

GOLD NANOPARTICLES BIOSYNTHESIS USING *ZINGIBER OFFICINALE* AND THEIR IMPACT ON THE GROWTH AND CHEMICAL COMPOSITION OF LENTIL (*LENS CULINARIS* MEDIC.)

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Abstract

The synthesis of gold nanoparticles (AuNPs) using *Zingiber officinale* has been reported in the study, and the impact of the different concentrations (ppm) of gold nanoparticles is investigated. Five different concentrations (0, 10, 25, 50 and 100 ppm) of gold nanoparticles were applied to examine physiological (including seeds germination, plant height, leaf number and biomass production) and biochemical parameters (including total soluble sugars, total soluble phenols, total chlorophyll, carotenoids, total soluble amino acids and protein contents) of the seedlings of *Lens culinaris* as a model of Legumes crop. GNPs were synthesized and characterized through Uv-visible spectra, transmission electron microscopy, XRD and FTIR. Generally, all growth and biochemical parameters were increased as a result of treatment with gold nanoparticles by using low concentrations only at 5ppm and 10ppm, while at high concentrations the effect is reversed.

Key words: Gold nanoparticles, Physiological, Biochemical parameters, *Lens culinaris*.

Introduction

Lentils (*Lens culinaris*) belong to legume family. Lentils have up to 60 g/100g carbohydrates, 28.7-31.5% proteins, which is considerable among legumes. Lentils have high amount of phytochemical compounds like Polyphenols. Polyphenols are secondary metabolites that have major role in tissue protection against free radicals; therefore lentils may drop the rates of cancer, diabetes, Parkinson, heart failure and Alzheimer (National Nutrient Database, 2013; Maninder *et al.*, 2007; Anderson *et al.*, 2002). Utilizing plant extracts for gold nanoparticles biosynthesis has gained importance in recent years due to the enhancement of chemical, biological, and physical properties of these particles (Daniel & Astruc, 2004). Metal nanoparticles are very important due to their unusual size and shape dependant properties (Kamat, 2002) and are found to have potential applications in biological and chemical sensing of single molecules (Giljohann *et al.*, 2010), controlled release of biologically relevant molecules (Ghosh *et al.*, 2008), catalysis (Lewis 1993), immunoassays (Lee *et al.*, 2004), medicine (Cole *et al.*, 2015), cosmetology (Saha *et al.*, 2011), biology (Murphy *et al.*, 2008), and pharmacology (Hainfeld *et al.*, 2008). Recently, biosynthesis of nanoparticles by plant is gaining importance due to its simplicity and eco-friendliness. Biosynthesis of gold nanoparticles by plants such as lemongrass (Shankar *et al.*, 2004), *Aloe vera* (Chandran *et al.*, 2006), alfalfa (Gardea-Torresdey *et al.*, 2002), neem (Shankar *et al.*, 2004), tamarind (Ankamwar *et al.*, 2005), *Cinnamomum camphora* (Huang *et al.*, 2007), Emblic (Ankamwar *et al.*, 2005), *Mangifera indica* leaf (Philip, 2010 a), *Hibiscus rosasinensis* (Philip, 2010 b), *Murraya koenigii* leaf (Philip *et al.*, 2011) *Ocimum sanctum* (Philip & Unni, 2011) and *Psidium guajava* (Praba *et al.*, 2016) have been reported. However, ionic forms of gold have been shown to have cytotoxicity on various cell types and adverse effects on red blood cells (Garner *et al.*, 1994). Also it has been reported that the synthesized gold nanoparticles conventionally i.e. citrate lead to

capped gold nanoparticles aggregated in physiological conditions hindering its in vivo applications (Joanne *et al.*, 2011; Nam *et al.*, 2009). Therefore, an attempt has been made for green synthesis of gold nanoparticles by chemical reduction technique using *Zingiber officinale* extract which can act both as reducing and stabilizing agent. *Z. officinale* belonging to the family Zingiberaceae is a common constituent of diet worldwide and considered as a more potent anti-platelet agent than aspirin (Nurtjahja-Tjendraputra *et al.*, 2003), with anti-inflammatory and analgesic properties similar to nonsteroidal anti-inflammatory drugs, without the side effects of gastrointestinal bleeding and ulcer formation (Black *et al.*, 2010). Therefore, gold nanoparticles synthesized with *Z. officinale* extract are stable could be highly beneficial for drug delivery, gene delivery and biosensor applications where there is a direct contact of these nanoparticles with blood.

Materials and Methods

AuNPs synthesis and characterization: The green synthesis of AuNPs was synthesized by *Zingiber officinale* and characterized by UV-Visible Spectroscopy (Victoria, Australia) in the wavelength range of 200 to 1000 nm and transmission Electron Microscopic (TEM, JEM 1400) was viewed at 100 kV. The size distribution of the nanoparticles was determined using the ImageJ 1.45s software and the data were plotted as histograms. Scanning electron microscopy (SEM) was performed using a QUANTA 250 instrument equipped with an energy dispersive X-ray spectroscope (EDS). Thin films of the sample were prepared by placing a few drops of the solution on a carbon coated copper grid and drying it under a mercury lamp for 5 min. The thin films thus formed were examined using the SEM. EDS analysis was performed for analyzing the elemental composition of gold nanoparticles. Differential light scattering (DLS) was conducted on a ZEN 3600 HT Laser Malvern Zetasizer Nano series instrument (Malvern Instruments, UK) to measure the zeta potential. The changes in chemical bonds and composition were determined using

(FTIR) Fourier transform infrared spectroscopy model Nicolet 6700 spectrometer with resolution of 4 cm⁻¹ (Thermo Electron Corporation, USA). Measurements were performed in the range of 400–4000 cm⁻¹.

Germination and growth parameters: All seeds of Lentils (*Lens culinaris*) were collected from Al- Riyadh market and kept in the dark at 4C until use. For each individual treatment ten seeds were soaked for 1 h in 5 mL of either 5, 10 or 25, 50, 100 ppm of gold-nanoparticles, and a deionized water as control without surface sterilization (Yin *et al.*, 2012). One piece of filter paper was put into each petri dish, and 4 ml of the appropriate treatment test solution was added. Ten seeds were then transferred onto the filter paper, then Petri dishes were covered; three replicates of each treatment were intended. Seed was considered to have germinated when the radicle or plumule emerged from the seed coat and seeds germination rate (GR) was calculated as the proportion of the total seeds that germinated. We transferred total seeds that germinated onto the pots then biomass, leaves number and plant height were measured after 15 days of incubation according to (Yin *et al.*, 2012):

(1) Treatment effects on germination (G)
(GT – GC)/GC

whereas (GT) is germination treatment and (GC) germination control

(2) Treatment effects on biomass production (B)
(BT – BC)/BC

whereas (BT) is biomass production treatment and (BC) biomass production control

(3) Treatment effects on leaves number (L)
(LT – LC)/LC

whereas (LT) is leaves number treatment and (LC) leaves number control

(4) Treatment effects on plant height (H)
(HT – HC)/HC

whereas (HT) is plant height treatment and (HC) plant height control.

Chemical analysis

Pigment contents: The photosynthetic pigments e.g., Chlorophyll (a,b) and Carotenoid have been extracted in 5 ml of acetone 80% by grinding treatedleaves using pestle and mortar. Thehomogenate wascentrifuged at 3000 g for 10 minat 4°C. The absorbance of the resultingsupernatant was taken at 480, 645 and 663 nm.Different pigments were estimated using thefollowing formula by (Barnes *et al.*, 1992):

Chl a (mg/l) = 12.7 (A663) – 2.69 (A645)

Chl b (mg/l) = 22.9 (A645) – 4.68 (A663)

Car (mg/l) = 1000 (A480) – 1.8Chl a - 85.02Chl b/198

Total soluble sugars: Total soluble sugars were measured by using phenol-sulfuric acid assay (Dubois *et al.*, 1956). The absorption was then determined by

spectrophotometry at 490 nm.

Total soluble phenols: Total soluble phenols were estimated by using Folin-Ciocaltue colorimetric methods by spectrophotometry at 530 nm (Snell & Snell, 1953).

Protein contents: Protein was determined according to Jackson (Jackson, 1973).

Total soluble amino acids: Total soluble amino acids were estimated by using the nine-hydrate color metric method, as described by Rosen (Rosen, 1957) and modified by (Seliem *et al.*, 1978).

Results and Discussion

Characterization of synthesized nanoparticles: The presence of AuNPs in the solution is confirmed by UV-Vis spectrophotometry, as shown in Fig. (1). The results show that a sharp peak appears at 533 nm, which indicates the formation of AuNPs. The obvious color change occurred within 24 h (Nath *et al.*, 2013;Coman *et al.*, 2014;Elavazhagan *et al.*, 2011).The morphology and particle size of the synthesized AuNPs have been investigated using TEM, and the results are presented in Fig. (2a). There is no aggregation observed in the spherical, tiny nanoparticles (5–10 nm) can be observed in Fig. (2b). The narrow size of the nanoparticles synthesized using *Z. officinale* is due to the presence of a large number of nucleation sites for AuCl₄ complexation. Furthermore, *Z. officinale* components can effectively prevent the aggregation of the nanoparticles. This observation is in agreement with earlier reports (Geraldes *et al.*, 2016), in which gold nanoparticles were synthesized using green tea, zimbro tea, and green coconut water. Also, AuNPs were synthesized using the flower extract of *Plumeria alba* (Mata *et al.*, 2016). The SEM images of the synthesized gold nanoparticles are presented in Fig. 3(a). It can be clearly noticed that at room temperature, the synthesized nanoparticles have a small size and are spherical in shape. The EDS spectrum (Fig. 3b) shows strong signals for gold nanoparticles in two different places of the sample, thus confirming their presence. Weak signals corresponding to other elements are also present in the spectrum. These can be due to the proteins, enzymes, and salts present in the plant extract (Gurunathan *et al.*, 2014). X-ray diffraction was used to confirm the crystalline nature of the particles. Fig. 4 shows a representative XRD pattern of the gold nanoparticles synthesized by the *Z. officinale* extract after the complete reduction of Au³⁺ to Au⁰. The diffraction peaks are 39 (111), 44.87 (200), 65.2 (220) and 77.13 (311) obtained are identical with those reported for the standard gold metal (Au⁰) (Joint Committee on Powder Diffraction Standards-JCPDS, USA) Thus, the XRD pattern suggests that the gold nanoparticles were essentially crystalline. Fig. 5 shows the FTIR spectrum of *Z. officinale*extract caped gold nanoparticles. The presence of band at 3478.18 cm⁻¹ is due to phenolic –OH stretching. The band at 1662.52 cm⁻¹ is due to C=O stretching Absorption was observed at at 1587.25 (stretching vibration of C=C), 1180.25, 1043.05 (stretching vibrations C=O), peaks at 860.18 cm⁻¹ and 725.49 are strong indications of heterocyclic compounds such as alkanoids, flavonoids and alkaloids, the active components of *Z. officinale*, which acts as the capping

agent. Zeta potential values are used as a hallmark indication of the stability of colloidal particles. Nanoparticles are considered to exist as stable colloids if their zeta potential is more than 25 mV or less than -25 mV (Lee *et al.*, 2016). The zeta potential of the AuNPs was -37 ± 0.2 mV (Fig. 6); the suspension of AuNPs in a buffer formed a stable colloid (well-dispersed) with no visible aggregation over 6 months.

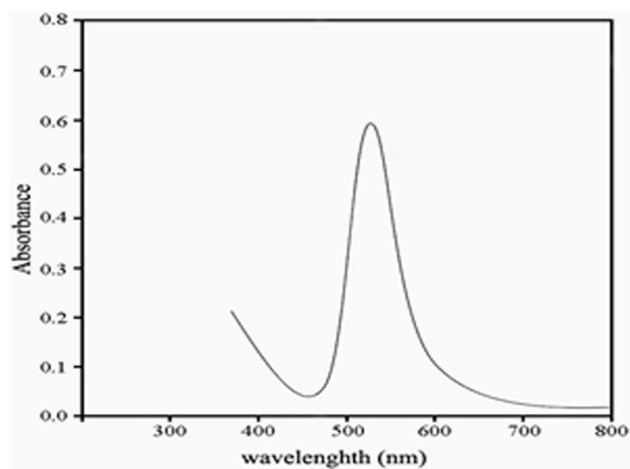


Fig. 1. Absorption spectra of gold nanoparticles prepared with *Z. officinale* extract.

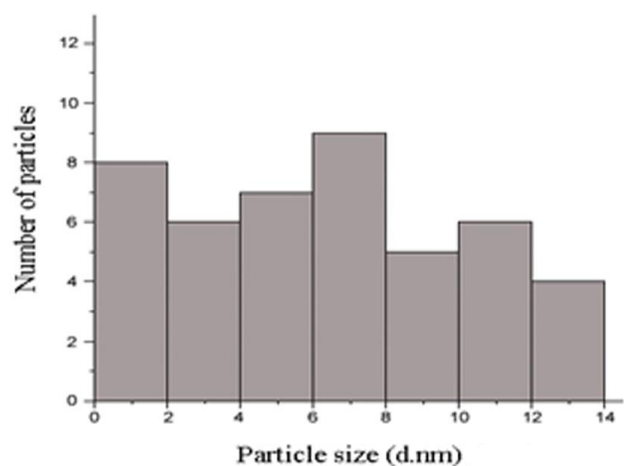
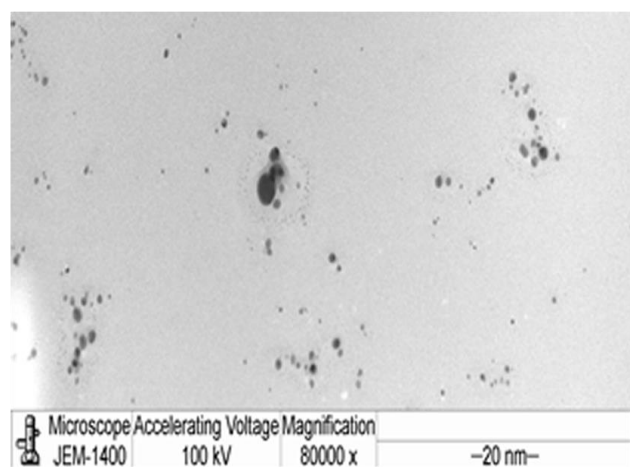


Fig. 2. TEM image of the Au-NPs forms using *Z. officinale* extract (A) and the histogram of the distribution of the particles size of Au-NPs (B).

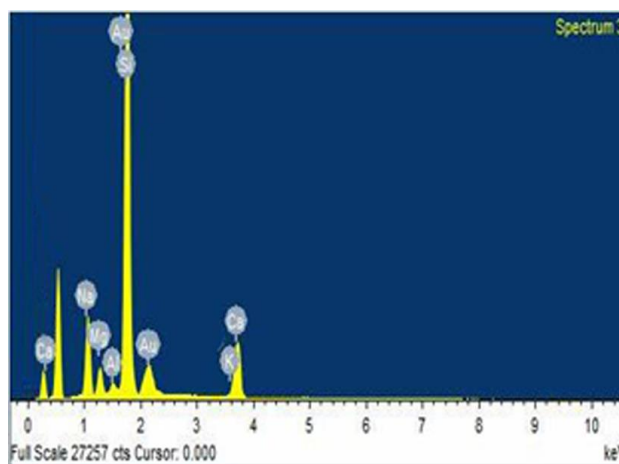
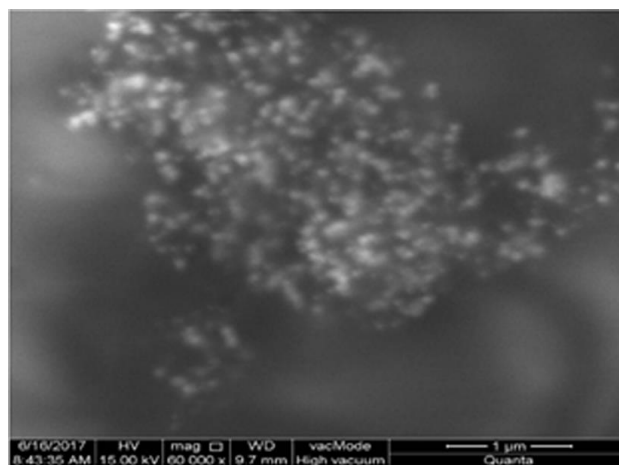


Fig. 3. SEM images of Au-NPs (A) and EDS spectrum of Au-NPs synthesized using *Z. officinale* (B).

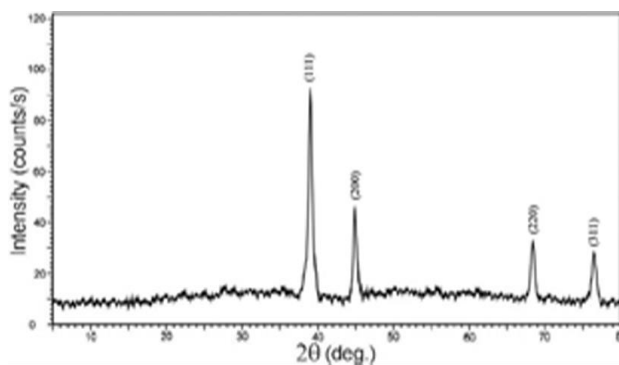


Fig. 4. XRD Pattern of Au-NPs synthesized using *Z. officinale*.

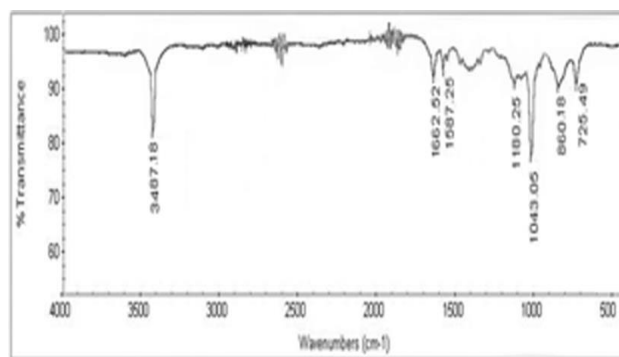


Fig. 5. FTIR spectra of Au-NPs synthesized using *Z. officinale*

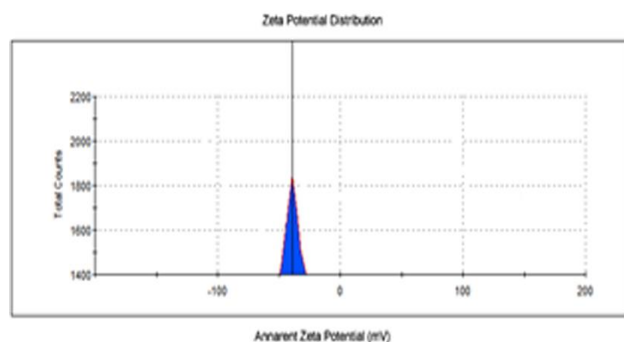


Fig. 6. Zeta potential image of Au-NPs synthesized using *Z. officinale*.

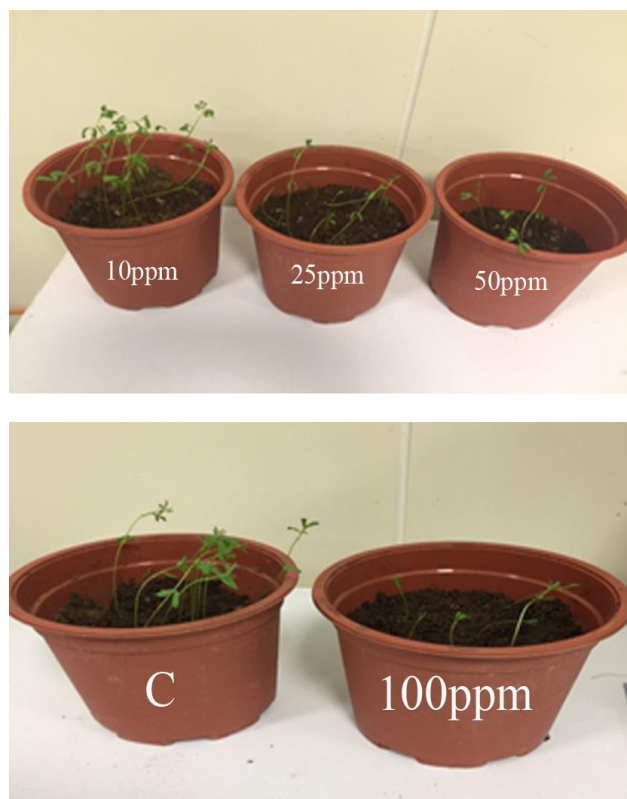


Fig. 7 (a) Effect of gold-nanoparticle treatment at 10, 25, 50 ppm on growth parameters, (b) Effect of gold-nanoparticle treatment at 100 ppm on growth parameters, (C) is control treatment.

Effect of Gold-nanoparticle treatment on germination and growth parameters:

Effect of AuNPs on seeds germination, plant height, leaves number and biomass production in the seedlings of *L. culinaris* were investigated under laboratory conditions showed in Table (1) and Figs. (7a & 7b). Germination responses differed between concentrations AuNP, exposure to 5 ppm AuNP had no effects when check with control while at 10 ppm no significant difference was recorded. Inhibition effects in seeds germination were recorded at 25, 50 100 ppm AuNP concentration. Generally, the germination rate responded inhibition to the highest dose (25, 50 and 100 ppm) led to 26.7%, 53.3% and 66.7% of control, respectively. Gold nanoparticle improvement in growth of *L. culinaris* seedlings was evident only at 10 ppm in plant height (23.23 cm), leaves number (17.67) and biomass

production (8.20 gm.) as compared to control treatment (17.63cm., 14 and 6.30gm.), respectively. Whereas this growth parameters responded negatively to the highest dose (25, 50 and 100 ppm) with difference significant.

The absorption of nanoparticles are differ from species to another (Ma *et al.*, 2010, Rico *et al.*, 2011). Zhu *et al.*, 2012 suggested that the absorption and distribution of nanoparticles depends on the surface charge of nanoparticles and plant species. Many researchers have shown that gold can accumulate, to varying degrees, by different plant species (Wilson Coral *et al.*, 2012). For example, exposure to particle of gold nanoparticles lead to improve seed germination in cucumber and lettuce (Asli & Neumann, 2009), *B. juncea* (Arora *et al.*, 2012) and *Gloriosa superba* (Gopinath *et al.*, 2014), Plant growth improvement is due to an increase in free radical pressure and increasing in antioxidant enzymes (Gongan *et al.*, 2014). It was observed that the treatment of the root with 10 ppm KAuCl₄ triggered a significant increase in length, but at higher concentrations a significant decrease was occurred (Poschenrieder *et al.*, 2013). Treatment with gold-nanoparticle motive decreasing in time required for seeds germination; this could be due to the increased permeability, facilitating the insertion of water and dioxygen into the cells, which expedite the metabolism and germination process (Zheng *et al.*, 2005). The number of leaves per plant were increased with nanoparticles treatment, cause regulated by a complex interaction of various genes whose expression is modify by growth hormones (Gonzalez *et al.*, 2010). (Seif *et al.*, 2011) increase in leaves number have been observed by inhibition of ethylene motion reduces the occasion of abscission. Consequently, gold-nanoparticles adverse could slow down the impact of ethylene, ensuing in an increase in leaf range of Brassica (Arora *et al.*, 2012). Gold-nanoparticle have been lead to improve in growth parameters of *Brassica* seedlings was obvious when the concentration was increased in plant height (from 8 to 9%), stem diameter and wide variety of branches according to plant. growing of gibberellic acid degree might be liable for shoot elongation (Stepanova *et al.*, 2007). The gold-nanoparticle treatment interferes with action of endogenous plant hormones, and induces changes in growth profile of the seedlings. Also it is possible that low dose turns on the functioning of hormones (Barrena *et al.*, 2009) whereas large doses negatively affects on plant growth and biomass production, probably adsorption of gold nanoparticles onto the cellular wall surfaces of primary root device cause decrease on pore length and inhibiting in water delivery potential, which purpose ultimately in lowering of increase parameters (Asli & Neumann, 2009; Feichtmeier *et al.*, 2015).

Actually, gold is an element required by way of plants as a hint; but, the absorption or insertion may additionally often display a few change in plant growth. Taylor and coworkers (Taylor *et al.*, 2014) have stated the effect of gold nanoparticles on physiological and genetic responses of *Arabidopsis thaliana* that its root length was reduced by 75 % at 100 mg L⁻¹ concentration. AuNPs set off toxicity in lettuce plants by inhibiting aquaporin function, which help within the transportation of huge variety of molecules (Shah & Belozerova, 2009).

Table 1. Effect of Gold-nanoparticle at 0, 10, 25, 50 and 100 ppm on germination rate, plant height, leaf number and Biomass production.

Gold nanoparticles conc. (ppm)	Germination (% inhibition)	standard error (SE)
0.0	0.0 ^a	± 3.00
5	0.0 ^a	± 2.84
10	0.5 ^a	± 2.71
25	26.7 ^b	± 1.71
50	53.3 ^c	± 1.13
100	66.7 ^d	± 1.19
LDS at 5%		0.726
Plant height (cm.)		
0.0	17.63 ^b	± 5.46
5	17.90 ^b	± 5.52
10	23.23 ^a	± 7.17
25	15.10 ^c	± 4.16
50	12.90 ^d	± 3.35
100	10.77 ^e	± 2.73
LDS at 5%		1.027
Leave number		
0.0	14.00 ^{bc}	± 4.33
5	14.33 ^{bc}	± 4.40
10	17.67 ^a	± 5.30
25	13.33 ^c	± 3.63
50	10.33 ^d	± 2.57
100	8.00 ^e	± 1.91
LDS at 5%		0.839
Biomass production (gm.)		
0.0	6.30 ^b	± 1.73
5	6.70 ^b	± 1.80
10	8.20 ^a	± 2.07
25	5.57 ^c	± 1.21
50	3.80 ^d	± 0.97
100	2.77 ^e	± 1.22
LDS at 5%		0.496

Effect of Gold-nanoparticle treatment on biochemical parameters: Data presented in Table (2) show that, the consequences suggest that AuNP nanoparticles cause growth the biochemical contents together with total soluble sugars, overall chlorophyll, carotenoids, general soluble amino acids and protein contents. A maximum increases were recorded in seedlings treated with 10 ppm AuNP 5.23, 2.66, 0.89, 3.25 and 3.83 mg/g f.wt respectively as compared with 2.94, 2.11, 0.74, 2.63 and 2.11 mg/g f.wt respectively for control treatment. In case of total phenols, the highest values were 3.74 mg/g f.wt at 100ppm followed by 2.96 mg/g f. wt at 50ppm cause raise in phenolic compound as a reaction to resistance high concentrations. The increasing in general chlorophyll contents leads to an increase inside the total photosynthate produced (Urbonaviciute *et al.*, 2006). It's been mentioned that nanoparticle dose should set off higher chlorophyll contents in *Asparagus* and *Sorghum* (An *et al.*, 2008; Namasivayam & Chitrakala, 2011). Purvis (1980) has said that higher ethylene reason more activity of chlorophyllase enzyme and destruction of internal chloroplast membranes. The implied inhibition of ethylene action through gold-nanoparticles is accountable for higher chlorophyll contents inside the handling seedlings. Absorbance by using chlorophyll molecules and for this reason accelerated the photochemical

response. This consequences in higher availability of lowering power (NADPH₂ and strength (ATP) to carry out CO₂ fixation (Krause *et al.*, 1998), the improved CO₂ fixation is evident by a 43% increase in the total sugar contents in the treated seedlings. Moaveni *et al.*, 2011, who reported that, TiO₂ nanoparticles could gain the amount of pigments and ease photosynthesis matter transportation by recovery in chlorophyll structure and light sorption in Barley. The contents of chlorophyll-a, chlorophyll-b, carotenoids total amino acids and total sugars were significantly increased with foliar application with varying TiO₂ nanoparticles concentrations (2, 4 and 6 mg/L) on coriander (Khater, 2015).

Antioxidant enzyme activity of plant reasonably increased in root and shoot which indicates that the Fe₃O₄ nanoparticles are not toxic to wheat plant under the given experimental conditions. Thus, plants protect cellular and sub-cellular system from the cytotoxic effects of active oxygen radicals with antioxidative enzymes (superoxide dismutase, SOD; catalase, CAT; peroxidase, POD and ascorbate peroxidase) and low molecular weight antioxidants (ascorbate, glutathione, proline, carotenoids, a-tocopherols and phenolics (Ozyigit *et al.*, 2016). Some researchers have reported that the gold nanoparticles inhibit plant growth (Binder *et al.*, 2007; Rodriguez *et al.*, 2007; Shah & Belozerovala, 2009; Taylor *et al.*, 2014).

Table 2. Effect of gold nanoparticle treatment at 0, 10, 25, 50 and 100 ppm. on chemical analysis.

Gold nanoparticles conc. (ppm)	Total soluble sugars mg/g f. wt	Total soluble phenols mg/g f. wt	Total Chlorophyll mg/g f. wt	Carotenoids mg/g f. wt	Total soluble amino acids mg/g f. wt	Protein contents mg/g f. wt
0.0	2.94	1.45	2.11	0.74	2.63	2.11
5	3.28	1.51	2.35	0.79	2.98	3.62
10	5.2	1.84	2.66	0.89	3.25	3.83
25	3.16	2.77	2.03	0.27	1.88	2.44
50	2.04	2.96	1.32	0.22	1.51	1.54
100	1.13	3.74	1.11	0.18	1.22	0.98

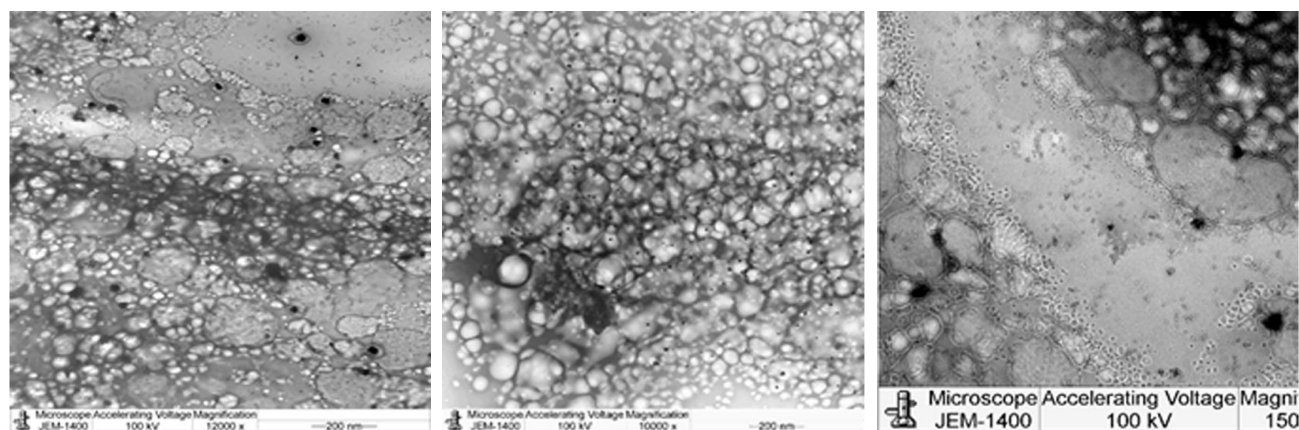


Fig. 8. TEM images of bio-accumulation of gold nanoparticles in roots at 25ppm (A), in roots at 100ppm (B), in leaves at 100ppm (C).

Intercellular accumulation of gold nanoparticles: The transmission electron microscopy (TEM) image of the plant treated with HAuCl_4 shows a deposit of gold. In this study, bio-accumulation of gold nanoparticles was found more in the roots than in the leaves because the roots directly contacted gold nanoparticles in the treatment solution. Thus, accumulation were increased with increasing concentration of applied AuNPs (Figs. 8A, 8B & 8C), highest cellular AuNP concentration was recorded at 100 ppm. Increase in intracellular CuO nanoparticle concentration, with increasing CuO nanoparticle exposure has been reported by (Zhou *et al.*, 2011). Brassica is known to be a metal hyperaccumulator with significant metal accumulation being reported for Zn (Rossi *et al.*, 2002) and nano-gold (Anderson *et al.*, 1998). These accumulated of gold-nanoparticles move through the vasculature of the plant (Sabo *et al.*, 2011) and translocations in different parts of a plant are not uniform.

Acknowledgement

This research project was supported by a grant from the Research Center of the Female Scientific and Medical Colleges, Deanship of Scientific Research, King Saud University.

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(Received for publication 14 March 2018)