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Environmentally friendly nano-selenium to improve antioxidant system and growth of groundnut cultivars under sandy soil conditions

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ABSTRACT

Selenium nanoparticles (SeNPs) as environmental friendly agent were applied for induces enhancement in the growth of three different groundnut (*Arachis hypogaea* L.) cultivars (NC, Gregory and Giza 6). The tested cultivars are different at biochemical and physiological levels. Foliar application of SeNPs (0, 20 and 40 ppm) was applied during vegetative stage. Foliar application of SeNPs (0, 20 and 40 ppm) was applied during vegetative stage. Effect of SeNPs on growth depends upon the used concentration of selenium nanoparticles and groundnut cultivar. Application of SeNPs improved growth of Gerogory cv. and Giza 6 cv., respectively, while the growth parameters of NC cv. were relatively negative affected by SeNPs treatments. The effects of SeNPs on growth of groundnut cultivars were linked with physiological and biochemical plant properties *i.e.* the changes in photosynthetic pigments, lipid peroxidation, antioxidants enzymes (catalase, peroxidase and ascorbic acid peroxidase), total phenols, total flavonoids and total soluble sugars. Generally, nano selenium act as stimulator and/or stressor enhanced the antioxidant defense systems in tested groundnut cultivars leads to the improvement of plant tolerance under sandy soil conditions.

1. Introduction

Nanotechnology has gain considerable attention in agriculture because of their high rate of absorption and penetration in plants. Moreover, extremely small size, structure and surface characteristics of nanoparticles result in unique physicochemical properties. In this way, nanoparticles of selenium had grown more attention due to its low toxicity, high bioavailability and strong ability to scavenge free radicals (Zhai et al., 2017). Selenium (Se) at low concentration is an essential element, at least 25 human selenopolypepties and enzymes-containing selenocysteine for human and animal body. In the environment, it exists as ionic selenite (Na_2SeO_3) and selenate, solid-state Se, selenocysteine and selenomethionine (Biswas et al., 2011; Bo Li et al., 2014). Selenium nanoparticles (SeNPs) can be synthesized through biological, physical or chemical methods. Chemical synthesis is mediated by precipitation, acid decomposition, and catalytic reduction using ascorbic acid, glucose, sulfur dioxide, and sodium dodecyl sulphate (Zhang et al., 2010; Dwivedi et al., 2011). In agriculture, different studies have shown that nanoparticles influence a plant's growth and development. There are many strategies of Se application in agriculture field, such as addition of Se to soil, soaking seeds in Se solution before sowing, hydroponic and aeroponic cultivation in a nutrient solution containing Se, and foliar application of plants with Se solution (El-Batal et al., 2016). Controlling of beneficial and economical crops pathogens are considered the wide world applicable field of using nanotechnology; from it, Elshahawy et al. (2018) applied the biogenic synthesized silver nanoparticles for controlling of Egyptian tomato pathogenic fungi. Producing of antimicrobial agents to control the pathogenic microorganisms especially appeared in agriculture field (Ali et al., 2016; Sultan et al., 2016; Darwesh et al., 2018). In order to improve the plant productivity, Arora et al. (2012), studied the effect of nanoparticles on *Brassica juncea* plants and Pallavi et al. (2016) studied on *Vigna sinensis* and *Brassica juncea*. Nanoparticles can regulate plant growth by modulating reactive oxygen species (ROS) dependent signaling pathway(s), which is dependent upon its pro-oxidant as well as antioxidant activities. Nanoparticles induced change in ROS levels in plants chloroplasts of might be associated with alteration in chloroplast functioning (Mittler, 2017). On the other side, nanoparticles improves the activity of antioxidant

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enzymes (López-Vargas et al., 2018), thus optimizations of doses and modes of nanoparticles applications are required, before its applications on crop plants.

Groundnut (*Arachis hypogaea* L.) as one of fundamental edible oil seed crops in the world, seed contains amounts of protein, lipid, carbohydrate and minerals (FAO, 2012). It has been gained more attention in Egypt and cultivated in the newly reclaimed sandy soil which faces many challenges as shortage of soil fertility, water resources limitation and increase of salinity. Groundnut growth is affected by many factors such as soil fertilities, varieties, water supply and plant population. Varieties of Egyptian groundnut differed in growth habit where some of them are erect and others are semi-spreading, so their root system may differ in volume and abilities to absorb nutrients. Recently this crop has been given great attention from Government as well as from the scientific institutes due to its suitability to cultivate in the new reclaimed sandy soils in Egypt, which located in Nubaria, Ismailia, Minia, Sharkia and Giza as well as Al-Tahady Sector, South Tahrir Province, Beheira Governorate. The sandy-textured soils were represented drought conditions on groundnut growth. The desert area represented by 96% from Egypt area, and they are extremely limited in plant nutrients and organic matter (El-Saady et al., 2014). By using recent irrigation system and application of growth regulators, these soils are proving to be most economically feasible soils for groundnut cultivation.

The current research aimed to study the effect of selenium nanoparticles (SeNPs) on improving plant tolerance of three different groundnut (*Arachis hypogaea* L.) cultivars (NC, Gregory and Giza 6) cultivated in sandy soil through enhancement of the growth and antioxidant status to overcome many challenges such as shortage of soil fertility, water resources limitation and increase of salinity.

2. Materials and methods

2.1. Selenium nanoparticles preparation

Stock solutions of 100 mM sodium selenite and 50 mM ascorbic acid were prepared in distilled H_2O. A ratio of 1:4 sodium selenite to ascorbic acid was reacted from the stock solutions. The ascorbic acid was mixed drop wise with the sodium selenite solution under magnetic stirring (600 rpm) at room temperature for 30 min. The mixtures were allowed to react till the colour changed from colorless to light orange. The morphology of the reductive SeNPs was spherical and size of 10–30 nm by High Resolution Transmission Electron Microscopy (HRTEM) JEOL (JEM-2100 TEM) (the picture not presented in this study). The produced SeNPs were applied to study their impacts on 3 different groundnut cultivars (NC, Gregory and Giza 6). After 30 days after sown, the plants were foliar sprayed with selenium nanoparticles at concentrations of 20 and 40 mg/L in addition to control (without treating).

2.2. The experiment preparation

A pot experiment was conducted during 2017, summer season, in the greenhouse of National Research Centre, Giza, Egypt. Seeds of groundnut cultivars (NC, Gregory and Giza 6) were sown in plastic pots (40 cm diameter and 40 cm depth) filled by sandy soil and arranged in factorial experiment in complete randomize design with 5 replicates. The soil texture is sandy, field capacity (16.6%), pH 8.2, EC (1.7 mmhos/cm), Cl^- 0.57, HCO_3^- 1.3, Na^+ 0.99, K^+ 0.30, Ca^{2+} 0.70 and Mg^{2+} 0.40. Phosphorus and potassium fertilizers were applied before sowing at a rate of 6.0 and 3.0 g/pot of calcium super phosphate (15.5% P_2O_5) and potassium sulphate (48–50% K_2O), respectively. Nitrogen fertilizer was applied as two equal portions at a rate of 0.60 g/pot for each in the form of ammonium nitrate (33.5% N) at 30 and 60 days after planting.

2.3. Growth parameters measurements

A 45 days after planting, a representative sample was taken from each treatment for recording some growth parameters: shoot height (cm), root length, fresh and dry weights of shoots/plant and fresh and dry weights of roots/plant.

2.4. Biochemical constituents

2.4.1. Photosynthetic pigments measurement

The photosynthetic pigments (chlorophyll “a”, chlorophyll “b” and carotenoids) were colorimetrically estimated according to Metzner et al. (1965). In brief, a known fresh weight of leaves was homogenized in 85% aqueous acetone for 5 min. The homogenate was centrifuged at 6000 rpm and the supernatant was made up to a volume of 25 ml with 85% acetone. The extract was measured against a blank of pure 85% aqueous acetone at two wavelengths 663 and 644 nm, using spectrophotometer. In case of carotenoids, the extract was measured at 452 nm. The concentration of the pigment fractions (chlorophyll “a”, chlorophyll “b” and carotenoids) were measured as µg/ml using the following equations:

$$\text{Chlorophyll a} = 10.3\ E_{663} - 0.918\ E_{644} = \mu g/ml$$

$$\text{Chlorophyll b} = 19.7\ E_{644} - 3.870\ E_{663} = \mu g/ml$$

$$\text{Carotenoids} = 4.2\ E_{452} - (0.0264\ \text{Chlorophyll a} + 0.426\ \text{Chlorophyll b}) = \mu g/ml$$

The concentrations of chlorophylls and carotenoids were represented by mg/g fresh weight.

2.4.2. Total soluble sugars measurement

A known weight of the sample was extracted by submersion of dry tissue in 10 ml of 80% (v/v) ethanol at 25 °C with shaking overnight, then filtered through a glass funnel. The residue was re-extracted by 80% ethanol and filtered again, and then the filtrate was made up to a known volume. Total soluble sugars were determined colorimetrically by the phenol-sulphuric acid as described by Dubois et al. (1951) as follows: a known volume (1 ml) of sugar extract was quantitatively transferred into a test tube and 1 ml of 5% aqueous phenol solution was added followed by 5 ml of concentrated sulphuric acid, mixed thoroughly and the tubes were left to cool at room temperature. Measurements of the intensity of the yellow-orange colour were carried out by using spectrophotometer at 490 nm. A standard curve was prepared using a known concentration of glucose. The blank was distilled water (1 ml) instead of sugar solution. Total sugars were expressed as g/100 g dry weight.

2.4.3. Total phenols measurement

Total phenols content was determined colorimetrically according to the method reported by Snell and Snell (1953). For estimation, 1 ml of ethanol extract was mixed with 10 drops of concentrated hydrochloric acid, heated rapidly in boiling water bath for 10 min, cooled, then 1 ml of Folin-Ciocalteau reagent and 1.5 ml of 14% sodium carbonate were added. The mixture was made up to 5 ml by distilled water, shaken well, and then kept in a boiling water bath for 5 min. The developed colour was measured using spectrophotometer at 650 nm against a reagent blank. Total soluble phenolic compounds were calculated as mg g^{-1} dry weight using standard curve with pyrogallol.

2.4.4. Total flavonoids measurement

Total flavonoids content in leaves were determined according to a colorimetric method described by Adom and Liu (2002). One g of sample was mixed with 20 ml of 80% chilled ethanol for 10 min. After centrifugation at 2500 × *g* for 10 min, the supernatant was removed and extraction was repeated once. Supernatants were pooled, evaporated at

45 °C to 5 ml, and reconstituted in 10 ml of water. The extracts were stored at - 40 °C until use.

Appropriate dilutions of sample extracts (2 ml) were reacted with 0.2 ml of 5% sodium nitrite, after 5 mints, followed by reaction with 0.2 ml of 10% aluminum chloride to form a flavonoid–aluminum complex. Solution absorbance at 510 nm was immediately measured and compared to that of catechin standards.

2.5. Assay of antioxidant enzymes activity

2.5.1. Enzymes extraction

MuKherjee and Choudhuri (1983) described the sample preparation procedures. A fresh leaf samples (250 mg) were frozen in liquid nitrogen and finely ground by pestle in a chilled mortar, the frozen powder was added to 10 ml of 100 mM phosphate buffer (KH_2PO_4/K_2HPO_4) pH 6.8. The homogenates were centrifuged at 20000 × *g* for 20 min. The supernatant was made up to a known volume with the same buffer and used as crude enzyme extract for the assay of different enzyme activities.

2.5.2. Peroxidase (POX, EC 1.11.1.7) assay

POX activity was determined using a mixture solution of 5.8 ml phosphate buffer (50 mM, pH 7.0), 0.2 ml of the enzyme extract and 2.0 ml of 20 mM H_2O_2. After addition of 2.0 ml of 20 mM pyrogallol, the rate of increment in absorbance as pyrogallol was determined by spectrophotometer within one mint at 470 nm (Darwesh et al., 2015). One unit of enzyme activity was represented the amount of the enzyme that stimulate the conversion of 1 µmol of H_2O_2 per minute at 25°C (Darwesh et al., 2019). The blank sample was made by using buffer instead of enzyme extract. The enzyme activities were expressed by U/hr/g FW.

2.5.3. Ascorbate peroxidase (APX, EC 1.11.1.11) assay

APX enzyme activity was assayed using the method of Darwesh et al. (2014), 10 ml of solution contained 5.5 ml of 50 mM phosphate buffer pH 7.0, 0.5 ml of the enzyme extract, 1.0 ml of 20 mM H_2O_2, 1.0 ml 20 mM EDTA and 2.0 ml of 20 mM L-ascorbic acid. The decline rate in absorbance as ascorbate oxidized was read at 290 nm with spectrophotometer ($\varepsilon = 2.8\ mM^{-1}\ cm^{-1}$). One unit of enzyme activity was calculated as the amount of the enzyme that catalyzed the diversion of micromole of H_2O_2 per minute at 25 °C.

2.5.4. Catalase (CAT, EC 1.11.1.6) assay

CAT enzyme activity was assayed according to the method of Chen et al. (2000). The reaction mixture with final volume of 10 ml containing 40 µl enzyme extract was added to 9.96 ml H_2O_2 phosphate buffer (pH 7.0) (0.16 ml of 30% H_2O_2 to 100 ml of 50 mM phosphate buffer). The decrement rate of H_2O_2 absorbance in 60 s was detected with spectrophotometer at 250 nm. Buffer was used instead of enzyme extract in the blank sample. One unit of enzyme activity was defined as the amount of the enzyme that reduced 50% of the H_2O_2 in minute at 25°C (Kong et al., 1999).

2.6. Lipid peroxidation

The level of lipid peroxidation was measured by determining the levels of malondialdehyde (MDA) content using the method of Hodges et al. (1999). A leaf sample (250 mg) was grinded in 10 ml of 5% trichloroacetic acid (TCA), then centrifuged at 15000 × *g* for 10 min. To 2.0 ml of the supernatant, 4.0 ml of 0.5% thiobarbaturic acid (TBA) in 20% TCA was added. The mixture was heated at 95°C for 30 min and then quickly cooled in an ice bath and centrifuged at 10000 × *g* for 10 min the absorbance of supernatant was recorded at 532 nm by spectrophotometer. The value for non-specific absorption at 600 nm was subtracted. By using absorption coefficient of 155 $nmol^{-1}\ cm^{-1}$, the MDA content was counted and represented as nmol (MDA) g^{-1} fresh weight.

2.7. Statistical analysis

Data were statistically studied as a spilt plot design (Snedecor and Cochran, 1990). The least significant differences (LSD) at 5% level of probability were accounted to compare the means of tested treatments when significant differences were detected ($P < 0.05$).

3. Results

3.1. Growth parameters

Data represented in Tabe (1) indicated that no significant differences ($P > 0.05$) between three groundnut tested cultivars (NC, Gerogry and Giza 6) were found on plant height and root length. In respect to fresh and dry weights of shoot and root systems per plant (g), the differences between groundnut cultivars were significant ($P < 0.05$). Gerogry cultivar showed the highest values of fresh and dry weights of root per plant as compared to other tested cultivars. However, Giza 6 recorded higher values of fresh and dry weights of shoot per plant as compared to these recorded by other tested cultivars.

The results represent in Table (1) showed that the effect of SeNPs on growth parameters of groundnut cultivars depended on the used concentration and plant cultivar. In NC cv., significant negative effects ($P < 0.05$) of SeNPs were observed on growth parameters, with the exception of shoot length, showed positive responses at 20 mg/L and 40 mg/L, reach 21.62% and 46.0%, respectively. In Gerogory cv., SeNPs treatments, especially at 20 mg/L improved shoot length 55.35% compared to the control value. Fresh and dry weights of shoots/plant were increased ($P < 0.05$) by 17.48% and 10.53%, respectively in response to 20 mg/L SeNPs, but it were declined as response to higher concentration (40 mg/L) by 41.6% and 29.0% respectively, compared to the control. SeNPs treatments especially at low concentration had

Table 1
Effect of selenium nanoparticles on growth parameters of some groundnut cultivars grown under sandy soil conditions.

Cultivars	Selenium (mg/L)	Plant height (cm)	Shoot fresh weight(g)	Shoot dry weight(g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
NC	**0**	37.00cd	130.30b	37.80b	15.00	4.35a	1.22a
	20	45.67b	92.30e	26.77e	11.00	2.20cd	0.62cd
	40	54.67a	75.50f	21.90f	14.00	3.60b	1.01b
Gregory	**0**	34.33c	113.00c	32.77c	12.00	4.50a	1.26a
	20	53.33de	132.75b	38.51b	11.67	1.25f	0.37f
	40	40.00c	97.50de	28.28de	12.00	1.90de	0.53de
Giza -6	**0**	35.00cd	143.80a	41.70a	9.00	1.55ef	0.43ef
	20	32.00e	102.20d	29.64d	11.33	1.85de	0.52de
	40	37.33de	146.90a	42.60a	13.70	2.67c	0.75c
LSD 0.05	**V**	**2.14**	**3.65**	**1.06**	**NS**	**0.27**	**0.08**
	Se	**2.14**	**3.65**	**1.06**	**NS**	**0.27**	**0.08**
	V x Se	**3.70**	**6.33**	**1.84**	**NS**	**0.47**	**0.13**

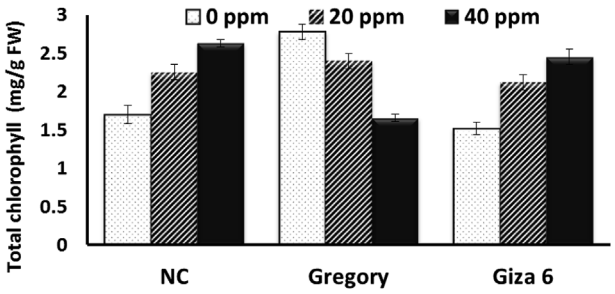

Fig. 1. Effect of selenium nanoparticles on total chlorophylls content in leaves of some groundnut cultivars grown in sandy soil. LSD at 0.05 for cultivar = 0.04, for SeNPs = 0.04, for interaction = 0.08, Vertical bars indicate ± SD.

significantly negative effects ($P < 0.05$) on fresh and dry roots weights of NC and Gerogory cultivars. Fresh and dry weights of roots/plant of 20 mg/L SeNPs-treated plants were significantly declined by 72.2% and 70.60%, respectively compared to the control treatment values.

In Giza 6 cv., the response of shoot system depended on the concentration of SeNPs. Foliar treatment with 20 mg/L SeNPs caused insignificant ($P > 0.05$) negative effects on shoot length, fresh and dry weights of shoot/plant while, 40 mg/L SeNPs caused insignificant positive effects ($P > 0.05$) as compared to the control plants. Moreover, foliar spray of SeNPs at 20 and 40 mg/L induced progressive and significant increases ($P < 0.05$) in the root/plant system. The higher increments are obtained at 40 mg/L SeNPs and reach 52.2, 72.26% and 74.42%, respectively for root length, fresh and dry weights of root/plant compared to the control treatment values.

3.2. Photosynthetic pigments

Data illustrated in Fig. (1) indicated that significant differences ($P < 0.05$) between the three groundnut cultivars were found on total chlorophyll and carotenoids contents. Gerogry cultivar showed the highest values of total chlorophyll and carotenoids content as compared to other tested cultivars. Moreover, the results indicated that total chlorophyll in NC and Giza 6 cultivars significantly increased ($P < 0.05$) with increasing SeNPs concentration, the highest increments were obtained at 40 mg/L SeNPs and represent by 42.9% and 48.0% in NC and Giza 6 cvs., respectively compared to the corresponding controls. On the contrary, application of SeNPs, especially at high concentration causes significantly decline ($P < 0.05$) in chlorophyll content by 40.3% in Gregory cv., compared to the corresponding control.

Carotenoids content of different groundnut cultivars in response to SeNPs treatments were illustrated in Fig. (2). The total carotenoids content was markedly increased ($P < 0.05$) with increasing the concentration of SeNPs in NC and Giza 6 cvs. The highest increment

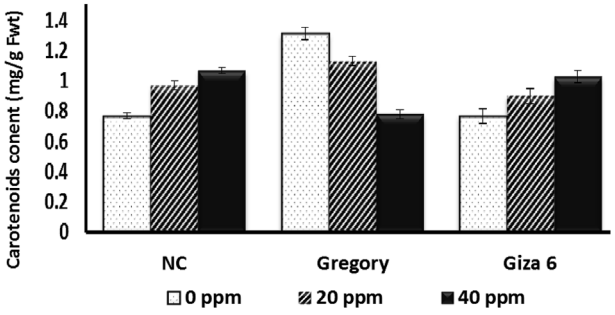

Fig. 2. Effect of selenium nanoparticles on carotenoids content in leaves of some groundnut cultivars grown in sandy soil. LSD at 0.05 for cultivar = 0.05, for SeNPs = 0.05, for interaction = 0.10, Vertical bars indicate ± SD.

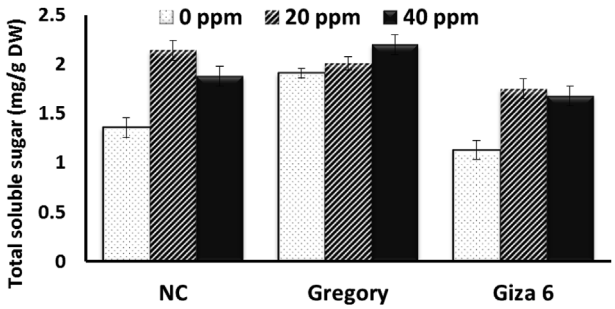

Fig. 3. Effect of selenium nanoparticles on total soluble sugars content in shoots of some groundnut cultivars grown in sandy soil. LSD at 0.05 for cultivar = 0.12, for SeNPs = 0.12, for interaction = 0.21, Vertical bars indicate ± SD.

percentages reached 39% for NC cv. and 33.77% in 40 mg/L SeNPs treated Giza 6 plants as compared to the control. While in Gregory, total carotenoids content was significantly decreased ($P < 0.05$) with increasing SeNPs concentration, the decrement were 13.7 and 40.46% below the control value.

3.3. Total soluble sugars (TSS)

The results presented in Fig. (3) cleared that significant differences ($P < 0.05$) between three groundnut tested cultivars (NC, Gerogry and Giza 6) were found on total soluble sugars (TSS) content. Gerogry cultivar showed the highest values of TSS as compared to other tested cultivars. Concerning, the effect of SeNPs, data showed that in all cultivars, TSS content increases with increasing the concentration of SeNPs treatments, the highest increment percent was recorded in NC cv., treated with 20 mg/L SeNPs comparing with the corresponding control value. However, no significance differences between the increases in TSS of Gregory and Giza 6 plants treated with low and high concentration of SeNPs.

3.4. Total phenols

The results presented in Fig. (4) cleared that significant differences ($P < 0.05$) between three groundnut tested cultivars (NC, Gerogry and Giza 6) were found on total phenols content of leaves. Gerogry cultivar showed the highest values of total phenols as compared to other tested cultivars. Changes in total phenols content of leaves depend on the concentration of SeNPs and groundnut cultivar were illustrated. In NC and Giza 6 cvs., foliar application of SeNPs leads to a marked increase ($P < 0.05$) in total phenols content as compared to control treatment value. This increase is more obvious with the higher concentration of SeNPs (40 mg/L). However, no significance differences between the increases in total phenols of NC and Giza 6 plants treated with tested concentrations of SeNPs. On the contrary, in Gregory cv., both SeNPs

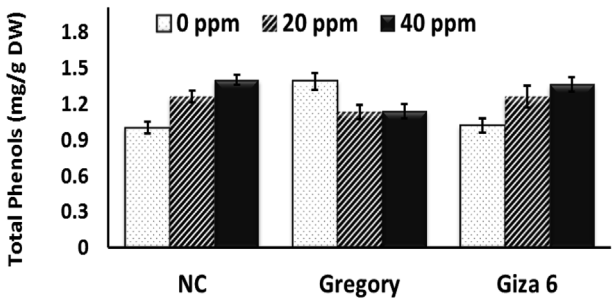

Fig. 4. Effect of selenium nanoparticles on total phenols content in shoots of some groundnut cultivars grown in sandy soil. LSD at 0.05 for cultivar = 0.08, for SeNPs = 0.08, for interaction = 0.12, Vertical bars indicate ± SD.

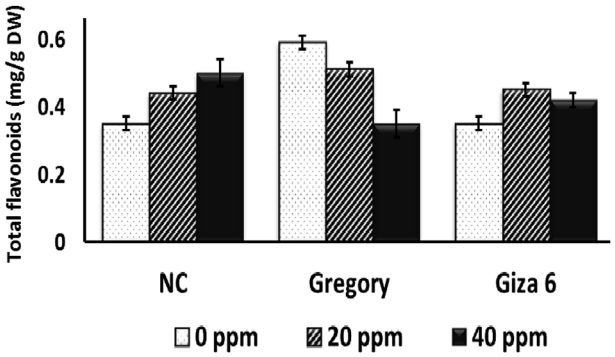

Fig. 5. Effect of selenium nanoparticles on total flavonoids content in shoots of some groundnut cultivars grown in sandy soil. LSD at 0.05 for variety = 0.05, for SeNPs = 0.05, for interaction = 0.08, Vertical bars indicate ± SD.

treatments lead to marked equal decreases ($P < 0.05$) in total phenols content in leaves as compared to control treatment value.

3.5. Total flavonoids

The results presented in Fig. (5) cleared that significant differences ($P < 0.05$) between three groundnut tested cultivars (NC, Gerogry and Giza 6) were detected in total flavonoids content of leaves. Gerogry cultivar showed the highest values of total flavonoids as compared to other tested cultivars. The application of SeNPs induced significant increments ($P < 0.05$) in total flavonoids contents of different tested groundnut cultivars. Concerning NC cv., application of SeNPs, especially at high concentration (40 mg/L) seemed to be the most effective treatment for increasing total flavonoids, however reached 42.86% comparing the untreated plants. On the other hand, in Giza 6 cv., the increment of total flavonoids are much more pronounced in response to low concentration (20 mg/L) and represented by 28.57%. While in Gregory, total flavonoids content decreases with increasing SeNPs concentration, the maximum decrement are 40.68% below the control treatment value in response to 40 mg/L SeNPs.

3.6. Antioxidant enzymes activity

3.6.1. Peroxidase enzyme activity

Data in Fig. (6) cleared that Giza 6 plants showed the highest POX activity with a significant difference compared to other tested cultivars. However, no significant differences between NC and Gerogry cultivars in their peroxidase enzyme activity (POX). Regarding the effect of SeNPs on POX activity, it is cleared that all SeNPs-treated plants, especially at high concentration showed significantly increase ($P < 0.05$) in POX activity. The highest increment percent recorded 241.7% in NC cv., treated with 40 ppm SeNPs compared to the control value.

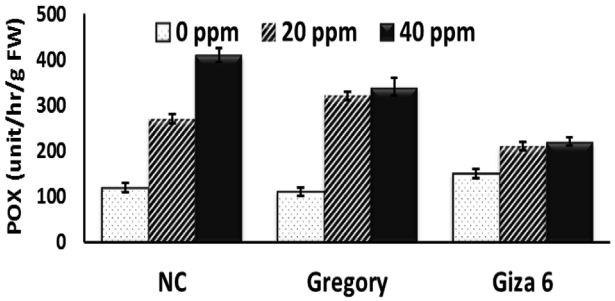

Fig. 6. Effect of selenium nanoparticles on peroxidase enzyme activity in leaves of some groundnut cultivars grown in sandy soil. LSD at 0.05 for cultivar = 8.3, for SeNPs = 8.3, for interaction = 14.5, Vertical bars indicate ± SD.

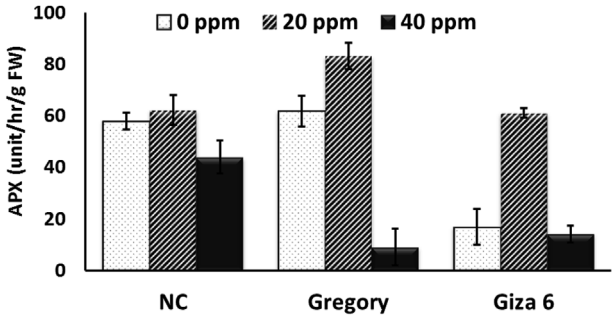

Fig. 7. Effect of selenium nanoparticles on ascorbate peroxidase enzyme activity in leaves of some groundnut cultivars grown in sandy soil. LSD at 0.05 for cultivar = 12.0, for SeNPs = 12.0, for interaction = 20.0, Vertical bars indicate ± SD.

3.6.2. Ascorbate peroxidase enzyme activity

Data in Fig. (7) cleared that significant differences ($P < 0.05$) between three groundnut tested cultivars (NC, Gerogry and Giza 6) were determined in ascorbate peroxidase enzyme activity (APX). Gerogry cultivar showed the highest activity of APX in comparison with other tested cultivars. In concerning the interaction between tested SeNPs and the different cultivars, it was revealed that activity of APX was significantly increased in 20 mg/L SeNPs treated Gregory and Giza 6 plants but significantly decreased in all 40 mg/L SeNPs treated plants compared to the corresponding control values.

3.6.3. Catalase

Data in Fig. (8) cleared that significant differences ($P < 0.05$) between three groundnut tested cultivars (NC, Gerogry and Giza 6) were determined in catalase (CAT) enzyme activity. The highest activity of CAT was observed in Giza 6 cultivar in comparison with other tested cultivars. The results demonstrated that CAT enzyme activity was strongly decreased ($P < 0.05$) in all SeNPs -treated groundnut cultivars compared to that of the control treatment (Fig. 8).

3.7. Lipid peroxidation

The results in Fig. (9) showed that it was observed that significant ($P < 0.05$) differences in lipid peroxidation (MDA) among tested groundnut cultivars (NC, Gerogry and Giza 6). The lowest value of MDA was observed in NC cultivar in comparison with other tested cultivars. Additionally, the foliar application of SeNPs, especially at high concentration (40 mg/L) caused slightly increases in MDA content of NC and Gregory cultivars grown in sandy soil. On the other hand, spraying Giza 6 treated with SeNPs treatments; especially at 20 ppm SeNPs decreased MDA content by 16.70% compared to control value.

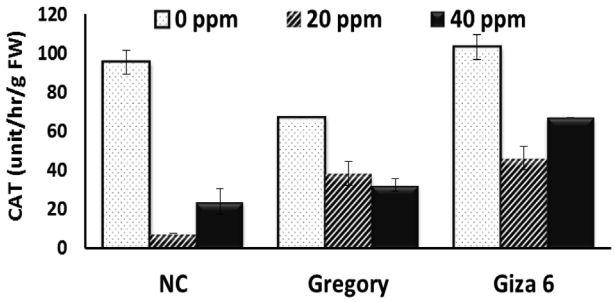

Fig. 8. Effect of selenium nanoparticles on catalase enzyme activity in leaves of some groundnut cultivars grown in sandy soil. LSD at 0.05 for cultivar = 9.0, for SeNPs = 9.0, for interaction = 17.0, Vertical bars indicate ± SD.

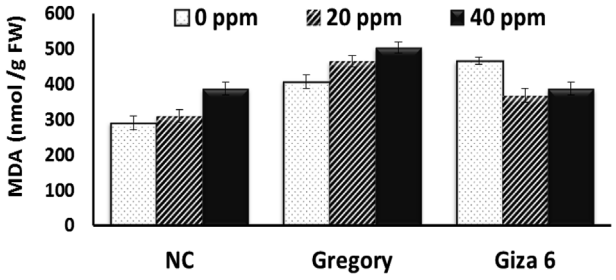

Fig. 9. Effect of selenium nanoparticles on lipid peroxidation in leaves of some groundnut cultivars grown in sandy soil. LSD at 0.05 for cultivar = 21.0, for SeNPs = 21.0, for interaction = 55.60, vertical bars indicate ± SD.

4. Discussion

The results declared that application of SeNPs improved growth parameters of groundnut cultivars; Gerogory and Giza 6. In this respect, Arora et al. (2012) suggested that the changes in the growth of *Brassica* seedlings exposed to gold NPs might be due to action of plant hormones. Moreover, Sharma et al. (2012) reported that the AgNPs treatment improved the growth by modification of the antioxidant systems of *Brassica* seedlings grown *in vitro*. Nanoparticles promoted photosynthesis and nitrogen metabolism and in turn improved growth of spinach at 20 mg/L TiO_2 (Gao et al., 2008). Additionally, they improved the plant growth and development by increasing transpiration rate, net photosynthetic rate, stomatal conductance, effective photochemical efficiency, PSII potential activity, actual photochemical efficiency, photochemical quench and electron transport rate (Siddiqui et al., 2014). Nanoparticles enhance root growth by inhibiting ethylene signaling in *Crocus sativus* (Rezvani et al., 2012). On the other hand, the growth parameters of NC cv. were relatively negative affected by SeNPs treatments, the growth reduction may be due to the conversation of metabolic energy and metabolites from the processes involved in the normal growth to ameliorate the oxidative stress effects imposed by SeNPs. Metal nanoparticles may be move through the symplastic or apolastic routes to the roots and finally move and distribute to the stems and leaves through the xylem and phloem (Wang et al., 2012). So, it could be suggested that the differential response of plant cultivars and/or plant parts may be depending on the mobility of SeNPs in the plant, plant hormonal action and/or protein synthesis.

Chlorophyll and carotenoids are considered as the central part of the energy of green plant system; therefore, any alteration in their levels causes parallel effect on the metabolism of the plants. Data in the present investigation indicate that total chlorophyll increased with increasing SeNPs concentration in both cultivars (NC and Giza 6). These results agree with Salama (2012) who found that metallic nanoparticles increased chlorophyll contents and carotenoids of *Brassica juncea*. An increase in chlorophyll may be attributed to SeNPs effect on protection of certain chloroplast enzymes involved in the biosynthesis of photosynthetic pigments (Ragavan et al., 2017), especially, selenium acts as the catalytic centre of selenoproteins, such as glutathion peroxidase, hence it is important in the scavenger of free radicals and thus protect photosynthetic apparatus (Gupta and Gupta, 2016). On the other side, the decline of total chlorophyll content in SeNPs treated Gregory cv., may be explained by SeNPs treatment induces certain oxidative stress which caused peroxidation of chloroplast membrane and causes degradation of photosynthetic pigments. Moreover, SeNPs protects the chloroplast by increasing the activity of antioxidant enzymes, especially peroxidase. Moreover, Mohsenzadeh and Moosavian (2017) reported that the reduction in the amount of chlorophyll occurs because of degradation of the precursor of chlorophyll pigments.

The accumulation of total soluble sugars by all SeNPs treated groundnut plants might be due to maintain osmotic potential in the vacuoles and cytoplasm at an optimal level for cell metabolism, hence protected cell structures from free radicals. Additionally, Ze et al., (2011), reported that metal nanoparticles might promote light absorption by chloroplast through up regulation of genes related to light harvesting complex II, which in turn, increased levels of soluble sugars. These led us to conclude that SeNP might be acting as an activator in photosynthesis which in turn change total carbohydrates content (Smirnoff, 2011). Phenols are considered the cooperative network, using a chain of several redox reactions. Total phenols contents were increased in NC and Giza 6 cultivars treated with SeNPs, especially at high concentration. The significant positive correlation between SeNPs and total phenols contents can help to induce protective mechanisms against the membrane damages. These results are harmony with Walaa et al. (2010), who illustrated that the role of Se may be linked to activation of phenylalanine ammonia lyase (PAL) which control phenolic compounds synthesis. On the contrary, in Gregory cv., both SeNPs treatments lead to a marked decrease in total phenols content as compared to control value. This might be due to the increment POX enzyme activity which catalyzes the oxidation of phenolic compounds using H_2O_2 as the oxidizing agent (Hiraga et al., 2001). In this respect, Mekki and Hussein (2017) revealed that changes in total phenols content of groundnut cultivars, NC, Giza 6 and Gregory in response to foliar application of ascorbate (known as antioxidant compound) depended on its effect on sugar biosynthesis which refer to a direct relationship between sugar and phenolic compound biosynthesis.

Application of SeNPs induced the accumulation of total flavonoids in groundnut cultivars (NC and Giza 6 cultivars). These results are similar to the study of López-Vargas et al. (2018) on tomato plants. Foliar spray of SeNPs could generate certain stress in groundnut cultivars. This produces an increase in ROS, which activates flavonoids act as antioxidant defense mechanism, due to strongly control the free radical formation (Rizwan et al., 2017). On the other hand, total flavonoids decrease in SeNPs treated plants of Gregory cv. compared to the control. This result may be due to the difference in response degree of each cultivar to selenium nanoparticles. SeNPs treatment causes an overproduction of ROS in treated Gregory cultivar, resulting in a decrease of flavonoids.

APX, POX and CAT are enzymes that stimulate the diversion of H_2O_2 to water and O_2 (Gratao et al., 2005). The balance between ROS generation and activities of antioxidant enzymes determine whether oxidative signaling and/or damage will occur (Moller et al., 2007). Treatment with 20 ppm SeNPs results increment of POX and APX activities associate with decrement of catalase activity. Application of 40 ppm SeNPs produces decline in APX and CAT activities, this may be due to the high activity of POX enzyme. In this concern, Raigond et al. (2017) reported that metal nanoparticles increase antioxidant enzymes such as POX and APX in potato plants. The increase in POX and APX might be considered as a key point for the decomposition of H_2O_2, especially under CAT inactivation. Concerning the decrease in CAT enzyme activity, Xue et al. (1993) found that Se compounds direct dismutation of $O_2^{\bullet -}$ and $OH^{\bullet}$ into H_2O_2 followed by H_2O_2 scavenging by CAT enzyme. It appears that the decline in CAT activity may be due to the mediated role of selenium in detoxification mechanisms against the oxidative stress. Lipid peroxidation of cell membranes ends by formation of malondialdehyde (MDA) considered as a biochemical signal to determine the membrane injury by free radicals (Smirnoff, 1995). In current study, it appears that an increase in MDA content of NC and Gregory SeNPs treated plants. This indicated that SeNPs caused specific stress resulted in induction of enzymatic (POX in Gregory cv.) and non-enzymatic antioxidant compounds (such as phenols, flavonoids and carotenoids in NC cv.). On the other hand, SeNPs decreased MDA content in treated plants of Giza 6 cv., the reason behind positive effect of SeNPs on these plants may be due to specific response of Giza 6 cv. to SeNPs concentration through promotion of photosynthetic pigments as well as non-enzymatic antioxidant compounds synthesis.

5. Conclusion

In conclusion, nano selenium act as stimulator and/or stressor enhanced the antioxidant defense systems in tested groundnut cultivars leads to the improvement of plant tolerance under sandy soil conditions.

Conflicts of interest

Authors declare that there are no conflicts of interest.

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