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UPTAKE OF COPPER OXIDE NANOPARTICLES BY DIFFERENT TISSUES OF FENUGREEK (*TRIGONELLA FOENUM-GRAECUM* L.) PLANT

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Abstract

The present study gave a scientific insight about the role of Copper (II) oxides (CuO) nanoparticles (NPS) in the seeds germination of fenugreek (*Trigonella foenum-graecum* L.) plants when placing them on the surface of solidified Murashige and Skoog (MS) medium supported with (0, 25, 50, 75, 100, 150) ¼g/mL of those particles. The results showed superiority 75 ¼g/ml in induced of the seeds germination percentage with 100% after 2 days. The same concentration above has encouraged the best morphological and vegetative growth of the seedlings through the average length of their root and shoot system with 3.5 and 5.0 cm, respectively after 20 days. Also, this study success to initiate hairy roots on seedlings stems segments inoculated by *Agrobacterium rhizogenes* ATCC15834 with transformation percent reached up to 58.79% after 8 days. Free-bacteria hairy roots cultures produced when the hairy roots formed were excised and placed on agar-solidified MS medium contain antibiotic Cefotaxime at 250 mg/L to eliminate bacteria. The Scanning Electron Microscopy (SEM) technique explained the clearly ability of the transgenic hairy roots for uptake those NPS from its medium and accumulated those nanoparticle on their cells surface more than other seedlings tissues.

Key words: Copper oxide, nanoparticle, fenugreek

Introduction

Nanoparticles (NPS) can be define as the particles having < 100 nm size and comparatively large surface area to volume ratio nanoparticles used for several application including: food technology, agriculture, medicine, pharmaceuticals, electronics in addition to protection of environment. (UlAin *et al.,* 2018). The interaction between plant cells and NPS lead to change in biochemical reaction responsible for regulating gene expression and this will lead to enhance growth and development of the plant according to their type and concentration of NPS (Madbouly, 2018). Plant biotechnology and agricultural research are being harnessed with the new approach in nanotechnology. Many research works on exploitation of growth promoting effect of nanoparticles have been achieved in the last decade and developed new strategies in the field of Nano-agriculture (Hatami and Ghorbanpour, 2014; Kannah *et al.,* 2019).

Hairy-root cultures developed via genetic transformation through *A. rhizogenes* infection (Gelvin, 2009). During the infection process, *A. rhizogenes* transfers a segment of DNA (T-DNA) to the plant genome, it's a portion of root-inducing plasmid (pRi) of (Giri and Narasu 2000). Formation of hairy roots initiates after the integration of T-DNA with the plant genome (Niazian *et al.,* 2017). Hairy roots have several advantages such as genotype and phenotype stability, fast and indefinite *in vitro* growth (Bahari *et al.,* 2020). Due to the last characteristic mentioned, HR cultures have been called "phytochemical factories" since their biosynthetic capacity is similar to the native plant root; moreover, they often accumulate phytochemicals at a higher level than undifferentiated cultures (Gelvin, 2009). Researchers reported that the majority of metals accumulated in the roots in the many plants (Carbonell-Barrachina *et al.,* 1994; Pickering *et al.,* 2000), especially for the organic and metallic pollutants (Coniglio *et al.,* 2008).

Fenugreek (*Trigonella foenum-graecum* L.) is a valuable plant belong to family leguminosae and used medicinally in different countries and area source of many potent and powerful drugs (Kor *et al.,* 2013).

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This study investigated the ability of different tissues of fenugreek (*Trigonella foenum- graceum* L.) plants to uptake and accumulation Copper II oxide nanoparticles at different concentration and role of those nanoparticles in the seeds germination and speed, seedlings growth.

Materials and Methods

Surface sterilization of fenugreek seeds and cultured on solid MS medium supplemented with CuO NPS

Seeds of fenugreek (*Trigonella foenum- graceum* L.) plants surface sterilized by soaking in ethanol 96% for two minutes flowed by 3% sodium hypochlorite (NaOCl) with stirred for 5 minutes, then finally rinsed with sterilized distilled water three times, one minute (Al-Mahdawe, 2013).Sterilized seeds placed on the surface of solidified MS (Murashige and Skoog,1962) medium and supplemented with (0,25,50,75,100, 150) µg/ml of CuO NPS individually. Samples maintained in culture room at 22±2°C in the dark. After produced the complete seedlings were transported to the same condition but in 1500 lux with 16 hours light/ 8 hours dark.

Hairy root culture establishment

Stems segments of fenugreek (*Trigonella foenum-graceum* L.) excised form seedlings grown of MSO at 15 days and inoculated with *Agrobacterium rhizogenes* ATCC15834 (supplemented from Prof. Dr. Jutta Ludwig-mueller, Technical university Dresden, Germany) suspension incubated for 48 hours, used direct injection (Al-Mallah and Mohammed, 2012). After hairy root produced excised and placed on solidified MS medium and subculture every 20 days for its cultures formed. When contaminated formed, add the antibiotic Cefotaxime at 250 mg/L (AL-Mallah *et al.,* 1987) for eliminate from bacteria. All samples maintained at the same conditions above.

For study the behavior of hairy roots with CuO NPS, put tuft from it on the MS medium supplemented with the concentration (0, 25, 50, 75, 100, 150) µg /ml of CuO NPS individually.

Agropine test to improve the genetic transformation

Used the paper electrophoresis at method described by (Tepfer and Tempe, 1981) to improve the synthesized Agropine by transformed hairy roots.

Characterization of seeds germination, seedlings growth and hairy roots growth

Determinate of the seeds germination percent and its speed, length of root and shoot after 10, 20 days and addition, noticed the behavior of hairy roots grow on MS medium contain different concentration of CuO NPS.

Preparation of samples for Scanning Electron Microscope (SEM)test

→ Shoot of seedlings grown on MSO medium alone (control).

→ Roots, shoot and leaves of seedlings grown on MSO medium supplemented with 75 µg/ml of CuO nanoparticles (which the best for its growth).

→ Transgenic hairy roots grown on MSO medium supplemented with 75, 10µg /ml of CuO nanoparticles

Table 1: Effect of different concentration of CuO nanoparticles on the seeds germination percent and its speed of fenugreek plant cultured on MS solid medium.

MS+CuO (µg / ml)	Percent of seeds germination (%)	Speed of germination (days)
0 (Control)	90	3
25	80	3
50	90	2
75	100	2
100	80	3
150	60	5

Each value represents the rate of five replicates.

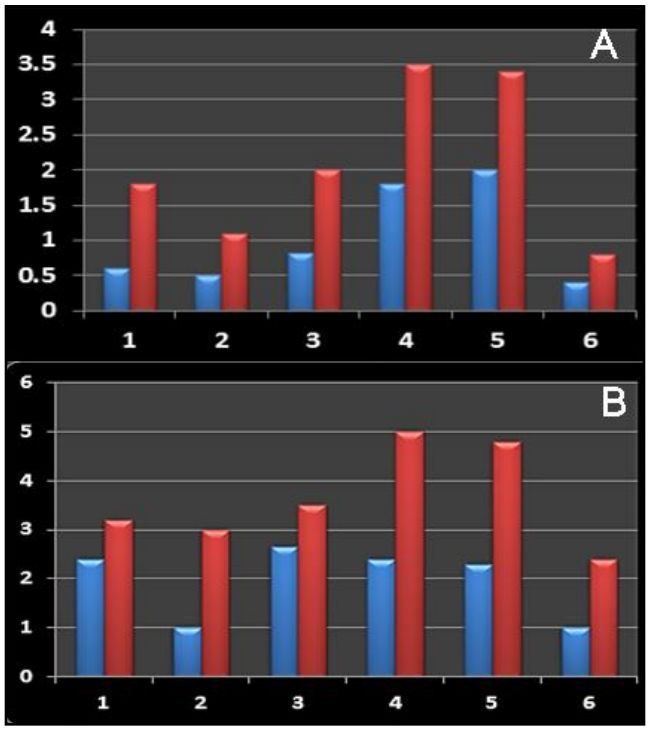

Fig. 1: Average of root system length (A) and shoot system length (B) after 10 days, 20 days of fenugreek seedlings grown on MS medium supplemented with different concentration of CuO nanoparticles:
1- MS (control) 2- MS+25 µg/ml3- MS+50 µg/ml 4- MS+75 µg/ml5- MS+100 µg/ml6- MS+150 µg/ml
Each value represents the rate of five replicates

(which the best for its growth).

were dried at 80 °C individually using oven (Gallenkamp Oven BS model, England) for 24 hours (Yahya and Al-Salih, 2014).

Scanning Electron Microscope (SEM) (VEGA/ TESCAN; Czech, Republic) found in CAC laboratory for chemistry analysis center, Baghdad, Iraq was used to detect nanoparticles in above samples.

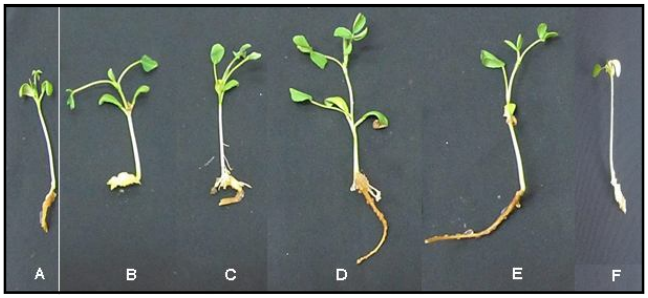

Fig. 2: A morphological comparison between the seedling grown on MS solid medium supplemented with different concentrations of CuO nanoparticles after 20 days.
1- MS (control)2- MS+25 µg/ml3- MS+50 µg/ml 4- MS+75 µg/ml5- MS+100 µg/ml6- MS+150 µg/ml.

The Results

Effect of CuO NPS on percent and speed of fenugreek plant seeds germination

The percent of fenugreek seeds germination and its speed of germination contrasted according to concentration of CuO (0, 25, 50, 75, 100, 150) µg/ml which used in this study table 1.

The results showed superiority of MS medium contain 75 µg/ml on the other concentration of CuONPS in encourage the percent of seed germination with 100% after 2 days, when comparing with MS medium alone (control), where the percent reached to 90% after 3 day.

Then the medium supplemented with 50 µg/ml where stimulated 90% of speed germination after 3days. while the concentration 150 µg/ml when added to MS medium inhibited the percent and speed of germination with 60% after 5 days.

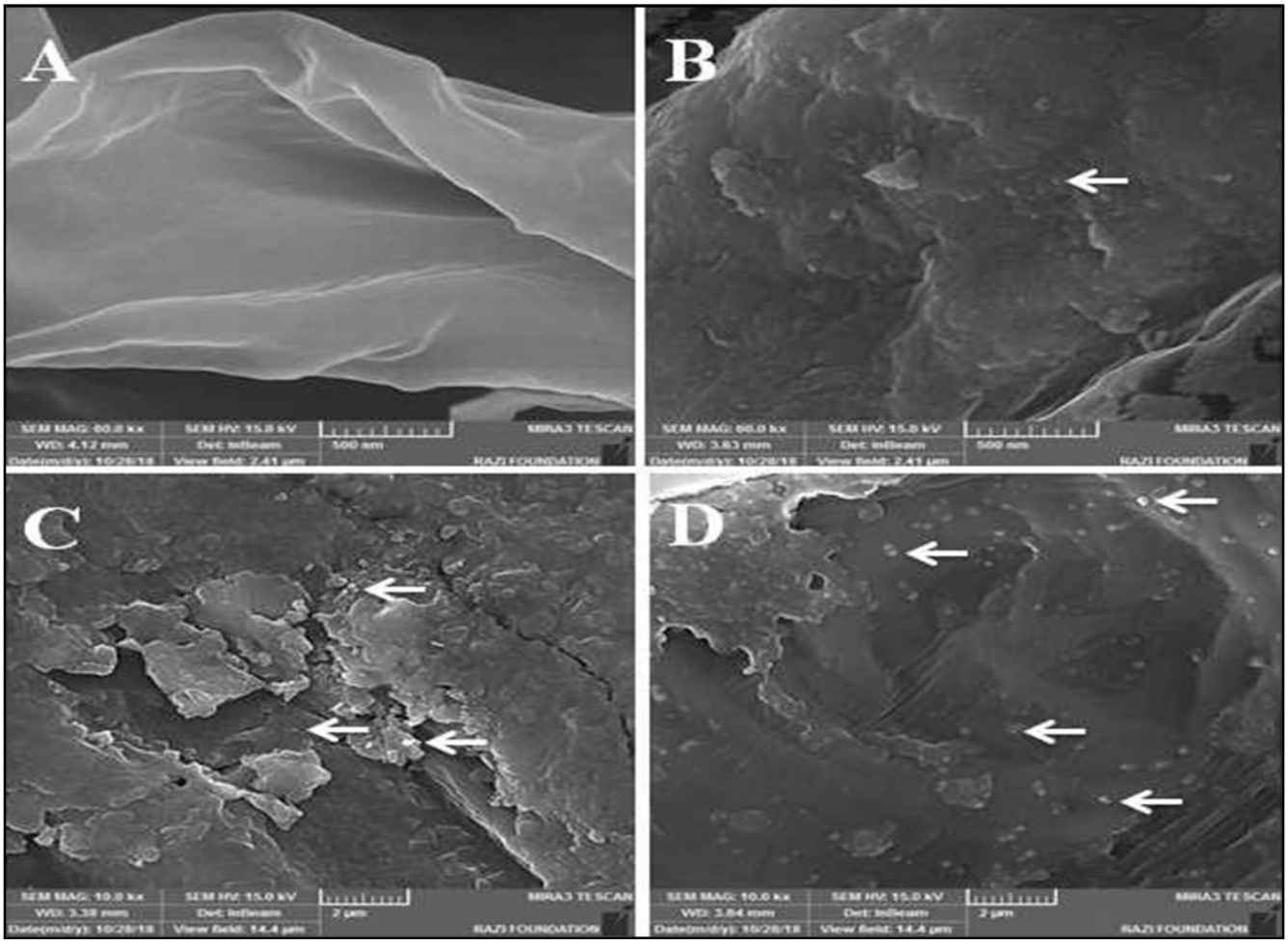

Fig. 3: SEM photography for different parts from seedlings of *Trigonella foenum-graecum* after 20 days.
A- Shoot grown on MS medium (control) B- Root grown on MS + 75 ¼g/ ml of CuO C- Stem grown on MS + 75 ¼g/ ml of CuO D- Leaf grown on MS + 75 ¼g/ ml of CuO (arrows referred to the CuO nanoparticles)

Effect the CuO NPS concentration on growth of the fenugreek plant seedlings

The results explained the important role of CuO NPS in stimulated and developed of fenugreek plant seedlings with identical stimulated both of the root and shoot system after 20 days (Fig. 1: A+B).

The data of the (Fig. 1: A+B) refers that the best average for root and shoot length of seedlings were grown on MS medium contained 75 µg/ml with reached to 3.5, 5 cm respectively after 20 days (Fig. 1:4 in A+B).

Then MS medium contain 100 µg/ml, where the average of length were 3.4, 4.8 cm respectively (Fig. 1:5 in A+B).Comparing with MS medium alone (control), where reached to 1.8, 3.2 cm respectively (Fig. 1:1 in A+B). MS medium contain 50, 150 µg/ml of CuO NPS inhibit the grown of seedlings after 20 days (Fig. 1:2 and 6 in A+B).

All of the pervious data associated with the appearance characteristics of seedlings which grown on MS medium supplemented with CuO NPS Fig. 2.

Production of the hairy root cultures

Hairy roots initiated on stems at inoculated sites.It began with the emergence of small structures that later developed into white-colored hair roots after 8 days, with It number and length increased with the time, the percent was 58.79%. Free- bacteria hairy roots cultures production when their hairy roots excised and cultured on solid MSO medium contain antibiotic Cefotaxime. This hairy roots cultures were negatively geotropism, branched and fast growing.

Improve the genetic transformation by Agropine test

The paper of electrophoresis showed the separated of black spot from the hairy roots samples corresponding in its site to the site of spot produced from standard Agropine with lacking of any spot separation from normal roots samples.

Effect the CuO NPS concentration on growth the hairy roots

The growing hairy roots on the MS medium supported by adding 75,100 µg/ml of CuO NPS were appearance clearly growth and increase of the roots branches and superior on the other concentrations in which the hairy roots showed weakness in its growth.

Detection for uptake the CuO NPS on the surface of different fenugreek plant tissues cells using Scanning Electron Microscope (SEM)

SEM examinations of different parts of seedlings grown on MS contain CuO NPS showed the ability of its cells for uptake those particles from the media where contained 75 µg/ml and accumulated on the surface, this explained in the Fig. 3. Data referred the superiority of leaves cells for accumulated CuO NPS on its cells surface (Fig. 3-D), more than root and stem cells. When the control samples were lacking from any particles on its surface (Fig. 3-A).

SEM examinations also showed the distinguish ability

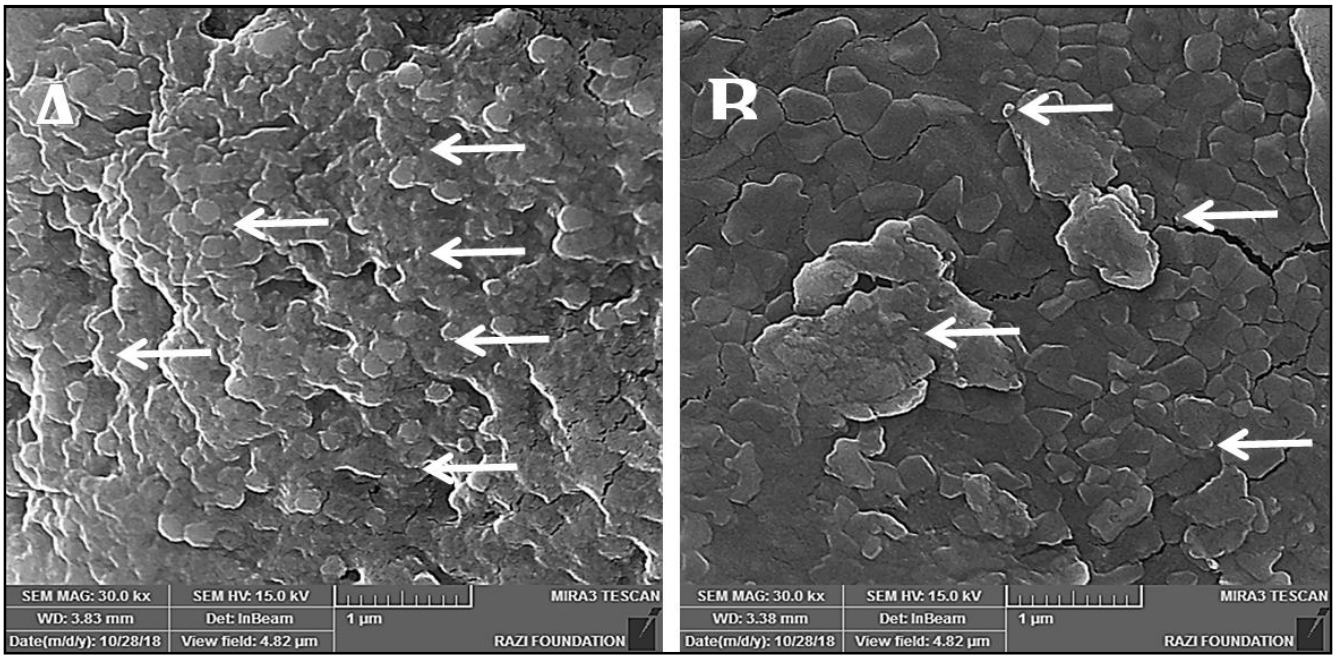

Fig. 4: SEM photography for hairy roots cells grown on the MS medium supplemented with CuO NPS concentration. A- MS + 75 ¼g/ ml of CuO B- MS + 100 ¼g/ml of CuO (arrows referred to the CuO nanoparticles)

of the hairy roots for uptake the copper nanoparticles at 75,100 µg /ml (Fig. 4-A, B) and accumulated it's on the surface of the hairy roots cells more than other seedlings tissues. With superiority the hairy roots at 75 µg /ml in accumulation (Fig. 4-A).

The Discussion

The high percent of germination and its speed of fenugreek (*Trigonella foenum-graecum*) plants seeds when grown on media containing CuO NPS at optimal concentration 75 µg/ml within superiority in length average of shoot and root of seedlings, may be belong to the role of nanoparticles in encourage the embryo activation and important enzymes for germination, addition to the enhanced uptake of water and nutrient. (Adhikari *et al.,* 2012; Hojjat and Hojjat, 2015; Liu *et al.,* 2016). The effect on the early stages of plant growth may be followed by similar enhancements at later stages as well (Yaseen and Wasan, 2015; Yahya and Mohammed, 2019), with the biological mineralization of copper and its activation as a growth catalyst as an electron transport carrier during the electron transport chain (Dhoke *et al.,* 2013).

The effect of nanoparticles copper II oxide in the growth of seedling with increasing the cell division explained the increasing of protein content, with found that some minerals, including Cu, Ni, Mn, Zn are secondary nutrients have an important role in the plants growth involved in many cellular functions such as protein production, Photosynthesis and metabolism of IAA (Sinhal, 2007; Yahya, 2019; Yhaya, 2020).

The induced of hairy roots by Ri plasmid of *Agrobacterium rhizogenes* R1601, due to success transferred of T-DNA genes from bacteria to the plant cell genome, conjugation and gene expression (Tzfira and Citovsky, 2008; Gelvin, 2009; Mohammed and Masyab, 2020).

Using SEM examinations, the presence of CuO NPS on the surfaces of fenugreek plant seedlings cells assure the ability of those cells for uptake and accumulation of this nanoparticles (Dhoke *et al.,* 2013). As in the ability of grass plants to polarize and biotransformation of nanoparticles of zinc, copper and iron oxides on the surfaces of vegetative cells associated with increased biomass (Sharma and Pathak, 2014; Al-Taee, 2014). Hairy roots provide a large surface area due to fast growth and highly branched nature and hence contact between the contaminants and tissue in comparison to naturally growing roots thus providing reliable and reproducible experimental system to study the pollutants and their response to toxic substances (Meagher, 2000; Suza *et al.,* 2008).

Conclusion

This study found that the ability of fenugreek (*Trigonella foenum-graecum*) tissues to uptake and accumulate nanoparticles, with superiority of hairy roots on other tissues, from above we can consider this tissues and its plant regeneration as a vital model for phytoremediation programs to clean the contaminated copper as a heavy metals. And the role of copper II oxide nanoparticles in enhanced seed germination, its speed and growth of seedlings.

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References

Adhikari, T., S. Kundu and A.K. Biswas (2012). Effect of copper oxide nanoparticle on seed germination of selected crops. *J. Agri. Sci. Technol.*, **2:** 815–823.

Al-Mahdawe, M.M., M.K. Al-Mallah and A.O. Al-Attrakchii (2013). Somatic embryogenesis and plant regeneration from cotyledonary node's calli of Trigonella *Foenum-graecum* L. *J. Biotech. Res. Center*., **7(3):** 29-35.

Al-Mallah, M.K., M.R. Davey and E.C. Cocking (1987). Enzymatic treatment of clover root hairs removes abarrier to *Rhizobium* host specificity. *Bio. Technology,* **5:** 1319-1322.

Al-Mallah, M.K. and A.A. Mohammed (2012). Transfer of Ri T-DNA genes of *Agrobacterium rhizogenes* R1601 via direct microinjection and Co_cultivation to carrot, *Daucus carota* L. tissue and production of transformed hairy root cultures. *Iraqi J. Biotech.,* **11:** 227-239.

Al-Taee, R.T.Y. (2014). Inclusion of nanoparticles of copper and zinc oxides in seedlings and callus of wild Prosopis plant. PhD thesis, College of Science, University of Mosul.

Bahari, Z., S. Sazegari, A. Niazi and A. Afsharifar (2020). The application of an Agrobacterium-mediated in planta transformation system in a *Catharanthus roseus* medicinal plant. *Czech J. Genetics and Plant Breeding, Czech J. Genet. Plant Breed.,* **56(1):** 34-41.

Carbonell-Barrachina, A., F.B. Carbonell and J.M. Beyeto (1994). Effect of arsenite on concentrations of micronutrients in tomato plants grown in hydroponic culture. *J. Plant Nutrit.,* **17:** 1887–1903.

Coniglio, M.S., V.D. Busto, P.S. González, M.I. Medina, S. Milrad and E. Agostini (2008). Application of *Brassica napus* hairy root cultures for phenol removal from aqueous solutions. *Chemosphere,* **72:** 1035–1042.

Dhoke, S.K., P. Mahajan, R. Kamble and A. Khanna (2013). Effect of nanoparticles suspension on the growth of mung (*Vignaradiata*) seedlings by foliar spray method. *Nanotech. Develop*., **3:** 1-5.

Gelvin, S.B. (2009). Agrobacterium in the genomics age. *Plant Physiol.,* **50:** 1665-1676.

Giri, M.A. and L. Narasu (2000). Transgenic hairy roots: recent trends and applications. *Biotech. Adv.,* **18:** 1–22.

Hatami, M. and M. Ghorbanpour (2014). Defense enzyme activities and biochemical variations of *Pelargonium zonale* in response to nanosilver application and dark storage. *Turk. J. Biol*., **38:** 130-139.

Hojjat, S.S. and H. Hojjat (2015). Effect of nano silver on seed germination and seedling growth in fenugreek seed. *Internat. J. Food Engineering*., **1(2):** 106-110.

Kannah, A.M.A., A.J.Z. Al-Hayani and M.I. Al-Jubouri (2019). The biological ability of *Chara* sp. algae to absorb nickel from the environment. *MJBMB.,* **2:** 12-16.

Kor, N.M., M.B. Didarshetaban and H.R.S. Pour (2013). Fenugreek (*Trigonella foenum-graecum* L.) As a valuable medicinal plant. *Internat. J. Advan. Biol. Biomed. Res.*, **1(8):** 922-931.

Liu, R., H. Zhang and R. Lal (2016). Effects of stabilized nanoparticles of copper, zinc, manganese and iron oxides in low concentrations on lettuce (*Lactuca sativa*) seed germination: nanotoxicants or nanonutrients. *Water, Air, Soil Pollut*., **227:** 42.

Madbouly, A.K. (2018). Nanoparticles as novel plant growth promoters. *Novel Res. Microbiol. J.,* **2(4):** 61-64.

Meagher, R.B. (2000). Phytoremediation of toxic elemental and organic pollutants.*Curr. Opin Plant Biol*., **3(2):** 153-62.

Mohammed, A.A. and H.M. Masyab (2020). Genetic transformation of *Nigella sativa* L. plants with *Agrobacterium rhizogenes* 35S *GUS* R1000 and estimation of Thymoquinone level in transformed hairy roots cultures. *Plant Archives,* **20(1):** 3649-3652.

Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiol. Plant*., **15:** 473-479.

Niazian, M., S.A.S. Sadat-Noori, P. Galuszka and S.M.M. Mortazavian (2017). Tissue culture-based Agrobacterium-mediated and in planta transformation methods. *Czech J. Genet. Plant Breed.,* **53:** 133-143.

Pickering, I.J., R.C. Prince, M.J. George, R.D. Smith, G.N. George and D.E. Salt (2000). Reduction and coordination of arsenic in Indian mustard. *Plant Physiol.,* **122:** 1171–1177.

Sharma, S. and H. Pathak (2014). Basic techniques of phytoremediation. *Internat. J. Sci. Engineering. Res*., **5(4):** 584-605.

Sinhal, V.K. (2007). Phytotoxic and cytogenetic effects of Zn^{2+} and Pb^{2+} in *Vicia faba*. *Poll. Res*., **26:** 417-420.

Soni, S. and S. Jain (2014). A review on phytoremediation of heavy metals from Soil by using plants to remove pollutants from the environment. *Internat. J. Advan. Res*., **2(8):** 197-203.

Suza, W., R.S. Harris and A. Lorence (2008). Hairy roots: from 3618 Pradip Hirapure *et al.,* high-value metabolite production to phytoremediation. *Electron. J. Integr. Biosci.,* **3(1):** 57–65.

Tepfer, D.A. and J. Tempe (1981). Production of di-agropine par des vaccines formess sous I, action *Agrobacterium rhizogenes*. Acad. Sci. Paris. Ser. III, 292:212-218.

Tzfira, T. and V. Citovsky (2008). Agrobacterium mediated genetic transformation of plants: biology and biotechnology. *Current Opinion in Biotechnology,* **17:** 147-154.

Ul Ain, N., I. Ul Haq, B.H. Abbasi, R. Javed and M. Zia (2018). Influence of PVP/PEG impregnated CuO NPS on physiological and biochemical characteristics of *Trigonella foenum-graecum* L. *The Institution of Engineering Technol*., **12(3):** 349-356.

Yahya, R.T. (2019). Effect of copper oxide nanoparticles in some biomolecules content of *Vicia faba* L. plants tissues. Eco. Env. and Cons. (S9-S13).

Yahya, R.T. (2020). Morphological and physiological response of *Lupinus albus* plants tissues for treatment to zinc oxide nanoparticle. *Plant Archives,* **20(1):** 3465-3468.

Yahya, R.T. and H.S. Al-Salih (2014). Uptake of Zinc Nanoparticles by *Prosopis farcta* L. Plants Callus Cultures. *Eng. Tech. J*., **32:** Part (B), (3), 616-623.

Yahya, R.T. and A.A. Mohammed (2019). Role of Zinc Oxide (ZnO) Nanoparticles in Germination and Growth of Chickpea (*Cicer arietinum* L.) Plant Seedlings Under Water Stress Effect. Published in 6th nternational Conference of Biotechnology, Environment and Engineering Sciences (ICBEI) 28-29 December 2019, Alexandria-Egypt.

Yaseen, A.A.M. and M.H. Wasan (2015). Response of sunflower (*Helianthus annuus* L.) to spraying of nano silver, organic fertilizer (Algastar) and salicylic acid and their impact on seeds content of fatty acids and vicine. *American J. Experiment. Agricult*., **9(1):** 1-12.