In a study carried out with *Vicia faba*, it was showed that the NPQ' values increased in the leaves containing the Ag NPs, which demonstrates that NPs may cause an increase in the dissipation of light energy by non-photochemical forms, instead of photochemical processes (Falco et al. 2020). However, after 72 h, a reversal in the behaviour of the NPQ occurred, where a significant decrease after 120 h was observed; this may be due to the formation of the first leaves and the transformation of the seed into seedling, being the plant able to performing self-adjustment. Morphological or physiological adjustments, presented by plants under stress, allow them to maintain their metabolism and promote the conditions that enable growth even under continued stress (Bohnert et al. 1995).

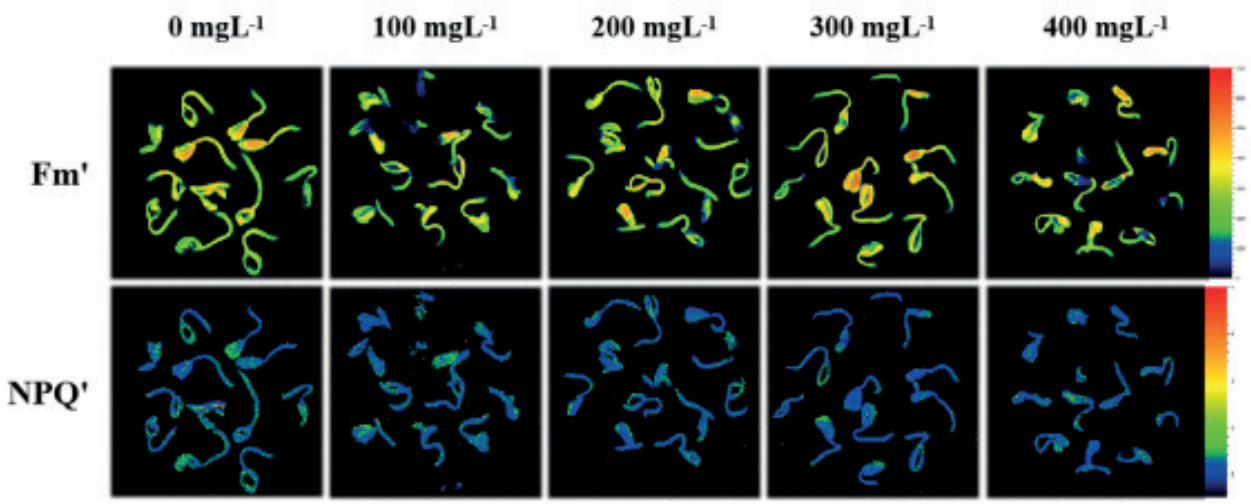

Figure 3. Fm' and NPQ' images, 120 h after soaking in 0, 100, 200, 300 and 400 mgL^{-1} of CuO NPs, obtained by means of chlorophyll *a* fluorescence imaging.

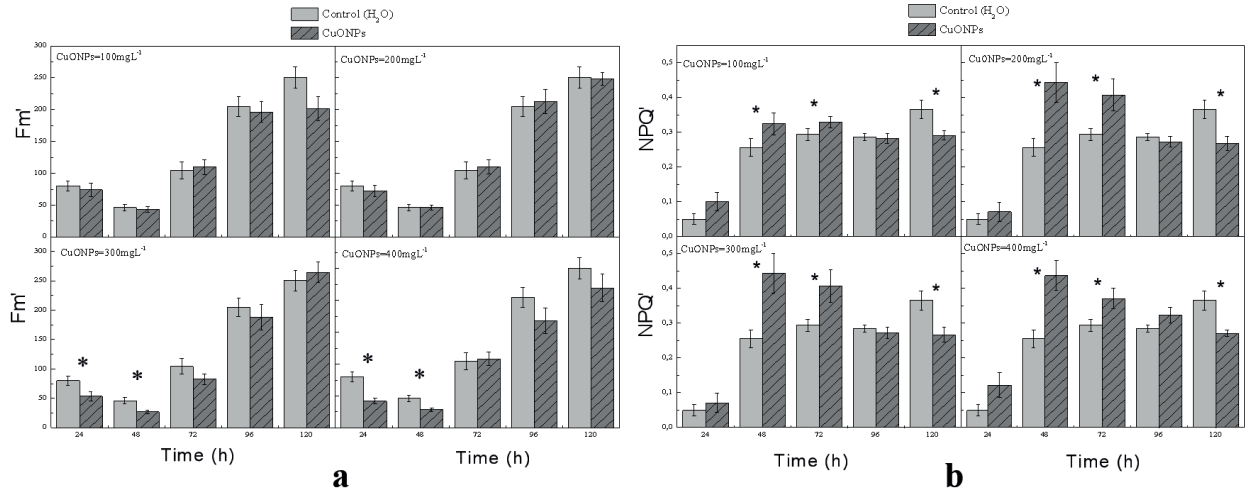

Figure 4. (a): Means of Fm' of 12 seeds of *Sesbania virgata*, as a function of time, after soaking for 12 h in an aqueous solution of 0, 100, 200, 300 and 400 mgL^{-1} CuO NPs (< 50 nm). * Significant difference (t-test, $p < 0.05$, n = 12). (b): NPQ' means of 12 seeds of *Sesbania virgata* as a function of time after being exposed to 0, 100, 200, 300 and 400 mgL^{-1} CuO NPs (< 50 nm). * Significant difference (t-test, $p < 0.05$, n = 12).

Stationary fluorescence

The stationary fluorescence measurements were performed on cotyledons, the first leaves that emerge from the embryo. The samples presented two emission bands in the red and far-red range, when excited at 405 nm, with peaks at 685 and 735 nm, respectively. These fluorescence bands are due to the Chl molecules present in the photosystem II and photosystem I, located in the thylakoid membranes of chloroplasts (Mishra & Gopal 2010). Based on the spectrum, an increase in fluorescence intensity of Chl was observed with treatment at 100 and 200 mgL^{-1} CuO NPs and a suppression at the concentrations of 300 and 400 mgL^{-1}, when compared with the control (H_2O Milli-Q). These results are presented in Figure 5. These results may be attributed to the consumption of chlorophyll molecules as

reserves, because as this ratio increases, the concentration of chlorophyll is lower, owing to the selective re-absorption of red relative to far-red fluorescence by chlorophyll molecules (Cerovic et al. 1999, Caires et al. 2010). In a study with rice (*Oryza sativa* L.) exposed to CuO NPs under hydroponic condition, it was showed a reduction of the pigment content in the leaves, including chlorophyll *a*, chlorophyll *b*, and carotenoids. According to the authors, CuO NPs, especially at a concentration of 250 mgL^{-1}, affected the growth and development of rice seedlings, probably due to oxidative damage and disturbance of chlorophyll and carotenoid synthesis (Yang et al. 2020).

The fluorescence ratio F685/F735 provides information about the physiological state and the chlorophyll content of plants (Buschmann 2007). This relationship is used to quantify the maximum efficiency of PS II. In the analysis of the chlorophyll content, the fluorescence ratio is an indicator of oxidative stress (Lu et al. 2000). The analysis of F685/F735, i.e. the fluorescence intensity of the characteristic peaks of chlorophyll *a* (in Figure 5a and 5b), shows that there were changes in the chlorophyll content of seeds treated with CuO NPs, differences were not significant.

Root size

The analysis of the average root length of 12 seeds of *S. virgata* germinated with CuO NPs demonstrated that there was a root length inhibition. An average length of 18.6 and 18.9 mm was determined to the roots subjected to the CuO NPs at 100 and 200 mgL^{-1}, respectively. These results show a significant reduction of the root length (t-test, $p < 0.05$, $n = 12$) when compared to the control group (H_2O), which presented an average length of 23.4 mm. At the higher concentrations of 300 and 400 mgL^{-1}, roots had an average length of 21.2 mm and 19.9 mm, respectively, which showed a reduction in the length of the roots, but there was no significant difference (t-test, $p < 0.05$, $n = 12$) when compared to the average of the root of the control group (H_2O). A representative image showing the root length inhibition is shown in Figure 6. The present findings corroborate previous studies showing that, when subjected to stress induced by nanoparticles, plants tend

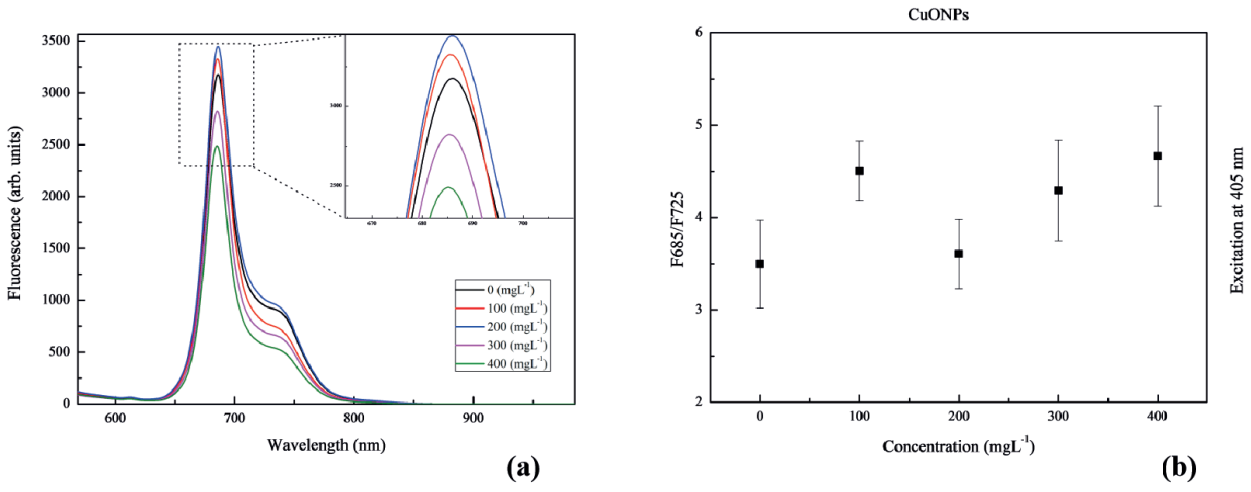

Figure 5. (a) Fluorescence intensity of seeds of *Sesbania virgata* treated with different concentrations of CuO NPs, with 405 nm excitation, at 120 h of germination; (b) F685/F735 ratio according to the CuO NP concentration, with excitation at 405 nm and 120 h of germination.

to decrease the size of the shoot or the root (Rousseau et al. 2013). Other authors corroborate to the present phytotoxicity observation. For instance, Pelegrino et al. (2020) described the effects of the CuO NPs on *Lactuca sativa* L. They observed that CuO NPs over 40 mg^{-1} inhibited the seed germination and root growth. Yang, et al. (2020) evaluated the phytotoxicity of CuO NPs (sized <50 nm) in rice (*Oryza sativa* L.), under hydroponic condition. They showed seven days of exposure to 62.5, 125 and 250 mgL^{-1} of CuO NPs significantly suppressed the growth rate of rice seedlings compared to the control. In addition, physiological indexes associated with antioxidants, including membrane damage and antioxidant enzyme activity, were also detected.

The results obtained in experiments with CuO NPs on seeds of *S. virgata* showed that the values of Fm‘ and NPQ’ increased during germination, as expected, as the photosynthetic apparatus is still developing; however, when evaluating and comparing with control seeds, only NPQ’ increased. The fact that non-photochemical quenching does not increase dissipation was evident when we analysed the temperature plot as a function of time, since there was an increase in the temperature of the seeds with CuO NPs, especially in the period of 72 h, as shown in Figure 2 and Figure 4. This fact may indicate that seeds are dissipating energy in the form of heat, mainly due to the process of oxidative stress that produces free radicals, which are oxidant species that induce oxidative reactions and heat dissipation (Junfei et al. 2017, Müller et al. 2001). As for the chlorophyll content of the cotyledon leaves of *S. virgata*, after 120 h of germination, there was an increase in chlorophyll fluorescence intensity in photosystem II at lower concentrations of nanoparticles and a suppression of chlorophyll activity at higher concentrations of nanoparticles, which suggests that CuO NPs were able to penetrate the seeds. In a study on pumpkin plants (*Cucurbita maxima*) grown in an aqueous medium containing iron oxide nanoparticle, the plants were able to absorb, translocate and accumulate nanoparticles in plant tissues (Zhu et al. 2008). When analysing the F685/F735 ratio (Figure 4b) although this value was higher for the seeds treated with CuO NPs, there was no significant difference in relation to the other treatments.

This more intense activity may possibly relate to a lower concentration of nanoparticles in solution with less surface activity of the nanoparticles available for induction of oxidative stress *in situ*. As the concentration of nanoparticles increases, there is a tendency for agglomeration to occur, with a loss of surface area and reduction in the reactivity of metallic nanoparticles due to the decrease in surface area and reactivity; furthermore, agglomeration

Figure 6. Size of root at *Sesbania virgata,* with different concentrations of CuO NPs. Photo: Eliene Santos.

inhibits permeation in the body. Plants block the channels and/or reduce the intensity of oxidative stress reactions with a consequent reduction in the production of radical species, which are strong oxidants. In view of the foregoing, the suppression of chlorophyll in plants of *S. virgata* may related to the NP-induced inhibition of biochemical processes of plants, causing changes in their photosynthetic activities and, for this reason, decreased fluorescence yield of dissipated chlorophyll. With regard to root development, we observed a decrease in length at all concentrations of NPs when compared to the control, with significant differences in the treatments at 100 and 200 mgL^{-1}.

CONCLUSION

The present investigation showed that CuO NPs caused a significant alteration in the temperature of the seeds and a reduction in the root length of the seedlings, indicating metabolic damage and changes in energy dissipation and plant growth. The data demonstrated that all observed changes promoted by NPs were concentration-dependent. Nevertheless, it is important to stress that, for all tested concentrations of NPs, our results also revealed a trend of plant recovery after 72 h of NPs exposition. Based in these findings, we can conclude that the formation and operation of the photosynthetic apparatus of the seedlings were impacted when submitted to CuO NPs, which affects the development and growth of the plants, especially the root development. In summary, the present study demonstrates that NPs affect the photosynthetic performance of the *S. virgata* seedlings, which indicates that CuO NPs may present a potential risk to plant organisms. Finally, our results also suggest that the non-invasive optical techniques have great potential to be applied as analytical tools for detecting physiological impact of NPs on plants during their early stage of development.

REFERENCES

ANDRADE HP. 2010. Cienciométrica global analysis in bioindicators: an overview of the trends in the years 1998 to 2007. Dissertation (Master's thesis), Catholic University of Goiás (UFG), Department of Biological Sciences, Goiás.

ANDRADE J, TAVARES S & MALHER C. 2007. Phytoremediation: the use in improving environmental quality. São Paulo: Office of Texts, p. 176.

ARAÚJO EC, MENDONÇA AV, BARROSO DG, LAMÔNICA KR & SILVA RF. 2004. Morphological characterization of fruits, seeds and seedlings of *Sesbania virgata* pers. Brazilian Seed Journal 26(1): 105-110.

BETTINELLI M, BEONE GM, SPEZIAS S & BAFFI C. 2000. Determination of heavy metals in soil and sediments by microwave-assisted digestion and inductively coupled plasma optical emission spectrometry analysis. Anal Chim Acta 424: 289-296.

BHATTACHARJEE S. 2016. DLS and zeta potential - What they are and what they are not? J Control Release 235: 337-351.

BOHNERT HJ, NELSON DE & JENSEN RG. 1995. Adaptations to environmental stresses. Plant Cell 7: 1099-1111.

BRAYNER R, FIÉVET F & CORADIN T. 2013. Nanomaterials: a danger or a promise? A chemical and biological perspective. Springer, London. doi: 10.1007/978-1-4471-42133.

BUCKERIDGE MS & DIETRICH SMC. 1996. Mobilization of the raffinose family oligosacharides and galactomannan in germinating seeds of *Sesbania marginata* Benth. (Leguminosae - Faboideae). Plant Sci 117: 33-43.

BUSCHMANN C. 2007. Variability and application of the chlorophyll fluorescence emission ratio red/far-red of leave. Photosynth Res 92: 261-271.

CAIRES ARL, SCHERER MD, SANTOS TSB, PONTIM BCA, GAVASSONI WL & OLIVEIRA SL. 2010. Water stress response of conventional and transgenic soybean plants monitored by chlorophyll a fluorescence. J Fluorescence 20: 645-649.

CAMARGOS VN, CARVALHO ML, ARAUJO M, MAGALHAES DV & LINHARES FH. 2008. Overcoming dormancy and seed physiological quality assessment of *Sesbania virgata*. Ciênc Agrotec 32(6): 1858-1865.

CARNIEL BF. 2013. Evaluation of environmental and social impacts of the use of nanotechnologies in agriculture:

a methodological proposal. 2013.189 f. Dissertation (Master's degree in Biotechnology) Federal University of São Carlos (UFSCar). São Carlos, São Paulo.

CEROVIC ZG, SAMSON G, MORALES F, TREMBLAY N & MOYA I. 1999. Ultraviolet-induced fluorescence for plant monitoring: present state and prospects. Agronomie 19: 543-578.

DELARMELINA WM ET AL. 2014. Different substrates for seedling production of *Sesbania virgata*. Forest and Environment 21(2): 224-233.

FALCO WF, BOTERO ER, FALCÃO EA, SANTIAGO EF, BAGNATO VS & CAIRES ARL. 2011. *In vivo* observation of chlorophyll fluorescence quenching induced by gold nanoparticles. J Photochem Photobiol A Chem 225: 65-71.

FALCO WF, SCHERER MD, OLIVEIRA SL, WENDER H, COLBECK I, LAWSON T & CAIRES ARL. 2020. Phytotoxicity of silver nanoparticles on Vicia faba: evaluation of particle size effects on photosynthetic performance and leaf gas exchange. Sci Total Environ 701: 134816.

JUNFEI G, ZHENXIANG Z, ZHIKANG L, YING C, ZHIQIN W, HAO Z & JIANCHANG Y. 2017. Photosynthetic properties and potentials for improvement of photosynthesis in pale green leaf rice under high light conditions. Front Plant Sci 8: 1082-1096.

KASS MEl, BROHAN L, GAUTIER NØB, BÉCHU S, DAVID CØ, LEMAITRE N & RICHARD-PLOUET M. 2017. TiO_2 anatase solutions for electron transporting layers in organic photovoltaic cells. Chemphyschem 18(17): 2390-2396

KERBAUY GB. 2008. Plant Physiology. Rio de Janeiro: Ed. Guanabara Koogan SA, 431 p.

KLAINE SJ, ALVAREZ PJJ, BATLEY GE, FERNANDES TF, HANDY RD, LYON DY, MAHENDRA S, MCLAUGHLIN MJ & LEAD JR. 2008. Nanomaterials in the environment: behaviour, fate, bioavailability, and effects. Environ Toxicol Chem 27: 1825-1851.

LU CM, CHAU CW & ZHANG JH. 2000. Acute toxicity of excess mercury on the photosynthetic performance of cyanobacterium, *S. platensis* - assessment by chlorophyll fluorescence analysis. Chemosphere 41: 191-196.

MISHRA KB & GOPAL R. 2010. Detection of nickel-induced stress using laser induced fluorescence signatures from leaves of wheat seedlings. Int J Remote Sens 29(1): 157-173.

MOSA KA, EL-NAGGAR M, RAMAMOORTHY K, ALAWADHI H, ELNAGGAR A, WARTANIAN S, IBRAHIM E & HANI H. 2018. Copper nanoparticles induced genotoxicty, oxidative stress, and changes in superoxide dismutase (SOD) gene expression in Cucumber (*Cucumis sativus*) plants. Front Plant Sci 9: 872. doi: 10.3389/fpls.2018.00872.

MÜLLER P, LI XP & NIYOGI KK. 2001. Non-photochemical quenching. A response to excess light energy. Plant Physiol 125(4): 1558-1566.

NAIR PMG & CHUNG IM. 2015a. The responses of germinating seedlings of green peas to copper oxide nanoparticles. Biology Plantarum 59: 591-595.

NAIR PMG & CHUNG IM. 2015b. Changes in the growth, redox status and expression of oxidative stress related genes in chickpea (*Cicer arietinum* L.) in response to copper oxide nanoparticle exposure. J Plant Growth Regul 34(2): 350-361.

NAIR PMG & CHUNG IM. 2017. Evaluation of stress effects of copper oxide nanoparticles in *Brassica napus* L. seedlings. Biotech 7(5): 293-301.

PELEGRINO MT, KOHATSU MY, SEABRA AB, MONTEIRO LR, GOMES DG, OLIVEIRA HC, ROLIM WR, JESUS TA, BATISTA BL & LANGE CN. 2020. Effects of copper oxide nanoparticles on growth of lettuce (*Lactuca sativa* L.) seedlings and possible implications of nitric oxide in their antioxidative defense. Environ Monit Assess 192(4): 232.

POMPÊO MLM. 2008. Monitoring and management of aquatic macrophytes. Ecology Brasiliensis 12(3): 406-424.

POTT A & POTT VJ. 1994. Pantanal Plants. EMPRAPA/CPAP/SPI, Corumbá, 320 p.

QUEIROZ AM, MEZACASA AV, GRACIANO DE, FALCO WF, M'PEKO JC, GUIMARÃES JFC, LAWSON T, COLBECK I, OLIVEIRA SL & CAIRES ARL. 2016. Quenching of chlorophyll fluorescence induced by silver nanoparticles. Spectrochim Acta A Mol Biomol Spectrosc 168: 73-77.

ROUSSEAU C, BELIN E, BOVE E, ROUSSEAU D, FABRE F, BERRUYER R, GUILLAUMÈS J, MANCEAU C, JACQUES MA & BOUREAU T. 2013. High throughput quantitative phenotyping of plant resistance using chlorophyll fluorescence image analysis. Plant Methods 9: 17.

SEO M, NAMBARA E, CHOI G & YAMAGUCHI S. 2009. Interaction of light and hormone signals in germinating seeds. Molecular Biology Plant, Dordrecht 69(4): 463-472.

SHAW AK & HOSSAIN Z. 2013. Impact of nano-CuO stress on rice (*Oryza sativa* L.) seedlings. Chemosphere 93(6): 906-915.

SHUGUANG J, WEIJUN S & YANG Z. 2009. Enhanced adaptability of *Sesbania rostrata* to Pb/Zn tailing via stem nodulation. J Environ Sci 21: 1135-1141.

SIMONIN M, CANTAREL AAM, CROUZET A, GERVAIX J, MARTINS JMF & RICHAUME A. 2018. Negative effects of copper oxide nanoparticles on carbon and nitrogen cycle microbial

activities in contrasting agricultural soils and in presence of plants. Front Microbiol 9: 3102.

STETEFELD J, MCKENNA SA & PATEL TR. 2016. Dynamic light scattering: a practical guide and applications in biomedical sciences. Biophys Rev 8: 409-427.

TONINI PP, LISBOA CGS, SILVA CO, MAZZONI-VIVEIROS SC & BUCKERIDGE MS. 2007. Testa is involved in the control of storage mobilisation in seeds of *Sesbania virgata* (Cav.) Pers., a tropical legume tree from of the Atlantic Forest. Trees 21: 13-21.

VAN NL, MA C, SHANG J, RUI Y, LIU S & XING B. 2016. Effects of CuO nanoparticles on insecticidal activity and phytotoxicity in conventional and transgenic cotton. Chemosphere 144: 661-670.

YANG B ET AL. 2003. Growth and metal accumulation in vetiver and two *Sesbania* species on lead/zinc mine tailings. Chemosphere 52(15): 93-106.

YANG Z, XIAO Y, JIAO T, ZHANG Y, CHEN J & GAO Y. 2020. Effects of Copper oxide nanoparticles on the growth of rice (*Oryza sativa* L.) seedlings and the relevant physiological responses. Int J Environ Res Public Health 17(4): 1260.

ZANANDREA I ET AL. 2009. Tolerance of *Sesbania virgata* plants to flooding. Aust J Bot 57: 661-669.

ZHU H, HAN J, XIAO JQ & JIN Y. 2008. Uptake, translocation, and accumulation of manufactured iron oxide nanoparticles by pumpkin plants. J Environ Monitor 10(6): 713-717.

How to cite

SANTOS ES, GRACIANO DE, FALCO WF, LIMA CAIRES AR & DE ARRUDA EJ. 2021. Effects of copper oxide nanoparticles on germination of *Sesbania virgata* (FABACEAE) plants. An Acad Bras Cienc 93: e20190739. DOI 10.1590/0001-3765202120190739.

Manuscript received on June 26, 2019;
accepted for publication on June 30, 2020

ELIENE S. DOS SANTOS[1]
https://orcid.org/0000-0002-0136-3183

DANIELA E. GRACIANO[1]
https://orcid.org/0000-0001-5559-6129

WILLIAM F. FALCO[1]
https://orcid.org/0000-0003-2744-8668

ANDERSON R. LIMA CAIRES[2]
https://orcid.org/0000-0002-2602-9480

EDUARDO J. DE ARRUDA[1]
https://orcid.org/0000-0002-9835-467X

[1]Universidade Federal da Grande Dourados, Faculdade de Ciências e Tecnologia, Rodovia Dourados/Ithaum, Unidade Km 12-2, Caixa Postal 364, 79804-970 Dourados, MS, Brazil

[2]Grupo de Óptica e Fotônica, Universidade Federal de Mato Grosso do Sul, Instituto de Física, Caixa Postal 549, 79070-900 Campo Grande, MS, Brazil

Correspondence to: **Eduardo José de Arruda**
E-mail: ejarruda@gmail.com

Author contributions

ELIENE SILVA DOS SANTOS: Conceptualization, Investigation, Methodology, Writing - review & editing. DANIELA ESPANGUER GRACIANO: Investigation, Methodology, Writing - review & editing. WILLIAM FERREIRA FALCO: Formal analysis. ANDERSON RODRIGUES LIMA CAIRES: Conceptualization, Funding acquisition, Investigation, Methodology, Writing - review. EDUARDO JOSÉ DE ARRUDA: Formal analysis, Resources, Supervision, Writing - review & editing.