Materials Letters 313 (2022) 131756

Contents lists available at ScienceDirect

Materials Letters

journal homepage: www.elsevier.com/locate/matlet

Impact of biosynthesized CuO nanoparticles on seed germination and cyto-physiological responses of *Trigonella foenum-graecum* and *Vigna radiata*

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ARTICLE INFO

Keywords:
Abrus precatorius
CuO nanoparticles
Cytotoxicity
Protein profile
Seed germination

ABSTRACT

Copper oxide nanoparticles (CuO·NPs) biosynthesized from plant extracts is emerging nanotechnology for medical and environmental applications. In the current study, the leaf extract of a medicinal plant, *Abrus precatorius* was used as a reducing agent for CuO·NPs biosynthesis and the formation of NPs was confirmed with various techniques. CuO·NPs (0, 10, 100, 200, 300, 400 and 500 mg/L) prepared were subjected to seed germination, seedling characteristics and cyto-physiological assay using *Trigonella foenum-graecum* (fenugreek) and *Vigna radiata* (green gram). Altogether, the CuO·NPs uptake and accumulation, chromosomal aberrations, and mitotic index in the roots of fenugreek and green gram seedlings were contingent on the concentration of CuO·NPs. A maximum of 80% and 86.6% mitotic index was recorded for fenugreek and green gram, respectively at 10 mg/L CuO·NPs, while it was 42.3% and 63.6% in 500 mg/L CuO·NPs. Protein expression of the CuO·NPs treated seedlings showed great variation. The findings imply that higher concentrations of CuO·NPs negatively impact the germination and cyto-physiology of fenugreek and green gram.

1. Introduction

Green synthesis is one of the emerging approaches for the synthesis of metal NPs and their composites. Many of the metals were used for NPs synthesis due to their properties and applications [1,2]. Though metal-based NPs and nanocomposites are applied widely, their impact and toxicity are not evaluated recurrently which requires prompt attention [3]. *Abrus precatorius* L. of Fabaceae (Fig. 1a) has a long history of medicinal uses [4] which has also been used for ZnS·NPs synthesis [5]. The toxicity of ZnS·NPs from *A. precatorius* leaves (APL) was evaluated in earthworms and the study reported that ZnS·NPs can hinder the enzymatic and protein profile of coelomic fluid [5]. Cu in the soil is vital for plant metabolism as it assists respiration and photosynthesis. Cu deficiency or excess levels in soil negatively impacts plant growth and metabolism [6]. Plant extract mediated CuO·NPs find wider applications in biomedical and environmental remediation fields [7]. CuO·NPs exposure to low concentrations are known to improve catalase and superoxide dismutase in rice seedlings while higher concentrations (250 mg/L) had detrimental effects apart from the reduced chlorophyll and carotenoid contents [8]. However, the toxicological evaluation of specific CuO·NPs in plants is limited. Hence, the present study was purposed to investigate

(i) synthesis and characterization of CuO·NPs using the extract of APL
(ii) impact of APL:CuO·NPs impact on seed germination of economically important legume crops, *Trigonella foenum-graecum* L. (fenugreek) and *Vigna radiata* (L.) R.Wilczek (green gram)
(iii) the accumulation and uptake levels of NPs on root tissues, cytological responses, and protein profiling of fenugreek and green gram.

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https://doi.org/10.1016/j.matlet.2022.131756
Received 19 November 2021; Received in revised form 8 January 2022; Accepted 17 January 2022
Available online 21 January 2022

2. Materials and methods

APL extract preparation and CuO·NPs synthesis, characterization and seed germination study methods were given in Supplementary data (SD.1). The details of analyses of CuO·NPs accumulation and uptake rates are provided in Supplementary data (SD.2). The cytological and protein profiling studies were adopted as per the procedures described in Supplementary details (SD.3).

3. Results and discussion

3.1. Characterization of CuO·NPs

The development of reddish-brown at the end of biosynthesis indicates the formation of strongly prepared CuO·NPs. SEM images of CuO·NPs synthesized from APL extract recorded at magnification ranging from 5.62 to 9.24 kX clearly showed that the porosity in the agglomerated structure of particles CuO·NPs (Fig. 1b). This is in agreement with various crystal forms that have been found in the XRD analysis of CuO·NPs (Fig. 1c). The materials were well crystallized with sharp diffraction peaks at 2θ values of 20 θ, 27 θ, 30 θ, 35 θ, 40 θ, 43 θ, 57 θ, 60 θ and 45 θ, and these peaks corresponded to the crystal faces of Cu (Fig. 1d). Fig. 1(e) shows the successfully synthesized CuO·NPs with the absorbance peak between 400 and 590 nm which have attributed to their significant nature. The FTIR spectrum of CuO·NPs as in Fig. 1(f) shows the existence of seven leading peaks revealing O–H stretching vibrations (3336.21 cm^{-1}), C–H asymmetry (2920.62 cm^{-1}), C¼C protraction (1654.35 cm^{-1}), C¼C aromatic ring extension (1605.60 cm^{-1}), C–OH stretching vibrations (1453.52 cm^{-1}), and C–OH bending (1397.68 and 7162.29 cm^{-1}). Further, the existence of CuO was observed at a 624.57 cm^{-1} peak. These results are in harmony with previous studies [9,10].

3.2. Seed germination, CuO·NPs accumulation and uptake levels

Seedling root length lowered with the progressive CuO·NPs concentration for both fenugreek and green gram. CuO·NPs exhibited a bigger impact on green gram seedling length than fenugreek. The root length reduction was observed for green gram than fenugreek at 500 mg/L CuO·NPs concentration. The seed germination of fenugreek and green gram did not differ significantly ($P > 0.05$) between control (0 mg/L) and 10 mg/L CuO·NPs concentration while a significant reduction ($P < 0.05$) was found between higher concentrations (100 – 500 mg/L) (Fig. 2a-c). This indicates that low CuO·NPs concentrations were not affecting the seed germination. CuO·NPs accumulation in fenugreek and green gram roots was viewed as red fields in the CLSM images (Supplementary data, Fig. 1S). At 500 mg/L concentration of CuO·NPs, much noticed intracellular aggregates were in the size range of 300–500 nm. The highest level of CuO·NPs uptake was found in 500 mg/L at the absorbance of 0.27 and lower level concentration uptake in 10 mg/L at the absorbance of 0.06 for fenugreek. In green gram, the highest uptake level was found in 400 mg/L CuO·NPs at 0.086 absorbances, while a lower uptake level was recorded in 100 mg/L at 0.013 absorbances (Fig. 2d). The effect of CuO·NPs on seed germination is lower in higher concentration and *vice-versa* which could be attributed to the toxicity and accumulation of NPs [11].

3.3. Protein profiling and cytological analysis

The protein expression of fenugreek and green gram seedlings displayed variation in proteins between control and different CuO·NPs concentration with a range of 15–50 KDa protein bands (Fig. 3a-b). The number of dividing cells (DC)/300 cells at different CuO·NPs concentrations showed a maximum of 240 and 263 DC in 10 mg/L CuO·NPs for fenugreek and green gram, respectively. A downtrend of DC was recorded with the incremental variation of CuO·NPs. The DC/300 cells was in the order of 240 > 234 > 212 > 198 > 159 > 143 respectively in 10, 100, 200, 300, 400 and 500 mg/L CuO·NPs for fenugreek, while it was 263 > 245 > 220 > 217 > 209 > 191 for green gram. A similar declining trend in MI (%) of 80 > 78 > 70.6 > 60 > 53 > 42.3 was recorded in 10, 100, 200, 300, 400 and 500 mg/L CuO·NPs for fenugreek, and it was 86.6 > 87.6 > 81.6 > 73.3 > 72.3 > 69.6 > 63.6 for green gram. Chromosomal aberrations were observed for fenugreek and green gram root hairs of germinated seeds. Chromosomal break, chromosomal bridge, sticky chromosomes, c-metaphase and disturbed anaphase were the chromosomal aberrations recorded in the higher concentration of CuO·NPs. A percentage of 92.7 total chromosomal aberrations were recorded in 500 mg/L CuO·NPs fenugreek, whereas it was 80.7% for green gram (Table 1). Though DC and MI showed a non-significant ($P > 0.05$) difference between control (0 mg/L) and 10 mg/L CuO·NPs concentration for both fenugreek and green gram, the percentage of chromosomal aberration showed significant impact at P <

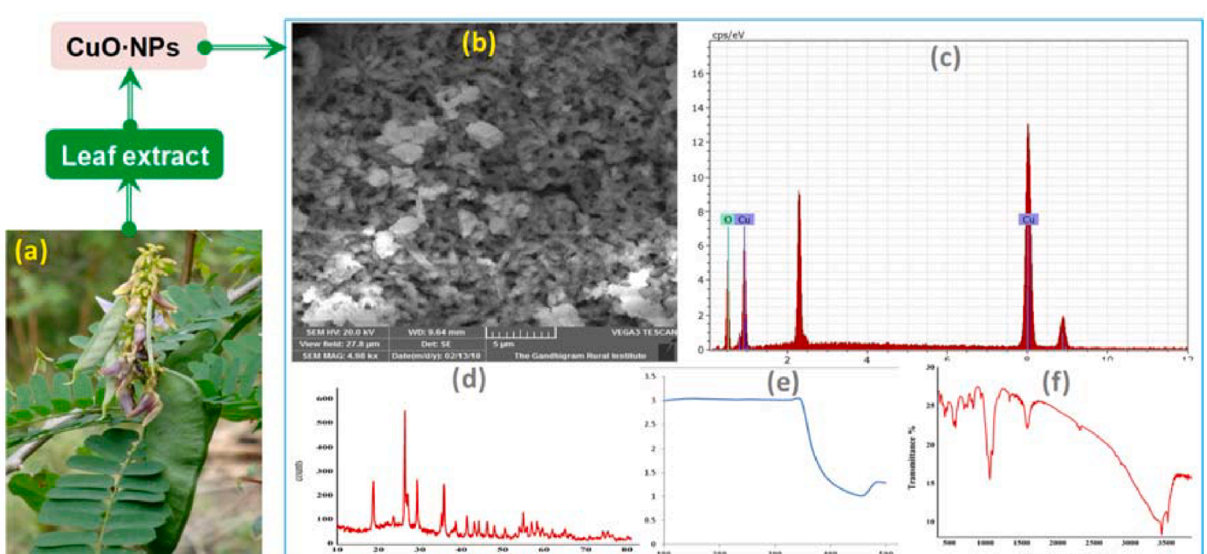

Fig. 1. Synthesis and characterization of CuO·NPs. (a) *Abrus precatorius*; (b) and (c) SEM and EDX image of CuO·NPs, respectively; (d) XRD pattern of CuO·NPs; (e) UV–Visible spectrum of CuO·NPs; (f) FTIR spectrum of CuO·NPs.

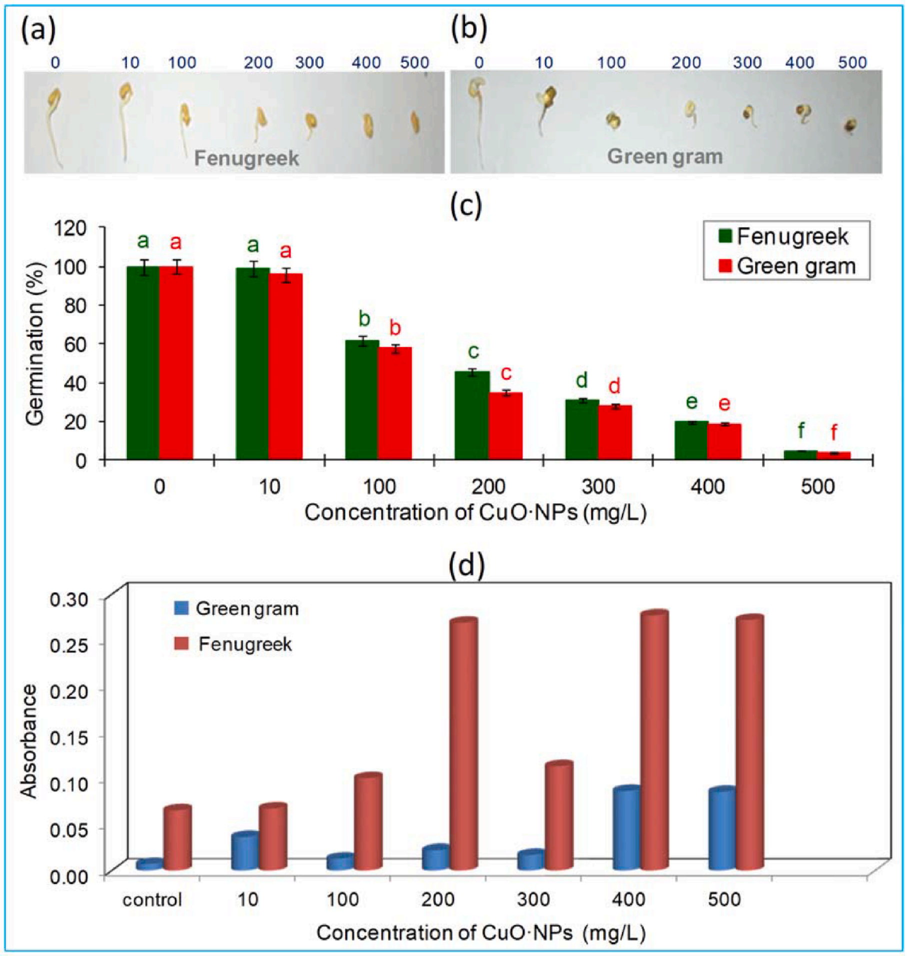

Fig. 2. Germination of (a) fenugreek and (b) green gram at different doses of CuO·NPs; (c) Seed germination percentage of fenugreek and green gram in different concentrations of CuO·NPs; Different alphabets on bars between treatments are significantly different at $P < 0.05$ by Duncan's multiple range test. (d) Uptake of CuO·NPs by fenugreek and green gram seedlings analyzed through AAS.

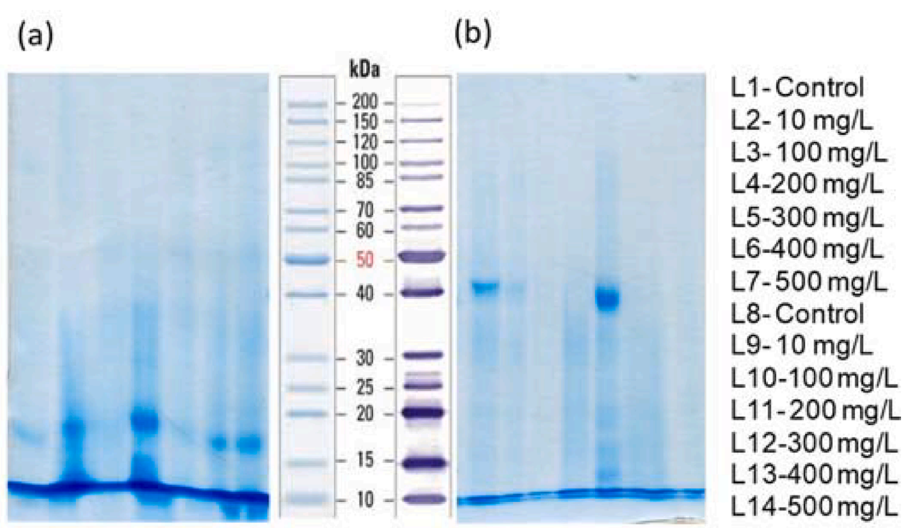

Fig. 3. Protein profile of (a) fenugreek and (b) green gram seedlings. Lanes L1 – L7: Fenugreek; Lanes L8 – L14: Green gram.

0.05. The concentration-dependent decrease of MI and increase in chromosomal aberrations is attributed to the entry of CuO·NPs into the roots followed by oxidative damage and cell cycle inhibition [12].

4. Conclusions

The biosynthesized APL:CuO·NPs demonstrated a concentration-dependent effect on the seed germination and seedling characteristics

Table 1
Cytotoxic effect of CuO·NPs on fenugreek and green gram seedling roots.

Conc. of CuO·NPs (mg/L)	Fenugreek				Green gram			
	No. of cells counted	No. of cells under division	MI (%)	Chromosomal aberration (%)	No. of cells counted	No. of cells under division	MI (%)	Chromosomal aberration (%)
0 (Control)	300	248[a]	82.6[a]	36.2[e]	300	260[a]	86.6[a]	20.3[e]
10	300	240[a]	80.0[a]	75.0[d]	300	263[a]	87.6[a]	45.6[d]
100	300	234[b]	78.0[a]	71.7[d]	300	245[b]	81.6[b]	61.2[c]
200	300	212[c]	70.6[b]	86.2[bc]	300	220[c]	73.3[c]	64.6[c]
300	300	198[d]	66.0[c]	88.0[b]	300	217[c]	72.3[c]	69.8[b]
400	300	159[e]	53.0[d]	82.0[c]	300	209[d]	69.6[c]	78.5[a]
500	300	143[f]	42.3[e]	92.7[a]	300	191[e]	63.6[d]	80.7[a]

The values with different superscript letters between treatments are significantly different at $P < 0.05$ by Duncan's multiple range test.

of fenugreek and green gram. The intracellular accumulation of CuO·NPs in the roots of seedlings was much pronounced above 400 mg/L concentration. The results indicate that the higher concentration of CuO·NPs impacts more on the number of DC, MI and the nature of chromosomes in fenugreek and green gram. The study indicates that higher concentrations of CuO·NPs are more toxic to plants, hence could be used in very low concentrations for environmental applications.

CRediT authorship contribution statement

Kurumban Kavitha: Methodology, Investigation, Software, Formal analysis. **James Arockia John Paul:** Methodology, Investigation, Software, Formal analysis. **Ponnuchamy Kumar:** Methodology, Writing – original draft, Data curation, Supervision. **Jayaprakasam Archana:** Methodology, Writing – original draft, Data curation, Supervision. **H. Faritha Begam:** Methodology, Writing – original draft, Data curation, Supervision. **Natchimuthu Karmegam:** Conceptualization, Methodology, Validation, Writing – review & editing, Supervision. **Muniyandi Biruntha:** Conceptualization, Methodology, Validation, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors are thankful to RUSA Scheme Phase 2.0 [F-24-51/2014–U, Policy (TNMulti-Gen), Dept of Edn., Govt. of India, dated 09.10.2018] for the facilities and support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.matlet.2022.131756.

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