Biotechnology Reports 24 (2019) e00377

Contents lists available at ScienceDirect

Biotechnology Reports

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

Evaluation of cytotoxicity, biochemical profile and yield components of groundnut plants treated with nano-selenium

Hebat-Allah A. Hussein[a,b,*], Osama M. Darwesh[c,**], Bahaa B. Mekki[d], Salwa M. El-Hallouty[e]

[a] Botany and Microbiology Department, Faculty of Science (Girls Branch), Al-Azhar University, Cairo, Egypt
[b] Biology Department, University College of Nairyah, Hafr El Batin University, Saudi Arabia
[c] Agricultural Microbiology Department, National Research Centre, Dokki, Cairo, Egypt
[d] Field Crops Research Department, National Research Centre, Dokki, Cairo, Egypt
[e] Pharmacognosy Department, National Research Centre, Dokki, Cairo, Egypt

ARTICLE INFO

Article history:
Received 10 May 2019
Received in revised form 19 July 2019
Accepted 8 September 2019

Keywords:
Cytotoxicity
Groundnut
Nano-Selenium (Nano-Se)
Protein signatures
Fatty acids composition

ABSTRACT

Knowledge about the risks of the nanoparticles application on the plant development and human health is still limited. Different concentrations of nano-selenium (0, 20 and 40 ppm) were applied to three different Egyptian groundnut (*Arachis hypogaea* l.) cultivars; (NC, Gregory and Giza 6) under sandy soil conditions at vegetative growth stage to investigate their effects on yield components, protein profile, fatty acids composition, total antioxidant content and cytotoxicity of yielded seeds. The results indicate that the tested Nano-Selenium (Nano-Se) concentrations improved yield components and seeds oil. However, Nano-Se altered protein signatures as well as fatty acids composition by increasing unsaturated fatty acids and/or decreasing saturated fatty acids as compared with control, the cytotoxicity assessments proved safety of the yield for human health.

1. Introduction

Groundnut (*Arachis hypogaea* L.) is an economic oilseed plant in the world; the seed provides 50–65% oil and 25–35% proteins, while the rest of the plant parts provide livestock fodder. The roots have nodules which provide nitrogen to soil, thus improves soil fertility. The stability and nutritional value of groundnut oil depend on the polyunsaturated fatty acids, certainly linoleic and linolenic acids [1]. The tested groundnut cultivars are different at physiology and morphology levels and possibly cultivated in Egyptian newly reclaimed sandy soil which faces shortage of soil fertility and water resources and salinity and/or drought stress [2].

Selenium (Se) is an essential micronutrient for humans, animals, and other organisms [3]. However, the role of nano-selenium (Nano- Se) in higher plants has not been illustrated distinctly. Earlier studies have evidenced that exogenously foliar application of nano-selenium enhanced the antioxidant potential in sweet basil (*Ocimum basilicum* L.) [4]; growth of tobacco (*Nicotiana tabacum* L.) [5]; groundnut (*Arachis hypogaea* L.) [2] and yield of mustard (*Brassica rapa* L.) [6]. In this concern, Djanaguiraman et al. [7] revealed that Se application decreased lipid peroxidation and cell membrane damage through increasing superoxide dismutase (SOD) and glutathione peroxidase (GPX) enzymes activities leading to prove its anti-oxidative activity. The increased growth in higher plants by Se application is through higher leaf photochemical efficiency [8].

Nanotechnology has a wide range of applications; one of them is nano-agriculture, which used to improve the productivity of plants and bio-controlling [9], fuel production [10], food industry [11,12], environment protection [13,14] and producing of antimicrobial agents [15,16]. Higher plants are different in ability of absorption and accumulation of synthetic engineered nanoparticles. The nanoparticles induce changes in several metabolic pathways, which finally affect plant growth and developments [17]. The effects of Nano-Se on different plant species can vary greatly with plant growth stages, method, and duration of exposure and depend on the nano-Se shape, size, chemical composition, concentration, surface structure, aggregation, and solubility [2]. Nano- Se has a potential antioxidant activity with reduced toxicity due to its redox state of zero (Se^0). Selenium nanoparticles are bright red and highly stable colloidal state has been synthesized to be used in nutrition and developed for applications in medical

* Corresponding author at: Botany and Microbiology Department, Faculty of Science (Girls Branch), Al-Azhar University, Cairo, Egypt.
** Corresponding author
E-mail addresses: hebahussein@azhar.edu.eg (H.-A.A. Hussein), darweshosama@yahoo.com (O.M. Darwesh).

https://doi.org/10.1016/j.btre.2019.e00377

therapy [18]. The biological activity of Nano-Se increases with their surface to volume ratio and decreasing particle size. Seleno-amino acids, seleno-methionine and seleno-cysteine, are accountable for the many biomedical effects of dietary Se, that is important in the scavenging of free radicals, protection against oxidative stress [19]. Nano-Se as a fertilizer can be used for increasing productivity of crop yield. Thus, it was revealed that there is a good opportunity for the intervention of Nano-Se in the area of nano-agriculture [20].

Our earlier study on groundnut plants (*Arachis hypogaea* L.) demonstrated that foliar application of Nano-Se improved plant growth, the leaf antioxidant peroxidase enzyme and decreased lipid peroxidation. However, there is still a gap of research interest in the effect of the presence of Nano-Se in living systems. For that, the present study evaluated the effects of Nano-Se on total antioxidant values, protein profiles, fatty acids composition in groundnut plants and their toxic effects on in human cell lines to validate weather yielded seeds originated from Nano-Se treated plants possess biosafety properties.

2. Materials and methods

A pot experiment was designed during summer season 2017 in the greenhouse of National Research Centre, Giza, Egypt to investigate the effect of selenium nanoparticles (Nano-Se) on yield and biochemical profile of three different groundnut (*Arachis hypogaea* l.) cultivars and biosafety of seed yields. 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 by factorial experiment in complete randomize design with 5 replicates for each treatment.

2.1. Selenium nanoparticles

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 mixture was allowed to react till the colour changed from colorless to light orange [2]. The produced selenium nanoparticles were characterized using spectrophotometer (Jenway UV/Visible- 2605 spectrophotometer, England) and transmission electron microscopy as described by Youssef et al. [21]. Nano-Se concentrations (0, 20 and 40 ppm) were prepared and applied two times over a period of two weeks as foliar spray on plants after 45 and 60 days, respectively from sowing.

2.2. Yield components

At the harvest time, 120 days after planting, yield components (number of pods per plant, pods weight per plant, number of seeds per plant, seed weight per plant, seed index and oil yield per plant) were estimated.

2.3. Oil content

The oil content of the seed yield was estimated according to the procedure of the A.O.A.S. [22].

2.4. Fatty acids composition

Quantitative estimation of fatty acid profile was detected by gas liquid chromatography. The lipid samples were methylated according to Slover and Lanza [23]. Each fatty acid percentage was calculated using the next equation:

$$Fatty\ acid\ \% = \frac{Area\ of\ each\ peak}{Total\ area\ of\ all\ peaks}\ x\ \ 100$$

2.5. Proteomic analysis

The plant tissue was frozen rapidly with liquid nitrogen to make the plant more fragile and ground before shaking in the buffer [24]. The dried samples were mixed with 1 ml of water-soluble protein extraction buffer in eppendorf tube and left in refrigerator overnight. The samples were vortexed for 15 s and centrifuged at 5000 rpm at 4 °C for 10 min. The supernatants containing water-soluble proteins were transferred to new eppendorf tubes and kept at deep-freeze until use for electrophoretic analysis [25]. To determine the relative molecular weight of extracted proteins, sodium m dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on a stacking and separating gel according to the method of Laemmli [26] using Mini-gel electrophoresis (BioRad, USA) [27]. The molecular weight of the isolated proteins was estimated in comparison to standard molecular weight markers (Marker, 11–245 kDa; Sigma, USA). The protein bands were visualized by staining with Coomassie Brilliant Blue G-250 (Sigma, USA) after documentation [28].

2.6. Antioxidant activity

To estimate the total antioxidant, groundnut plants seeds treated by Nano-Se was extracted in 80% acetone and detected depending on

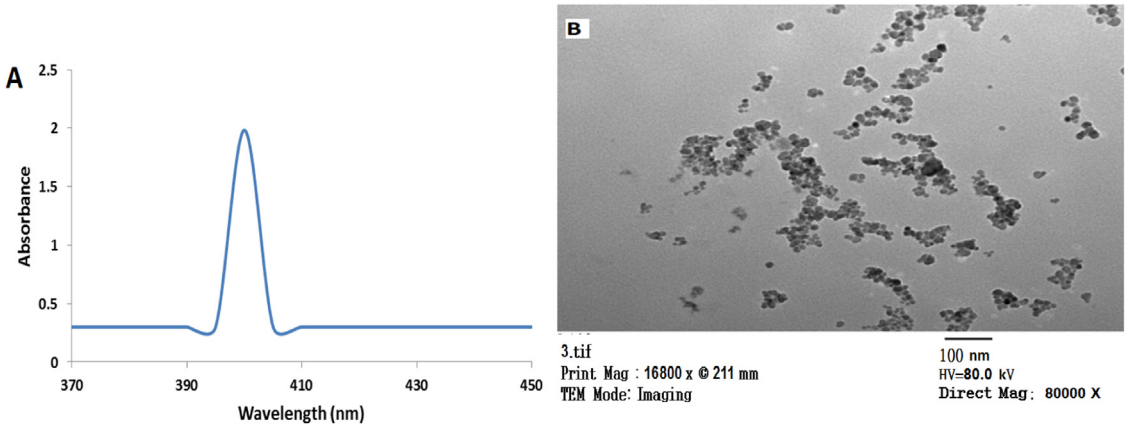

Fig. 1. A, UV/VIS absorption spectrum of selenium nanoparticles and B, TEM characterization of selenium nanoparticles.

Table 1
Effect of Nano-Se on yield components of some groundnut (*Arachis hypogaea* l.) cultivars cultivated in sandy soil.

Types of Groundnut Cultivars	Nano-Se (ppm)	Number of pods / plant	Pods weight (g/plant)	Seed weight (g/plant)	Number of seeds /plant	1000-seed weight (g)	Shelling %	Oil %	Oil yield (g/plant)
NC	0	16.00	21.31	13.76bc	22.32	61.11a	69.64a	55.00	7.57bcd
	20	28.00	22.02	13.37bc	22.33	61.15a	60.78ab	55.63	7.44cd
	40	39.00	36.23	20.46a	33.50	62.60a	56.59b	55.70	11.40a
Gregory	0	11.00	12.15	5.03d	17.33	29.06c	42.20d	51.99	2.62e
	20	33.00	27.82	15.31bc	28.50	58.34ab	54.93bc	55.55	8.50bc
	40	28.67	21.79	11.71c	21.33	54.65ab	54.38bc	52.29	6.13d
Giza -6	0	21.33	24.83	14.12bc	24.00	44.42b	56.28b	51.15	7.23cd
	20	31.33	30.19	15.59b	25.67	58.85a	51.44bcd	53.66	8.34bc
	40	44.00	36.51	17.00ab	37.33	65.87a	45.64cd	55.84	9.53ab
Means of Selenium	0	16.11c	19.43c	10.97c	21.22b	49.83b	56.04	52.72b	5.80c
	20	29.33b	24.67b	13.56b	23.11b	62.12a	55.53	53.86b	7.30b
	40	38.67a	33.52a	17.59a	33.11a	53.39b	52.87	55.69a	9.81a
LSD 0.05	V	3.81	3.70	2.12	4.14	8.22	6.02	1.20	1.20
	Se	3.81	3.70	2.12	4.14	8.22	NS	1.20	1.20
	V x Se	NS	NS	3.67	NS	14.24	10.42	NS	2.08

Table 2
Effect of Nano-Se on fatty acid composition of oil of some groundnut (*Arachis hypogaea* l.) cultivars cultivated in sandy soil. Data are represented as means ± SD.

Fatty acids composition		Palmitic (16:0)	Stearic (18:0)	Behenic (20:0)	Arachidic (22:0)	Lignoceric (24:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)
NC	**0**	12.58 ± 1.26	2.42 ± 0.84	2.06 ± 0.10	2.09 ± 0.32	1.25 ± 0.12	53.02 ± 1.04	30.22 ± 1.20	1.76 ± 0.24
	20	12.38 ± 1.00	2.74 ± 0.89	0.98 ± 0.12	–	–	50.48 ± 1.09	31.02 ± 1.13	1.32 ± 0.21
	40	12.71 ± 1.12	2.75 ± 0.25	––	—	—	44.53 ± 1.10	33.89 ± 1.42	1.24 ± 0.16
Gregory	**0**	13.45 ± 1.09	2.21 ± 0.43	0.97 ± 0.09	1.91 ± 0.64	1.09 ± 0.11	44.27 ± 1.20	34.12 ± 2.30	1.14 ± 0.33
	20	12.00 ± 1.47	1.86 ± 0.76	1.04 ± 0.11	1.75 ± 0.23	0.98 ± 0.22	51.15 ± 2.11	29.63 ± 3.00	1.21 ± 0.24
	40	10.60 ± 1.23	1.63 ± 0.86	1.57 ± 0.14	2.73 ± 0.43	1.34 ± 0.26	53.58 ± 2.32	26.11 ± 2.82	1.27 ± 0.18
Giza-6	**0**	11.62 ± 1.22	1.92 ± 0.73	1.34 ± 0.28	2.59 ± 0.22	1.27 ± 0.39	50.81 ± 2.04	28.34 ± 2.68	1.23 ± 0.20
	20	12.87 ± 1.25	2.02 ± 0.65	1.20 ± 0.30	2.75 ± 0.27	–	43.37 ± 1.75	36.55 ± 2.47	1.26 ± 0.43
	40	13.49 ± 1.60	1.76 ± 0.34	1.05 ± 0.24	2.05 ± 0.15	–	43.85 ± 1.39	36.68 ± 1.60	1.12 ± 0.65

Table 3
Effect of selenium nanoparticles on protein profile in yielded seeds of some groundnut (*Arachis hypogaea* l.) cultivars grown in sandy soil.

Types of Cultivar		**NC**			**Gregory**		**Giza 6**		
Nano- Se		**0**	**20 ppm**	**40 ppm**	**0**	**20 ppm**	**0**	**20 ppm**	**40 ppm**
No. of samples		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
No.	**M.W.**								
1	**115**	–	–	–	+	–	+	–	–
2	**109**	+	–	–	–	–	–	–	–
3	**99**	–	–	–	+	+	+	+	+
4	**97**	+	+	+	–	–	–	–	–
5	**85**	+	+	+	+	+	+	+	–
6	**75**	+	+	+	–	+	–	+	+
7	**64**	+	+	–	–	–	–	–	–
8	**59**	+	–	–	–	–	–	–	–
9	**53**	–	+	+	–	+	–	+	+
10	**50**	+	–	–	+	–	+	–	–
11	**45**	+	+	+	+	+	+	+	+
12	**40**	–	+	+	–	+	+	+	+
15	**35**	+	+	+	+	+	+	+	–
17	**30**	+	+	+	+	–	+	–	+
18	**27**	+	+	+	+	+	–	–	–
19	**25**	+	+	+	+	–	+	+	+
20	**22**	+	+	+	+	+	+	+	+
21	**18**	+	–	–	+	–	+	–	–
23	**15**	+	–	+	+	–	–	–	–
24	**14**	+	+	+	+	+	+	+	+

free radical scavenging by α-diphenyl-β-picrylhydrazyl [29]. Data represented as μg Trolox g^{-1} DW.

2.7. Cytotoxicity assessment

In this study, two human cell lines were used. A normal human cell line (BJ1) derived from healthy skin tissue and (Rep1) Retina, Eye; Pigmented Epithelium. Cell lines were obtained from Karolinska Center, Department of Oncology and Pathology (Stockholm, Sweden). All these cell lines are described as adherent cells, as they adhere to the surface of the flask in which they are grown [30].

Cell lines were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Lonza, Basel, Switzerland) and supplemented with 10% fetal bovine serum (Lonza) and 1% ampicillin and streptomycin. The cells were passaged using the following method.

The old media was decanted, and the cells were rinsed with 3 ml of trypsin (Lonza). Another 3 ml of trypsin (1X) was added to remove the adherent cells from the flask; then the flask was placed in the incubator for 5–15 min at 37 °C and 5% CO_2. The cells were constantly viewed under the inverted microscope until the cells were dispersed. DMEM's medium was added to the flask to complete the volume to 20 ml then 2–3 ml of FBS was added to inhibit the effect of trypsin as the prolonged exposure is harmful to the cells.

The anti-proliferative effects of the plant extracts were tested *in vitro* on the 2 cell lines, BJ1and RPe1 using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay [31]. The cells were cultured in 96-well plates, where each well contains 5×10^5 cells. For accuracy, a multi-channel pipette was used throughout the experiments. A 100 ppm final concentration of tested extracts was added in triplicates. The cells were treated for 48 h. 0.5% DMSO was used as a negative control. Cytotoxic was determined using MTT [3-(4, 5-dimethythiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay as described by Mosmann [32].

The assays were developed by removal of medium from each well after the incubation of the cells with and without the extracts. In each well, 40 μl of MTT (BioBasic, New York, USA) was added then the plates were incubated for 3–4 h. in 5% CO_2 incubator. After the incubation, 140 μl of sodium dodecyl sulfate (SDS) (solubilizing reagent) (ADWIC, Egypt) were added to each well and left for 20 min. The plates were then measured at 595 nm, which is the optimal absorbance according to multiple sources, on a microplate reader [31].

Percentage inhibition = 100 – (abs. of treated cells/abs. of untreated cells) x100

2.8. Statistical analysis

Data were statistically analyzed as a split plot design according to Snedecor and Cochran [33]. Data indicated mean values ± standard errors of the mean. Duncan's test was used to analyze the difference between treatments ($p < 0.05$ is considered as significance level).

3. Results and discussion

3.1. Nano-selenium preparation and characterization

Selenium is an essential element for plant growth. Also, the nano-scale of this element will be promising for increasing yield and immune system as well as plant fertility. For that, this study aimed to increase the plant yield and physicochemical properties beside the evaluation of cytotoxicity of nano-Se on plant crops. Nano-Se was prepared through chemical reduction of sodium selenite by ascorbic acid. One peak was detected at wavelength 400 nm described selenium formation at nano size (Fig. 1A). Engineered Nano-Se size was about 10–30 nm with red color and almost spherical morphology (Fig. 1B). Treatments were applied two times over a period of two weeks as foliar spray on plants after 45 and 60 days, respectively from sowing.

3.2. Yield components

Data represented in Table 1 showed that, the application of 20 ppm Nano-Se on NC plants, exhibited no significant effect on yield components, with the exception of No. of pods/plant, which showed a positive response ($P < 0.05$) represented by 75% compared to control. The magnitude response was much more pronounced with 40 ppm Nano-Se for increasing yield and its components. The increment represented by 143.8%, 70.0%, 48.7%, 50.1% and 53.2%, respectively for number of pods/plant, pods weight/plant, seeds weight/plant, number of seeds/plant and oil yield/plant compared to the unsprayed plants. While, shelling % was declined by 12.7% and 18.74% with 20 ppm and 40 ppm treatments, respectively compared to control.

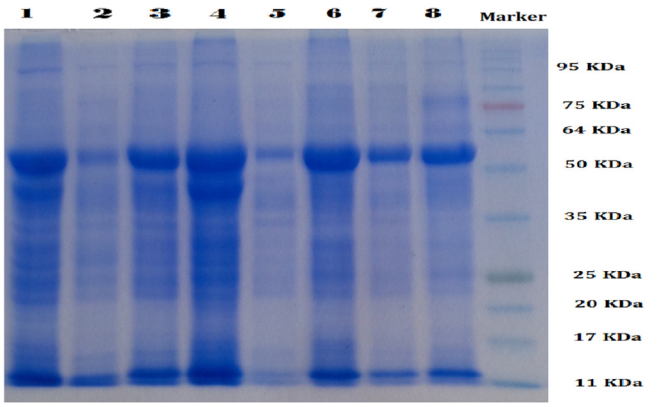

Fig. 2. Effect of selenium nanoparticles on protein profile in yielded seeds of some groundnut (*Arachis hypogaea* l.) cultivars grown in sandy soil. 1 = NC + 0 Nano-Se, 2 = NC + 20 ppm Nano- Se, 3 = NC + 40 ppm Nano-Se, 4 = Gregory + 0 Nano-Se, 5 = Gregory + 20 ppm Nano- Se, 6 = Giza 6 + 0 ppm Nano-Se, 7 = Giza 6 + 20 Nano-Se, 8 = Giza 6 + 40 ppm Nano-Se.

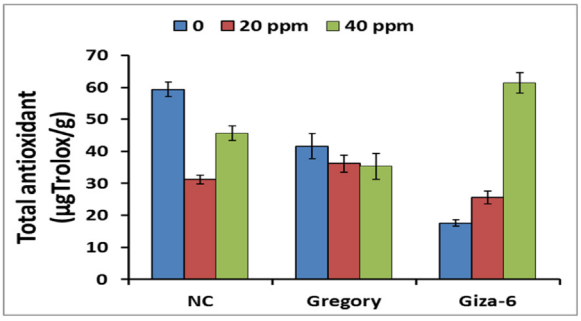

Fig. 3. Effect of selenium nanoparticles on total antioxidant in yielded seeds of some groundnut (*Arachis hypogaea* l.) cultivars grown in sandy soil. Data are represented as means ± SD of three replicates and show significance at $p < 0.05$.

On the other hand, in Gregory, 20 ppm Nano-Se seemed to be the most effective treatment for improving yield components ($P < 0.05$). The increments reached 200% in pods number/plant, 129.0%, in pods weight/plant, 204.4% in seeds weight/plant, 64.5% in seeds number/plant, 100.8% in 1000-seed weight, 30.17% in shelling % and 224.4% in oil yield/plant. Concerning Giza 6 var., 40 ppm Nano-Se was the best effective treatment for the enhancement of yield components ($P < 0.05$). The increments reached 106.28% in pods number/plant, 47.0%, in pods weight/plant, 20.40% in seeds weight/plant, 55.5% in seeds number/plant, 48.29% in 1000-seed weight and 31.81% in oil yield/plant over the controls. Similar results were obtained in study on *Arabidopsis thaliana* and *Brassica juncea* [34,35]. The role of Nano-Se in improvement of plant productivity might be due to the cellular ionic and osmotic balances which improve photosynthesis by increasing the abundance of green pigments resulting in decreased ROS levels, reactivation of antioxidants, and enhanced production of effective photochemical efficiency [36]. Moreover, Djanaguirama et al. [18] cited that Nano-Se significantly increased seed yield due to increment of photosynthetic rate and seed number per panicle. This was accomplished by decreased ROS content in the leaves along with higher antioxidant enzyme activities.

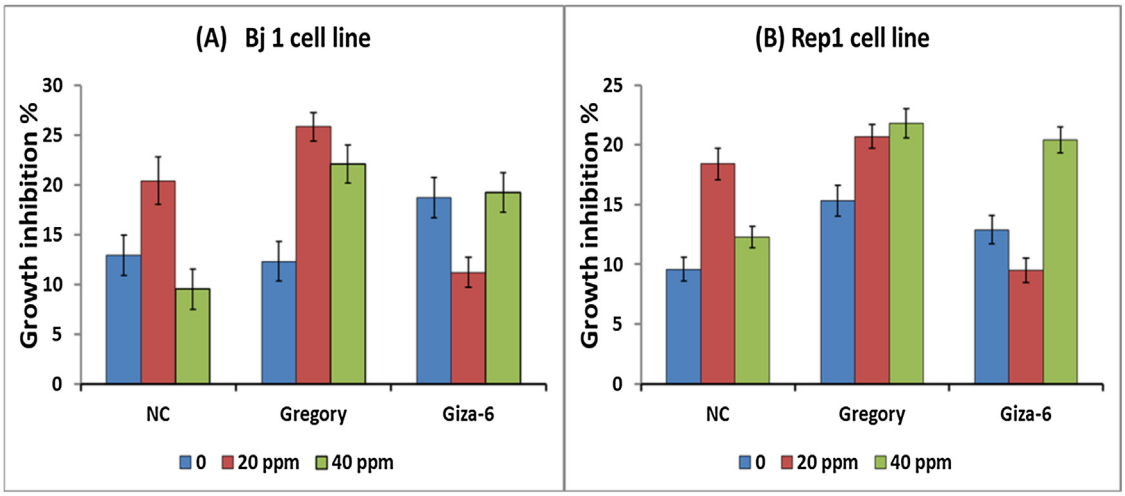

Fig. 4. (A) Effect of Nano-Se seeds extract on Bj1 cell line, normal human cell line derived from healthy skin tissue and (B) Effect of Nano-Se seeds extract on Rep1 cell line, normal human cell line derived from pigmented epithelium tissue of Retina Eye. Data are represented as means ± SD of three replicates and show significance at $p < 0.05$.

3.3. Fatty acids composition

The results presented in Table 2 declared that foliar spray of 20 ppm and 40 ppm Nano-Se on NC cv. plants resulted in significantly decrement in behenic acid by 52.43% and 100%, respectively compared to the control. Arachidic and lignoseric acids disappeared in all Nano-Se treated plants. On the other hand, Nano-Se treatment increased palmitic and stearic saturated fatty acids. The increase was directly proportional to the applied concentration of Nano-Se. Moreover, Nano-Se treatment, especially at 40 ppm increased the Linoleic unsaturated fatty acid by 12.14% but declined oleic and linolenic by 16.0% and 29.6%, respectively compared with untreated plants. Regarding Gregory var., 20 ppm Nano-Se decline palmitic, stearic, arachidic, and lignoseric acids evaluated by 10.78%, 15.84%, 8.38% and 10.1%, respectively but cause a slight increase in behenic acid compared to the seed of the control. Plants treated with 40 ppm show decrement in palmitic and stearic acids evaluated by 21.2% and 26.2%, respectively but increment in behenic, arachidic acid and lignoseric acids reached 61.86%, 42.93% and 22.94%. The linoleic (unsaturated fatty acid) was significantly decreased by 13.16%, and 23.48%, respectively in response to 20 ppm and 40 ppm Nano-Se compared to the control values. On the other hand, 20 and 40 ppm Nano-Se increased oleic acid by 15.54% and 21.0% respectively compared to the control value. In Giza 6, Nano-Se at high concentration decreased stearic, behenic, archidic and lignoseric acids by 8.33%, 21.64%, 20.84% and 100% as well as declined oleic and linolenic by 13.70% and 8.94% but increased linoleic by 29.43% compared to the corresponding control values. Interestingly, according to the present results, Nano-Se was found to have a positive impact on the oil quality of the studied groundnut cultivars, indicating a functional role of this element. Such positive role may be achieved by Se via protein-oil interaction [37] or via activation of appropriate enzymes.

3.4. Protein pattern

Comparison of the protein patterns of yielded seeds of Nano-Se treated groundnut cultivars revealed many differences in the protein bands (Table 3 & Fig. 2). In NC cv., Nano-Se concentrations (20 ppm and 40 ppm) caused disappearance of the proteins with molecular weight 109, 59, 50 and 18 KDa but induced the synthesis of new proteins with molecular weight 53 and 40 KDa. Only at low Nano-Se concentration, original proteins with molecular weight 40 and 15 KD were disappeared. On the other side, only at high Nano-Se concentration (40 ppm), protein band with molecular weight 64 KDa was missed. In Gregory cv., 20 ppm Nano-Se treatment caused disappearance of proteins with molecular weight 115KD, 50KD, 30 KD, 25 KD, 18 KD, 15KD and induced formation of proteins with molecular weight 75 KD, 53 KD and 40 KD. Unfortunately, protein bands of yielded seeds originated from 40 ppm Nano-Se treated plants were not analyzed. Regarding Giza 6, both Nano-Se concentrations (20 ppm and 40 ppm) caused loss of proteins with molecular weight 115, 50 and 18 KDa, in the same time resulted in formation of new proteins with molecular weight 75 KDa and 53 KDa. Only at 40 ppm Nano-Se, the proteins with molecular weight 35 and 85 KDa were missed. The depletion or synthesis of proteins as a result of Nano-Se application indicated that Nano-Se substitute sulphur element in S-containing proteins leading to incorrect folding and destroy protein structure [38].

3.5. Total antioxidant

The results presented in (Fig. 3) showed that total antioxidant in yielded seeds decreased significantly ($P < 0.05$) in NC cv. of groundnut plants and non-significantly ($P > 0.05$) in Gregory cv. by exposure to Nano-Se. on the other hand, in Giza 6 cv., total antioxidant increased ($P < 0.05$) in response to Nano-se application, especially at high concentration as compared with control values. These results may be due to the positive role of Nano-Se on lipid peroxidation and antioxidant enzymes, i.e., Catalase (Cat), ascorbate peroxidase (APX) and peroxidase (POX), as indicated by the increment in POX activities and the general decrement in Cat, APX [2].

3.6. Cytotoxicity of Nano-Se on two types of human Cell Line

The MTT results of the impact of seeds extract originated from Nano-Se treated plants on Bj1 cell line; normal human cell line derived from healthy skin tissue and Rep1 cell line, normal human cell line derived from pigmented epithelium tissue of Retina Eye are illustrated in Fig. 4(A&B). Most tested samples showed positive results in the growth inhibition percentages on Bj1 and Rep1cell lines, the positive response depending on the Nano-Se concentration and type of groundnut cultivar. Interestingly, the highest growth inhibition percentages in Bj1 and Rep1cell lines were detected ($P < 0.05$) with the seeds extracts of 20 ppm and 40 ppm Nano-Se treated Gregory cv. plants compared to those of other treated and untreated cultivars. In this concern, Nano-Se has ability

on increment the growth inhibition percentage of tested cell lines through induction of proteins with low molecular weight, increment of unsaturated fatty acids such as oleic and linolenic and decreased saturated fatty acids like palmitic and stearic fatty acids in yielded seeds of groundnut plant cv. Gregory. The results indicated that growth inhibition% of human cell lines associated with total antioxidant value in seed of Nano-Se treated plants and vice versa. Also, our results indicated that Nano-Se increases free radicles stress as indicated by the rise in total antioxidant and/or mission of low molecular weight proteins as well as changes in fatty acids composition, especially oleic and/or linolenic content. Additionally, Luo et al., 2012 recorded that Nano-Se inhibits the growth of human cells through induction of cell cycle arrest at S phase [39].

4. Conclusions

Nanoparticles of selenium have a potential antioxidant with reduced toxicity due to its redox state of zero (Se^0). Application of engineered Nano-Se in plant production and improvement has shown remarkable promising potential. Nano-Se showed higher inhibition of ROS which suggests the free radical scavenging efficiency of Nano-Se through increment of total antioxidant content reduced risk of Nano-Se on plants and human. Their exposure to plants has also altered fatty acid composition and protein signatures. Nano-Se may be applied in crop plants to increase the quality and quantity of yield associated with biosafety properties on plants and human health.

Declaration of Competing Interest

Authors declare that there are no conflicts of interest.

Acknowledgements

The authors express their sincere thanks to Faculty of Science (Girls Branch), Al-Azhar University and National Research Centre, Egypt, for providing all facilities to complete this work and deeply thanks to University College of Nairyah, Hafr El Batin University, Kingdom of Saudi Arabia for their supporting and providing.

References

[1] S.I. Ali, A.A. Mohamed, M.Y. Sameeh, O.M. Darwesh, T.M. Abd El-Razik, Gamma-irradiation affects volatile oil constituents, fatty acid composition and antimicrobial activity of fennel (*Foeniculum vulgare*) seeds extract, Res. J. Pharm. Biol. Chem. Sci. 7 (1) (2016) 524–532.
[2] H.A. Hussein, O.M. Darwesh, B.B. Mekki, Environmentally friendly nano-selenium to improve antioxidant system and growth of groundnut cultivars under sandy soil conditions, Biocatal. Agric. Biotechnol. 18 (2019) 1–7, doi: http://dx.doi.org/10.1016/j.bcab.2019.101080 101080.
[3] H.R. El-Ramady, E. Domokos-Szabolcsy, N.A. Abdalla, T.A. Alshaal, T.A. Shalaby, A. Sztrik, J. Prokisch, M. Fari, Selenium and nano-selenium in agroecosystems, Environ. Chem. Lett. 12 (2014) 495–510, doi:http://dx.doi.org/10.1007/s10311-014-0476-0.
[4] Z.O. Ardebili, N.O. Ardebili, S. Jalili, S. Safiallah, The modified qualities of basil plants by selenium and/or ascorbic acid, Turk. J. Bot. 39 (2015) 401–407, doi: http://dx.doi.org/10.3906/bot-1404-20.
[5] C. Jiang, C. Zu, J. Shen, F. Shao, T. Li, Effects of selenium on the growth and photosynthetic characteristics of flue-cured tobacco (*Nicotiana tabacum* L.), Acta Soc. Bot. Pol. 84 (2015) 71–77, doi:http://dx.doi.org/10.5586/asbp.2015.006.
[6] G.H. Lyons, Y. Genc, K. Soole, J. Stangoulis, F. Liu, R. Graham, Selenium increases seed production in *Brassica*, Plant Soil 318 (2009) 73–80, doi:http://dx.doi.org/10.1007/s11104-008-9818-7.
[7] M. Djanaguiraman, D. Devi, A. Shanker, J. Sheeba, U. Bangarusamy, Selenium an anti-oxidative protectant in soybean during senescence, Plant Soil 272 (2005) 77–86, doi:http://dx.doi.org/10.1007/s11104-004-4039-1.
[8] T. Feng, S. Chen, D. Gao, G. Liu, H. Bai, A. Li, L. Peng, Z. Ren, Selenium improves photosynthesis and protects photosystem II in pear (*Pyrus bretschneideri*), grape (*Vitis vinifera*), and peach (*Prunus persica*), Photosynthetica 53 (2015) 609–612, doi:http://dx.doi.org/10.1007/s11099-015-0118-1.
[9] I.E. Elshahawy, H.M. Abouelnasr, S.M. Lashin, O.M. Darwesh, First report of *Pythium aphanidermatum* infecting tomato in Egypt and controlling it using biogenic silver nanoparticles, J. Plant Prot. Res. 58 (2) (2018) 137–151.
[10] O.M. Darwesh, I.A. Matter, M.F. Eida, H. Moawad, Y. Oh, Influence of nitrogen source and growth phase on extracellular biosynthesis of silver nanoparticles using cultural filtrates of Scenedesmus obliquus, Appl. Sci. 9 (2019) 1–13, doi: http://dx.doi.org/10.3390/app9071465 1465.
[11] O.M. Darwesh, Y.Y. Sultan, M.M. Seif, D.A. Marrez, Bio-evaluation of crustacean and fungal nano-chitosan for applying as food ingredient, Tox. Rep. 5 (2018) 348–356.
[12] D.A. Marrez, A.E. Abdelhamid, O.M. Darwesh, Eco-friendly cellulose acetate green synthesized silver nano-composite as antibacterial packaging system for food safety, Food Packag. Shelf Life 20 (2019)100302.
[13] M.S. Hasanin, A.M. Mostafa, E.A. Mwafy, O.M. Darwesh, Eco-friendly cellulose nano fibers via first reported Egyptian Humicola fuscoatra Egyptia X4: isolation and characterization, Environ. Nanotechnol. Monit. Manag. 10 (2018) 409–418.
[14] O.M. Darwesh, I.A. Matter, M.F. Eida, Development of peroxidase enzyme immobilized magnetic nanoparticles for bioremediation of textile wastewater dye, J. Environ. Chem. Eng. 7 (2019)102805.
[15] H.E. Emam, O.M. Darwesh, R.M. Abdelhameed, In-Growth metal organic framework/synthetic hybrids as antimicrobial fabrics and its toxicity, Colloids Surf. B, Biointerface 165 (2018) 219–228.
[16] R.M. Abdelhameed, O.M. Darwesh, J. Rocha, A.M. Silva, IRMOF-3 biological activity enhancement by post-synthetic modification, Eur. J. Inorg. Chem. (2019) 1243–1249, doi:http://dx.doi.org/10.1002/ejic.201801442.
[17] F.K. El-Baz, K. Mahmoud, W.M. El-Senousy, O.M. Darwesh, A.E. El Gohary, Antiviral – antimicrobial and schistosomicidal activities of *Eucalyptus camaldulensis* essential oils, Int. J Pharm. Sci. Rev. Res. 31 (1) (2015) 262–268.
[18] M. Djanaguiraman, N. Belliraj, S.H. Bossmann, P.V. Prasad, High-temperature stress alleviation by selenium nanoparticle treatment in grain sorghum, ACS Omega 3 (3) (2018) 2479–2491.
[19] H. Wang, J. Zhang, H. Yu, Elemental selenium at nano size possesses lower toxicity without compromising the fundamental effect on selenoenzymes: comparison with selenomethionine in mice, Free Radic. Biol. Med. 42 (10) (2007) 1524–1533.
[20] M.S. Hasanin, O.M. Darwesh, I.A. Matter, H. El-Saied, Isolation and characterization of non-cellulolytic *Aspergillus flavus* EGYPTA5 exhibiting selective ligninolytic potential, Biocat. Agr. Biotechn. 17 (2019) 160–167, doi: http://dx.doi.org/10.1016/j.bcab.2018.11.012.
[21] A.M. Youssef, M.S. Hasanin, M.E. Abd El-Aziz, O.M. Darwesh, Green, economic, and partially biodegradable wood plastic composites via enzymatic surface modification of lignocellulosic fibers, Heliyon 5 (2019)e01332, doi:http://dx.doi.org/10.1016/j.heliyon.2019.e01332 1-22.
[22] A. O. C. S, Tentative Methods of the American Oil Chemists' Society, AOCS, Chicago, IL, 1998.
[23] H.T. Slover, E. Lanza, Quantitative analysis of food fatty acids by capillary gas chromatography, J. Am. Oil Chem. Soc. 56 (12) (1979) 933–939.
[24] R.J. Fido, E.N.C. Mills, N.M. Rigby, P.R. Shewry, Protein extraction from plant tissues. In protein purification protocols, Methods Mol. Biol. 244 (2004) 21–27.
[25] K.M. Barakat, M.Z. Mattar, S.Z. Sabae, O.M. Darwesh, S.H. Hassan, Production and characterization of bioactive pyocyanin pigment by marine *Pseudomonas aeruginosa* OSh1, Res. J. Pharm. Biol. Chem. Sci. 6 (5) (2015) 933–943.
[26] U.K. Laemmli, Cleavage of structural proteins during the assembly head of bacteriophage T4, Nature 227 (5259) (1970) 680–687.
[27] O.M. Darwesh, H. Moawad, O.S. Barakat, W.M. Abd El-Rahim, Bioremediation of textile reactive blue azo dye residues using nanobiotechnology approaches, Res. J. Pharm. Biol. Chem. Sci. 6 (1) (2015) 1202–1211.
[28] K.M. Barakat, S.W.M. Hassan, O.M. Darwesh, Biosurfactant production by haloalkaliphilic *Bacillus* strains isolated from Red Sea, Egypt, Egypt. J. Aquat. Res. 43 (2017) 205–211.
[29] W. Brand-Williams, M. Cuvelier, C. Berset, Use of free radical method to evaluate antioxidant activity, LWT-Food Sci. Technol. 28 (1) (1995) 25–30.
[30] A.A. Mohamed, S.I. Ali, O.M. Darwesh, S.M. El-Hallouty, M.Y. Sameeh, Chemical compositions, potential cytotoxic and antimicrobial activities of *nitraria retusa* methanolic extract sub-fractions, Int. J. Toxicol. Pharm. Res. 7 (4) (2015) 204–212.
[31] P. Samy, R.S. Ignacimuthu, A. Sen, Screening of 34 Indian medicinal plants for antibacterial properties, J. Ethnopharmacol. 62 (2) (1998) 173–182.
[32] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J Immun. Methods. 65 (1983) 55–63.
[33] O.M. Darwesh, M.F. Eida, I.A. Matter, Isolation, screening and optimization of L-asparaginase producing bacterial strains inhabiting agricultural soils, Biosci. Res. 15 (3) (2018) 2802–2812.
[34] V. Kumar, P. Guleria, V. Kumar, S.K. Yadav, Gold nanoparticle exposure induces growth and yield enhancement in Arabidopsis thaliana, Sci. Total Environ. 1 (2013) 462–468, doi:http://dx.doi.org/10.1016/j.scitotenv.2013.05.018 461–462.
[35] K.S. Siddiqi, A. Husen, Engineered gold nanoparticles and plant adaptation potential, Nanoscale Res. Let. 11 (1) (2016) 400–410, doi:http://dx.doi.org/10.1186/s11671-016-1607.
[36] M.H. Siddiqui, M.H. Al-Whaibi, M. Faisal, A.A. Al Sahli, Nano-silicon dioxide mitigates the adverse effects of salt stress on *Cucurbita pepo* L, Environ. Toxic. Chem. 33 (11) (2014) 2429–2437.

[37] N.A. Golubkina, T.V. Kiseleva, T.A. Krol, V.D. Strelets, Effect of sodium selenate on linseeds oil accumulation and its fatty acids composition, Fat Oil Ind. 3 (2012) 26–28.
[38] S. Kurokawa, M.J. Berry, Selenium. Role of the Essential Metalloid in Health. In Interrelations between Essential Metal Ions and Human Diseases, Springer, Dordrecht, 2013, pp. 499–534, doi:http://dx.doi.org/10.1007/978-94-007-7500-8_16.
[39] H. Luo, F. Wang, Y. Bai, T. Chen, W. Zheng, Selenium nanoparticles inhibit the growth of HeLa and MDA-MB-231 cells through induction of S phase arrest, Colloids Surf. B: Biointerface 94 (2012) 304–308.