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PHYSIOLOGICAL ANALYSIS OF CUO BULK AND NANOPARTICLES TO CASTOR (*RICINUS COMMUNIS* L.)

ABSTRACT

The rapidly increasing multifarious use of metallic nanoparticles in technology has necessitated evaluation of their impact on environmental, biotic and human health. The present study investigated the effects of different concentrations of bulk and nanosized CuO on seed germination and seedling growth of Ricinus communis in a randomized completely design with four replications. The experimental treatments included four concentrations of bulk CuO (10, 50, 100 and 500 ppm), four concentrations of nanosized CuO (10, 50, 100 and 500 ppm), and the control without CuO. The results indicate that only the weighted germination index and seedling dry biomass of Ricinus communis were significantly affected by the treatments. Other germination characteristics, plumule and radicle length, and seedling fresh weight were not significantly affected by bulk and nanosized CuO concentrations. It can be concluded that bulk and nanosized CuO in this cocentrations not toxic for germination and growth of *Ricinus communis*.

Keywords: bulk CuO; CuO nanoparticles; germination index; *Ricinus communis*; TTC test

INTRODUCTION

Nanotechnology has developed in industry rapidly. The value products of global market will be 2.6 trillion dollars in 2014 (Lux, 2006), based on nanotechnology. Possessing substantial impacts on economy, society, and environment have caused to generate both positive and negative responses from governments, scientists, and social media throughout the world (Yang *et al.*, 2006).

Nanoparticles closely interact with their surrounding environment and plants are an essential base component of all ecosystems. As a result nanoparticles will inevitably interact with plants and these interactions such as uptake and accumulation in plant biomass will greatly affect their fate and

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transport in the environment. Nanoparticles could also adhere to plant roots and exert physical or chemical toxicity on plants. Increasing numbers of publications have emerged recently concerning the interactions of nanoparticles with plants (Battke *et al.*, 2009; Lin and Xing 2007; Lin *et al.*, 2009). Most of these studies are focused on the potential toxicity of nanoparticles to plants and both positive and negative or inconsequential effects have been reported. In terms of metallic nanoparticles, copper nanoparticles were shown to be toxic to two crop species, mung bean (*Phaseolus radiatus*) and wheat (*Triticum aestivum*), as demonstrated by the reduced seedling growth rate (Lee *et al.*, 2008). Mung bean is more sensitive than wheat and the authors attributed this phenomenon to differences in root anatomy and architecture. Plants need only trace amounts of copper, and its increased concentrations are toxic for them. Free copper ions can unspecifically bind to thiol groups of enzyme proteins, which results in the loss of their secondary structure and, therefore, activity (Nekrasova and Maleva, 2007). Copper also exerts its toxic action through the Fenton reaction, i.e., generation of hydroxyl radicals catalyzed by the metal (Yruela, 2005). Copper at increased concentrations damages thylakoid membranes, thereby disturbing the functioning of photosystem II and the water oxidizing complex of chloroplasts (Pätsikkä *et al.*, 2002; Yruela, 2005). Of special interest are nanoparticles of metals, copper in particular, which enter the environment from certain natural sources as well as a result of industrial activities, as accidental pollutants (Gmoshinskii *et al.*, 2010).

Ricinus communis L., the castor oil plant, is a medicinal species of flowering plant in the spurge family, *Euphorbiaceae*. The oil from the seed is a very well-known laxative that has been widely used for over 2,000 years. The seed is used in Tibetan medicine, where it is considered to have an acrid, bitter and sweet taste with a heating potency. It is used in the treatment of indigestion and as a purgative (Zargari and Mir Heidar , 2011). The purpose of this study was to analyze effects of CuO bulk and nano particles on germination indexes and seedling growth of castor.

MATERIALS AND METHODS

Characterization of nano-sized and bulk CuO particles

CuO nanoparticles powder was supplied by Nutrient Company.The size and topography of CuO nanoparticles were determined by scanning tunneling microscope (STM) in the Central Laboratory of Ferdowsi University of Mashhad, Iran. X-ray diffraction (XRD) was carried out for determining of main and lateral phase of CuO nanoparticles. Bulk CuO was supplied by Merk Company.

Stock suspensions of 500 ppm bulk or nanosized CuO were prepared in deionized water and to avoid aggregation the suspensions were sonicated (GEX 750-5B Ultrasonic Processor VCX 750 Watt, 20 kHz, Cole Parmer, Vernon Hills, IL, USA) for 30 min, and suspensions with concentrations of 10, 50, 100 and 500 ppm were prepared. The pH after dispersion was 6.3. Total dissolved copper concentrations were measured to determine the role of soluble Cu in this experiment. Bulk and nanosized CuO suspensions at 10, 50, 100 and 500 ppm were centrifuged at 150 g for 10 min. The supernatants were subsequently filtered through 0.2-mm glass filters. The $Cu2^+$ concentrations in the filtrates were measured by atomic absorption spectrophotometry (AA-670, Shimadzu Company, Kyoto, Japan). The hydrodynamic diameters of bulk and nanosized CuO were analyzed by dynamic light scattering (DLS), using a particle size analyzer (VASCO 3, Cordouan, Pessac, France) at 25°C

Experimental Design and Data Observation

Experimental design was a randomized completely with four replications. The experimental treatments included four concentrations (10, 50, 100 and 500 ppm) of bulk and four concentrations (10, 50, 100 and 500 ppm) of nanosized CuO and untreated control (without any CuO types). The experiment was conducted in laboratory conditions with natural light and an average temperature of 25±1°C at the Faculty of Science, Mashhad Branch, Islamic Azad University, Mashhad, Iran, in 2014. *Ricinus communis* L. (var. 80-31) seeds were taken from the Pakan Bazr Company, Isfahan Province, Iran. One hundred seeds of similar size were randomly selected and placed on moistened paper as four groups of seeds in Petri dishes, and then 10 ml of each 8 concentration treatment was added to each Petri dish. For the control, only distilled water was added to Petri dishes. Germination tests were performed according to the rule issued by the International Seed Testing Association.

All concentrations of CuO and the control were run at the same time and consequently under equal light and temperature conditions. The number of germinated seeds was noted daily for 7 days. Seeds were considered as germinated when their radicle showed at least 1 mm length. In this study, we used following germination parameters: Germination percentage (GP, %), Relative germination percentage (RGP), Mean germination time (MGT), Germination index (GI) and Weighted germination index (WGI). Final percentage germination (GP) for each treatment was calculated after seven days. These parameters were also calculated from the formulas proposed by (Wu and Du 2007:

$$GP = \frac{GN}{SN} \times 100$$

where

GN is the total number of germinated seed, *SN* is the total number of seeds tested

Relative germination percentage (*RPG*) is equaled:

$$RGP = \frac{GP_{treatment}}{GP_{control}} \times 100$$

GI is a synthetic measure designed to reflect the synthetic germination ability including germination rate and germination numbers.

$$GI = \frac{(\sum(N - i) \times G_i) \times 100}{N \times GN}$$

where,

i is the number of days since the day of sowing and *Gi* is the number of seeds germinated on day *i*.

A weighted germination index (*WGI*) was calculated with maximum weight given to the seeds germinating early and less to those germinating late:

$$WGI = \frac{[N \times n_1 + (N - 1) \times n_2 + (N - 2) \times n_3 + \cdots]}{N \times N'}$$

where,

n1, n2, …, n7 are the number of seeds that germinated on first, second, and subsequent days until the 7th day, respectively; N is total days of experiment; N′ is the total number of seeds placed in incubation.

Vigour index (V_i) was calculated according to the formula:

$$V_i = GE \times SL$$

where,

GE germination [%] and *SL* seedling length [cm] (root + shoot).

After an incubation period of 7 days, plumule and radical length of seedlings were measured using a ruler. In order for dry biomass to be weighed, the 7-day seedlings were first weighed; then, having been placed in oven at 80°C for 48 h, they were weighed for a second time.

RESULTS

Characterization of bulk and nanosized CuO

STM images showed that nanoparticles CuO were 50 nm in size (Fig. 1, 2) and the bulk particles of CuO were 141 nm. The XRD patterns of CuO nanoparticles are shown in Fig. 3. XRD measurement showed that the CuO nanoparticles were composed of tenorite . The hydrodynamic diameters of the bulk and nanoparticles CuO were 945 and 578 nm, respectively, in deionized water (Table 1). In accordance with the Stokes-Einstein equation, the hydrodynamic diameter is larger than the actual particle size.

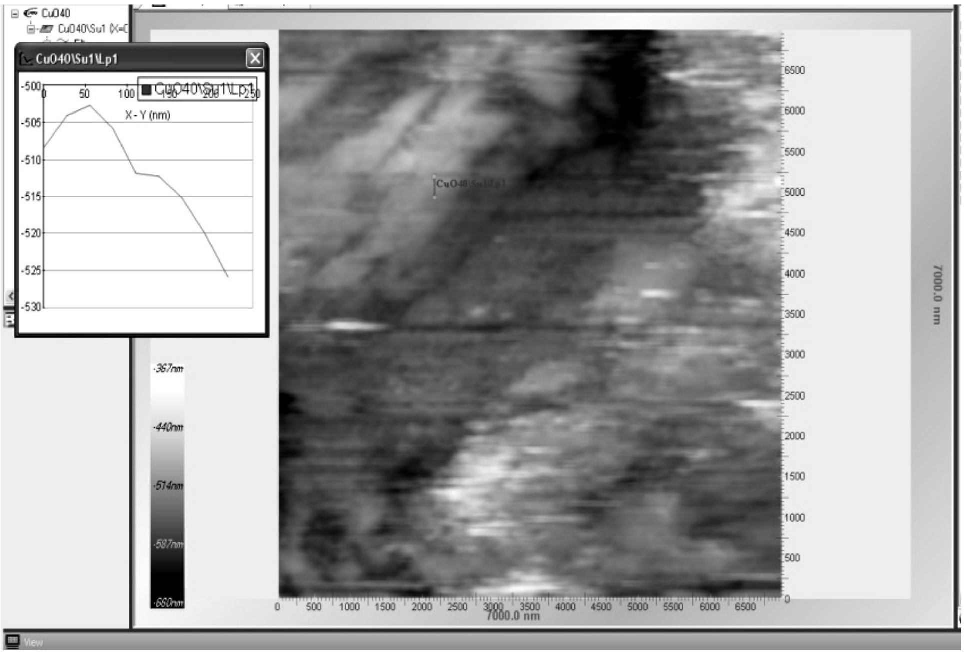

Fig. 1. Image of nanosized CuO by STM

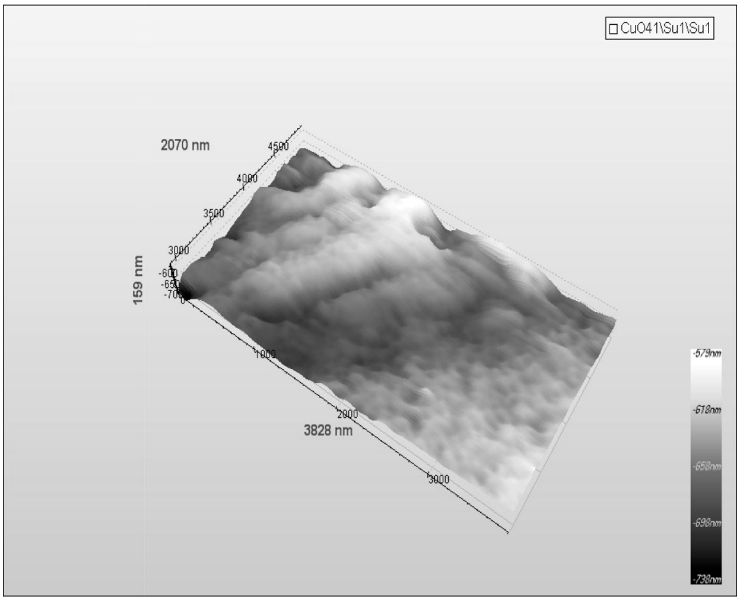

Fig. 2. Topographic image of nanosized CuO by STM

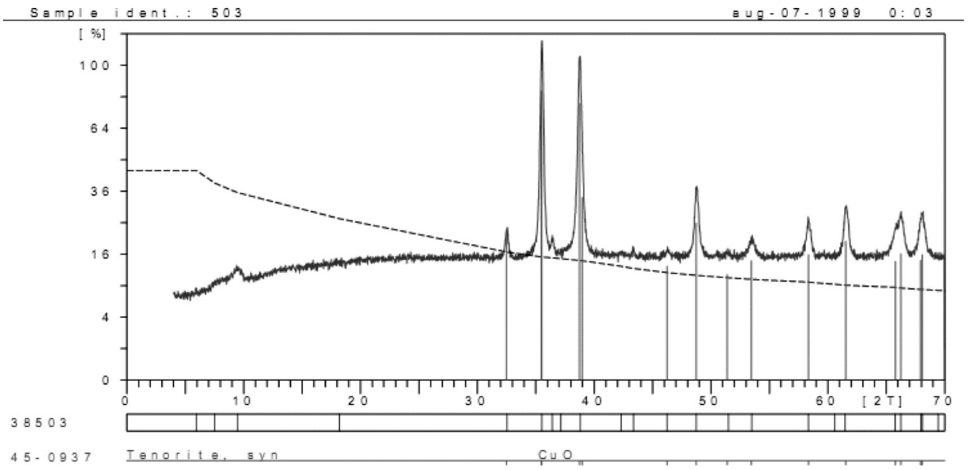

Fig. 3. XRD pattern of CuO nanoparticles

Table 1

Characteristics of bulk and nano-sized CuO particles used in this study (the particles were characterized in deionized water, pH 6.3

Particle	Purity [%]	Particle size [nm]	Hydrodynamic diameter [nm]
Bulk CuO	99.5	141	945 ±19
Nano-sized CuO	99.99	50	578± 22

Effect of bulk and nano-sized CuO on seed germination and seedling growth

After 7 days, the germination percentage of the caster seeds was calculated at each concentration of bulk and nanosized CuO. The seeds grown on control media without CuO recorded a germination percentage of 58%. The lowest germination percentage (47%) was recorded for 10 ppm of nanosized CuO (Table 2). Although the highest germination rate (7.77) was recorded for the 500 ppm nanosized CuO, it showed no significant difference with other treatments except for the 10 ppm 50 nanosized CuO and 100 and 500 ppm bulk CuO. The lowest mean germination time (5.10 d) was recorded for the 500 ppm nanosized CuO and the highest (5.43 d) for the 100 ppm bulk CuO treatment.

The 500 ppm nanosized CuO treatment significantly decreased mean germination time by 6% in comparison with the 100 ppm bulk CuO . The media containing 50 ppm bulk and nanosized CuO had a higher relative germination percentage (125.2 and 146.2, respectively) than the other treatments but this was not statistically significant (Table 2). The highest germination index (26.8) was recorded for the 500 ppm nanosized CuO treatment and the lowest (22.8) for the 100 ppm bulk CuO treatment. The 100 ppm bulk CuO treatment decreased the germination index by 7.8% in comparison with the control (Table 2). Different concentrations of bulk and nanosized CuO significantly affect the weighted germination index of caster seeds except for 50 and 100 ppm bulk CuO.

Table 2

Effect of different concentrations of bulk and nanosized CuO on seed germination of *Ricinus communis* L.

Concentration [ppm]	Germination [%]	RGP	Germination Rate [% × Day^{-1}]	MGT [Day]	GI	WGI
			Bulk CuO			
10	69 abc	113.7 a	6.48 abc	5.21 b	25.4 ab	0.39 a
50	73abc	125.2 a	6.58 abc	5.23 b	25.1 ab	0.39 a
100	55 abc	89.4 a	5.25 bc	5.43 a	22.8 b	0.36 ab
500	51 bc	82.5 a	5.01 c	5.17 b	26.0 ab	0.40 a
			Nano CuO			
	47c	85.5 a	4.90 c	5.20 b	23.7 ab	0.39 a
	79 a	146.2 a	7.67 ab	5.12 b	26.7 a	0.41 a
100	60 abc	114.2 a	6.35 abc	5.14 b	26.5 a	0.40 a
500	75 ab	114.2 a	7.77 a	5.10 b	26.8 a	0.41 a
Control	58abc		5.8 abc	5.26 ab	24.7 ab	0.35 b

Means in each column followed by similar letters are not significantly different at the 5% probability level using Duncan's multiple range test

The radicle and plumule length of all treatments of nanosized and bulk CuO was lower than of the control, but this effect was not significant(Table 3). All nanosized and bulk CuO treatments increased seedling fresh biomass unsignificantly. The highest seedling fresh biomass was recorded for the 50 ppm nanosized CuO.

The experimental treatments affected seedling dry biomass significantly. The lowest seedling dry biomass (0.002 g) was recorded for the 500 ppm bulk CuO and the highest was recorded for the 50 ppm nanosized CuO treatment (0.022 g). Bulk CuO treatments had no significant effect on seedling dry biomass.The 500 ppm Bulk CuO treatment decreased seedling dry biomass by 50% in comparison with the control (Table 3).

The vigor index was significantly affected by bulk and nanosized CuO concentrations(Table 3). The lowest and highest vigor index was recorded for the 500 and 50 ppm bulk CuO respectively, which changed the vigor index by 24.7% and 75%, respectively, in comparison with the control (Table 3).

Table 3

Effect of bulk and nanosized CuO concentrations on seedling growth of *Ricinus communis* L.

Concentration [ppm]	Plumule Length [cm]	Radicle Length [cm]	Seedling Fresh Biomass [g]	Seedling Dry Biomass [g]	Vigor Index
			Bulk CuO		
10	2.2 a	4.5 a	0.047 a	0.004 b	30.33 ab
50	2.7 a	5.7 a	0.052 a	0.003 b	59.00 a
100	2.1 a	4.3 a	0.056 a	0.004 b	39.33ab
500	2.2 a	4.8 a	0.040 a	0.002 b	25.33 b
			Nano CuO		
10	2.1 a	6.4 a	0.022 a	0.015 ab	52.00 ab
50	2.1 a	4.9 a	0.112 a	0.022 a	32.67 ab
100	2.7 a	5.9 a	0.042 a	0.003 b	40.67 ab
500	1.7 a	3.8 a	0.050 a	0.004 b	38.33 ab
Control	2.8 a	6.0 a	0.039 a	0.004 b	33.67 ab

Means in each column followed by similar letters are not significantly different at the 5% probability level using Duncan's multiple range test

The TTC tests showed that the effects of bulk and nanosized CuO on root tips did not vary by concentration (Fig. 4). After 24 h of treatment, all root tips were colored red.

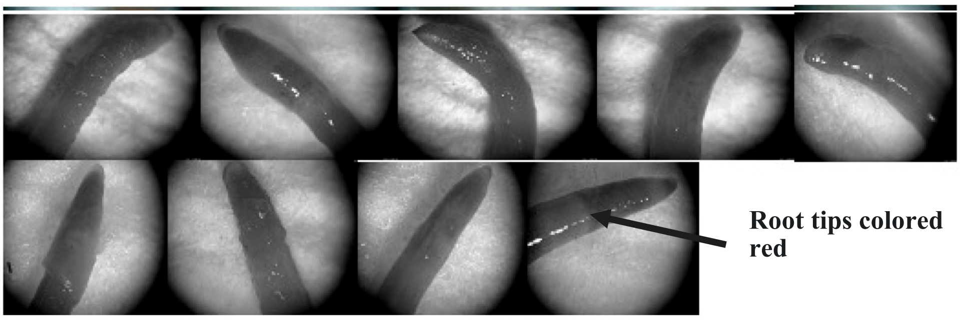

Fig. 4. TTC tests for different concentrations Of bulk and nano-sized CuO. Up from left to right: control, 10, 50, 100 and 500 ppm bulk Cuo, below from left to right: 10, 50, 100 and 500 ppm of nano CuO

To ensure that the observed effect is the result of nano CuO and that the solubility of nano CuO is negligible, the amount of Cu ion released from nano CuO during nanoparticle preparation was measured.The total soluble Cu2+ concentration released from the bulk and nanosized CuO was measured (Table 4) and it was found that these values were too low to be responsible for CuO nanoparticle or bulk phytotoxicity. Treatment with 500 ppm nanosized CuO , which released 4.42 ppm of soluble Cu, increased seed germination by 22.6% (Table 2) and decreased plumule elongation by 39% (Table 3). By contrast, exposure to equivalent concentrations of bulk CuO released 6.7 ppm of soluble Cu and resulted in 12% and 22% decreasing of germination and plumule elongation, respectively (Table 2 and 3). This experiment demonstrated that 4.42 ppm of Cu ion was not toxic to the test plants and even enhanced plant growth. Copper is an essential micronutrient, and it is necessary in low concentrations for plant growth, so these results suggest that the effect of nanosized CuO cannot be explained solely by the dissolved Cu2+ and that the particles themselves contribute to the effcts.

Table 4

Total dissolved Cu2+ released from nanosized and bulk CuO particles suspended in deionized water at pH 6.3

CuO concentrations [ppm]	Total dissolved Cu [ppm]	
	Nanosized CuO	Bulk CuO
10	0.19± 0.02	0.33± 0.04
50	0.6± 0.01	1.2± 0.02
100	1.2± 0.2	3.1± 0.09
500	4.42 ±0.16	6.7± 0.4

DISCUSSION

The world-wide use of nano-Cu can disturb the soil biological processes as well as the plant physiology /biochemistry, which in turn may affect human health. Being an essential micronutrient for plants, Cu at low concentration participates in photosynthetic electron transport, mitochondrial respiration, cell wall metabolism, hormone signaling, protein trafficking and iron mobilization, and significantly improves plant growth and development (Yruela, 2009).

The current study reports that nanosized CuO only significantly increased weighted germination index of castor plants and other germination characteristics and seedling growth were unaffected by nano CuO. Although, bulk CuO decreased germination indexes and radicle and plumule growth but had no significantly effect. It was concluded that the seed coat can probably act as protector for the embryo and totally guard the whole seed. The size of seeds could render more sensitivity to nanoparticles exposure (Shen *et al.*, 2010). This is because a large seed species has a lower surface to volume ratio than a small seeded species.The castor seeds are large and due to this, they are not sensitive to nanoparticles.

Our findings agree with Adhikari *et al.* (2012) where in both *Glycine max* and *Cicer arietinum*, germination was not checked up to 2000 mg × L^{-1} nano-CuO but the root growth was prevented above 500 mg × L^{-1} nano-CuO; the elongation of the roots was severely inhibited with increasing concentration of nano CuO as compared to the control. Also, Lin and Xing (2007) observed that five different nanoparticles at 2000 mgL^{-1} had little impact on the germination of six plant species. They observed that while carbon nanotube, Al_2O_3, and Al nanoparticles had no impact root elongation, ZnO nanoparticles dramatically reduced root growth for all five species, although corresponding bulk materials were not evaluated. Similarly Stampoulis *et al.* (2009) noted that zucchini seed germination was unaffected by bulk or nanoparticles Ag, Cu, Si, carbon nanotube, or ZnO at 1000 mg × L^{-1}. Alternatively, Yang and Watts (2005) showed that the root elongation of four agricultural crops was unaffected by alumina nanoparticles at 2–200 mg × L^{-1}. However, at 2000 mg × L^{-1}, root length and development of all species were reduced by 13%, although no direct comparison to bulk alumina particles was made. Canas *et al.* (2008) reported that the impact of carbon nanotubes at 750 mg × L^{-1} on the root growth of six crop plants was species-specific, with nanoparticles exposure inhibiting root elongation in some species (tomato) but enhancing growth(onion, cucumber) or having no effect in others (cabbage, carrots). Abdul Hafeez *et al.*(2015) showed that seed germination was not affect with 0.2 to 0.8 ppm but decreased significantly at 1.0 ppm of nano Cu. Whereas MS medium blended with low concentrations of nano Cu (0.2, 0.4, 0.6, and 0.8 and 1.0ppm) significantly increased leaf area, chlorophyll content, fresh and dry weight, and root dry weight as compared to control plants.

In another instance, nano CuO (100 mg × L^{-1}) did not affect germination, but inhibited growth of *Zea mays* seedlings (Wang *et al.*, 2012). In contrast, the dissolved Cu2+ ions and CuO bulk particles could not affect the Zea mays growth. Also, Nair and Chung(2014) reported root cell death of Arabidopsis thaliana was not observed in roots of plants exposed to 1 mg × L^{-1} nano CuO, however propidium iodide staining showed a dose- dependent increase in cytotoxicity in lateral root tips of plants which were exposed to 2.0,5.0, 10 and 20 mgL-1.

The results of the present study were contrary to the other investigations. Atha *et al.* (2012) showed that in the 10,100, 500 and 1000 mgL-1 nano-CuO and bulk-CuO exposed Raphanus sativus, the approximately three-fold total Cu accumulation in nano-CuO treated shoot (vs.bulk CuO) and the strong plant growth inhibition were credited to nano-CuO. root growth was inhibited 97% and that of shoot growth was inhibited 79%. Moon *et al.* (2014) revealed that significant inhibition of seed germination and root elongation of Cucumber (Cucumis sativus) seedlings treated with 100, 200, 400 and 600 mg × L^{-1} treatments of nano CuO. On the contrary, seed germination and shoot-to-root ratio were enhanced by nano-Cu application (Shah and Belozerova 2009).

Dimkpa *et al.* (2012) noted shoot length of wheat was reduced significantly by 13% and reduced root length by 59% under treatment of 500 mg × kg^{-1} nano CuO. Shaw and Hossain (2013) reported that different concentrations of CuO (0.5, 1 and 1.5 mM) inhibited of seed germination and seedlings growth of rice, significantly.

it is generally concluded that the nanoparticles effects on plants depended on the type, concentration, size, specific surface area and physiochemical properties of nanoparticles, plant species and plant age.

CONCLUSIONS

Overall experimental results showed that although presence of CuO nano and bulk particles not affects the seed germination and seedling growth of castor at different concentrations, significantly but the maximum germination percentage and rate, germination index and weighted germination index found at 500 ppm nano CuO. Plumule and radicle length of castor was unsignificantly inhibited by all of concentrations of CuO nano and bulk. Future studies should be directed towards understanding the mechanism of CuO nanoparticles effects.

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