EFFECT OF NANO-CHITOSAN ENCAPSULATED WITH ASCORBIC ACID AND GLUTATHIONE ON SOME BIOMOLECULES OF SUNFLOWER UNDER SALINE STRESS CONDITIONS

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A two seasoned 2020/2021-2021/2022 field experiment was performed in north Sinai governorate, Baloza experimental station, Egypt. Two sunflower varieties (Giza 102 and Sakha 53) were subjected to salinity stress and nano-chitosan encapsulated ascorbic acid (25.7 nm in size) treatments and modified ones with glutathione (48.8 nm in size) and 25 and 50 mg/L chitosan nanoparticles; CH NPs (CH25 and CH50) either individually or loaded with 1 mM glutathione (CH25@Glu and CH50@Glu), compared with the control. The DLS, SEM and FTIR were assigned for the prepared CH NPs. The significant highest increments in biological yield/feddan were obtained by CH50@Glu by Sakha 53 with the highest content of ῳ-3 fatty acid linolenic and low oleic acid, followed by CH25@Glu for the same variety. Contrarily, the highest biochemical parameters, such as antioxidant activity, were found in Giza 102 at CH25@Glu, followed by CH50@Glu, and the last dose of the same variety had the highest glutathione content. Sakha 53 variety treated with CH25 generated lesser harmful product, malondialdehyde. In the molecular level, there are no noticeable polymorphic changes in the plant genomic material with polymorphism average of 57.16% for Giza 102 and 46.16% for Sakha 53. So that, Sakha 53 is more stable than Giza102, it also has higher chlorophyll a content than Giza 102. The goal of this study was to compare the secondary metabolites, oil fatty acid composition, genetic uniformity, and productivity of two sunflower varieties under salinity stress and nano-CH treatments. Nano-CH encapsulated with ascorbic acid and loaded with glutathione can be used to maximize the utilization of Sakha 53 sunflower crop and this was related to some biochemical parameters (oil quality). The application of these recommendations in desert areas can play a role in reducing the food gap for edible oil in Egypt.

Keywords: phenolic acids, MDA, DNA, salinity, NPs, fatty acids

INTRODUCTION

Nanotechnology may be utilized to solve a variety of agricultural and environmental problems, as well as to address energy and resource restrictions, resource sustainability, overpopulation, and fertilizer application Parisi et al. (2015). Due to the obvious growing need for greater crop yields and more efficient ways for improving agricultural practices, the application of nanomaterials in agricultural research is on the rise (Gogos et al., 2012).

Evidently, nanoparticle (NP) molecules have the potential to have a significant impact on sustainable agriculture and precision agriculture by achieving maximum agricultural output (i.e., crop yields) while reducing inputs (i.e., fertilizers, pesticides, and herbicides) through environmental control and effective regulation. Because of their tiny size and increased surface area, NPs exhibit features that differ from bulk materials, such as enhanced solubility and surface reactivity (Cheng et al., 2014; Fraceto et al., 2016 and Seleiman et al., 2020).

Chitosan (CH) has developed as a biodegradable, nontoxic polymer with a wide range of agricultural and medicinal uses. Researchers have integrated chitosan-based NPs in a variety of products to improve their efficacy and biocompatibility as nanotechnology has emerged as a promising subject Bandara et al. (2020).

As a multi-purpose plant, sunflower (*Helianthus annuus* L.) is a species of the Asteraceae family grown commercially worldwide, offering a variety of nutritional and medicinal benefits. The sunflower seed, although used as a snack, salad garnish, and in some bakery goods, is primarily harvested for oil production, ranking in 4th position at world level (8% of 186 Mt oil in 2012) after palm (29%), soybean (22%), and oilseed rape (13%). The phenolic compounds, flavonoids, polyunsaturated fatty acids, and vitamins present in sunflower seeds and sprouts provide important antioxidant, antibacterial, anti-inflammatory, antihypertensive, wound-healing, and cardiovascular effects. In herbal remedies, it is used to cure a variety of diseases, including heart disease, bronchial, laryngeal, and pulmonary infections, coughs and colds, and whooping cough. These noteworthy medical, nutritional, and culinary properties have resulted in the sunflower and its constituent parts gaining historical and rising appeal across the world (Fowler, 2006; Bashir et al., 2015 and Nasim et al., 2016).

Sunflower has been established as a functional food or nutraceutical due to its beneficial health impacts, while its full potential has yet to be recognized. Sunflower contains dietary fiber, manganese, vitamins, tocopherols, phytosterols, triterpene glycosides, α-tocopherol, glutathione reductase, flavonoids, phenolic acids, carotenoids, peptides, chlorogenic acid, caffeic acid, alkaloids, tannins, and saponins, all of which contribute to their nutritional and functional development. Sunflower extract is considered to have anti-bacterial, anti-inflammatory, anti-cancer, and antioxidant properties

that protect human cells from damaging reactive oxygen molecules and pathogenic microbes. In addition, a pharmacological study on sunflower indicated that it has healing properties for a variety of disorders. Sunflower has a variety of health benefits, including blood pressure and diabetes control, skin protection, cholesterol reduction, and other functions (Adeleke and Babalola, 2020).

Salinity is one of the most common abiotic stresses that reduce agriculture productivity, and it is causing increased concern since saline conditions are becoming more common over the world, assisted by global warming. Salt has exacerbated almost 1.5 billion hectares of the world's rhizosphere, particularly in dry and semi-arid regions (Ittah et al., 2019). Food yield, quality, and quantity are all reduced as a result of salinity (Cuartero et al., 2006).

Ascorbic acid is one of the most significant antioxidants in plants, and it plays a crucial role in plant adaptability and resiliently to adverse environments. Ascorbic acid regulates the division and growth of the cells and improves the vegetative growth, which in turn is reflected in increasing yield and improving its quality (Paciolla et al., 2019).

Antioxidants are one of the most important components that protect plants from oxidative stress triggered by abiotic stressors. Glutathione (GSH; -glutamyl-cysteinyl-glycine) is a low molecular weight, water soluble thiol molecule found in most plant tissues and is one of the non-enzymatic antioxidants. Apart from its role in the storage and transport of reduced sulphur, GSH is involved in the detoxification of reactive oxygen species (ROS), either directly or indirectly. It functions as a cofactor in a variety of metabolic activities, interacts with plant hormones and signaling molecules, and stimulates signal transduction through its redox state (Foyer and Noctor 2005a and b).

Salinity, drought, high temperature, low temperature, and toxic metal stress are all abiotic stressors that glutathione improves plant tolerance to (Hasanuzzaman and Fujita, 2013). The focus of this research was to investigate how two sunflower (*Helianthus annuus* L.) varieties differed in antioxidant activity, biochemical components, phenolic profile, fatty acid composition, genetic uniformity and productivity under salinity stress. Also, a comparison was carried out between CH encapsulated ascorbic acid and coated in glutathione to maximize its benefits and reducing the adverse effects of salt stress in sunflower varieties.

MATERIALS AND METHODS

The soil of the experimental site was sandy (according to international textural grade) in texture, composing 89.12% sand, 6.34% silt and 4.54% clay with pH of 8.14, EC of 3.26 dS/m^2 and available nutrients of 25 mg/kg, 1.47 mg/kg P and 32 mg/kg K in the upper 0-30 cm soil layer (Table 1). The

mechanical and chemical analyses were performed according to Jackson (1958).

Table (1). Chemical analysis of the irrigation water and soil.

EC (dS/m^2)	pH	Chemical analysis of the irrigation water (well)							
		Cations (meq/L)				Anions (meq/L)			
		Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	CO_3^{2-}	HCO_3^{-}	Cl^{-}	SO_4^{2-}
7.96	7.91	1.09	1.79	5.06	1.23	=	1.20	7.23	0.72
Chemical analysis of the experimental soil									
EC (dS/m^2)	**pH**	**Ca^{2+}**	**Mg^{2+}**	**Na^{+}**	**K^{+}**	**CO_3^{2-}**	**HCO_3^{-}**	**Cl^{-}**	**SO_4^{2-}**
3.26	8.14	1.02	0.42	0.24	0.17	=	0.30	0.84	2.13

1. Planting and Treatments

Two varieties were used; Sakha 53 and Giza 102 and brought from Oil Crops Research Department, Field Crops Institute, Agricultural Research Center, Egypt, and cultivated in the middle of June at Baloza Research Station, North Sinai, Desert Research Center. Two foliar treatments were applied after 45 and 65 days from cultivation. The rate of seeds planting was 70 kg/faddan. The abbreviations of treatments were:

- Control
- 25 mg/L Chitosan NPs encapsulated ascorbic acid (CH25)
- 50 mg/L Chitosan NPs encapsulated ascorbic acid (CH50)
- 25 mg/L Chitosan NPs encapsulated ascorbic acid @ 1 mM glutathione (CH25@Glu)
- 50 mg/L Chitosan NPs encapsulated ascorbic acid @ 1 mM glutathione (CH50@Glu)

2. Preparation of Chitosan NPs Encapsulated Ascorbic acid and Conjugated with Glutathione

Chitosan NPs and encapsulated ascorbic acid were prepared using ionic gelation method (Calvo et al., 1997), with some modifications (Fig. 1). The benefits of ion gelatin method are its easy to process and conduct with simple steps, using aqueous environment, no heat consuming and can replace the risk of cross-linking reagents, it is also controllable and tunable as well as non-toxic.

Chitosan solution with concentration of 2000 mg/L in 1% acetic acid aqueous solution (1000 ml) was prepared. Ascorbic acid (4 g) was added to the CH solution under stirring for complete dissolving. Sodium tripolyphosphate (TPP) solution (0.66 g in 100 ml deionized water) was added dropwise to the above solution under magnetic stirring and after complete addition it was left under stirring for another 1 hour. By adding TPP anions to the as-prepared CH encapsulated ascorbic acid -GSH conjugate NPs, they were created. CH-based NPs were expected to develop via ionic gelatin between the positively charged amino groups of CH and the negatively

charged amino groups of TPP. However, it is possible that the amino group of the GSH moiety in the CH-GSH conjugate linked ionically with TPP anions, and this has to be investigated further.

For conjugation of glutathione in details, 1 mM glutathione, about 0.31 g was added to 1L CH NPs solution for each concentration and stirred for 1 hour. The obtained milky or white turbid suspension was indicated to the formation of CH NPs with their conjugated combination. The NPs were characterized using scanning electron microscope (SEM), dynamic light scattering (DLS) by Particle Sizing Systems, Inc. Santa Barbara, Calif., USA and Fourier Transform Infra-red (FTIR) measurement for the evaluation of their shape, size and function group as the previous literature of Mahdi et al. (2021a and b).

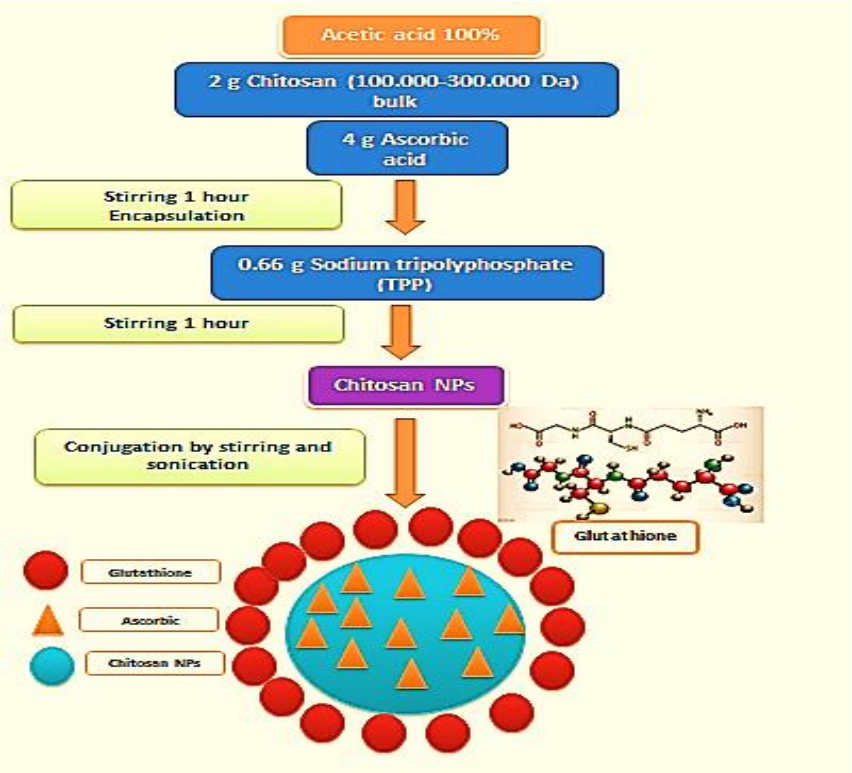


Fig. (1). Schematic diagram of chitosan nanoparticles encapsulated ascorbic acid and conjugated glutathione and the expected resulted molecules.

3. Biochemical Parameters

3.1. Lipid peroxidation assay

The level of lipid peroxidation in sunflower fresh leaf samples was measured in terms of estimating the end product, MDA (Heath and Packer, 1968) with some modifications. In brief, 0.5 g of plant fresh leaves was

homogenized in 2.5 ml of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 14,000 rpm, 4ºC for 15 min. Five % of thiobarbituric acid (TBA, 2.0 ml) was mixed with 20% TCA solution and the mixture was added to 0.5 ml of the liquid supernatant of leaf sample. The mixture was heated at 95°C for 30 min in a water bath and then incubated in an ice bath for 5 min. After centrifugation the supernatant was read at 532 nm and 600 nm for nonspecific turbidity of each sample also recorded and subtracted from the absorbance recorded at 532 nm. The concentration of MDA-TBA complex was calculated from MDA standard curve and converted to nmol/g fresh weight.

3.2. Antioxidant capacity

To investigate the scavenging potential of sunflower fresh leaves extract against the synthetic radical DPPH (2,2-di-phenyl-1-picrylhydrazyl), the following approach was followed: as a short explanation, 2 ml of a 0.004% solution of DPPH in absolute methanol was mixed with 0.5 ml of sunflower fresh leaves methanolic extract. The reaction solution was mixed and kept at room temperature for 30 min in the dark. The mixture was read at spectrophotometer, the absorbance was 517 nm according to the method of Oktay et al. (2003). The following formula was used to compute the DPPH radical scavenging rate:

$$RSC\ \% = (A_{blank} - A_{sample}) / (A_{blank}) \times 100$$

where RSC% = DPPH radical scavenging activity (%), Abs_{blank} is the absorbance of DPPH radical + methanol (2 ml DPPH and 0.5 ml methanol).

3.3. Glutathione reduced

In plant cells, glutathione is a tripeptide that interacts chemically with other free radicals. Under salinity stress, GSH protects the membrane structure by preventing acyl peroxide-induced lipid peroxidation (maintains membrane integrity). It aids in the detoxification of xenobiotics as a substrate for glutathione-S-transferase enzyme and a precursor of phytochelatin as a substrate for glutathione-S-transferase enzyme and a precursor of phytochelatines that function as heavy metals that bind peptides in plants. GSH is the most common non-protein thiol molecule found in plants and animals. Moron et al. (1979) method was used to determine GSH, which includes combining GSH with DTNB (5,5'-dithiobis nitro benzoic acid) to produce a yellow product that absorbs at 412 nm.

3.4. Peroxidase activity

About 0.5 g of fresh leaves was homogenized with 2.0 ml of 0.05 M sodium phosphate buffer (pH 7.0). The homogenate was centrifuged at 10.000 rpm at 4ºC for 10 min and the supernatant represented the crude enzyme source preserved at -20ºC until being used. Peroxidase was determined by using O–Dianisidine method according to Worthington Biochemical Corp (1972). Briefly, 1.5 ml of 0.01 M phosphate buffer (pH, 6.0), 10 µl of 1% O–dianisidine in absolute methanol was added and mixed; 0.1 ml of enzyme was added and mixed. Then 0.1 ml of 0.3% H_2O_2 was added; after mixing, the

increasing in absorbance was recorded kinetically at 460 nm for 3 min by a spectrophotometer as following: (change in Abs_{460}) / fresh weight / 3 min.

3.5. Chlorophyll content in leaves

Chlorophyll determination in leaves by Strain and Svec (1966), about, 1 g of leaves was extracted with acetone 90%. The extraction solution was kept in dark for 48 hours at room temperature. Samples were measured by spectrophotometer at 663, 645, chlorophyll a and b.

Chl a (µg/ml) = 11.64 (A663) - 2.16 (A645)

Ch b (µg/ml) = 20.97 (A645) - 3.94 (A663)

3.6. Fractionation of phenolic compounds by HPLC

The extraction was performed by 70% methanol according to Biswas et al. (2013). The system Thermo (Ultimate 3000) consisted of pump, automatic sample injector, and associated DELL-compatible computer supported with Cromelion7 interpretation program. A diode array detector DAD-3000 was used. The Thermo-hypersil reversed phase C18 column 2.5× 30 cm was operated at 25ºC. Mobile phase consists of 0.05% trifluoroacetic acid/acetonitrile (solvent A) and distilled water (solvent B). The UV absorption spectra of the standards as well as the samples were recorded in the range of 230–400 nm. Samples and standards solutions as well as the mobile phase were degassed and filtered through 0.45 µm membrane filter (millipore). Identification of the compounds was done by comparison of their retention's time and UV absorption spectrum with those of the standards.

Inj. Vol: 20 µl
Column: RP- C18
Column size: 2.5× 30 cm
Mobile phase: 0.05% trifluoroacetic acid/acetonitrile / H_2O
Flow rate: 1.0 ml/min
Temperature: 25°C
Detection: diode array

Time	Solvent (A)	Solvent (B)
0	18	82
5	20	80
12	40	60
20	18	82

3.7. Fatty acids fractionation by GLC

The fat content was determined using a Soxhlet extractor equipment and 250 ml of hexane at 60°C for 6 hours, followed by evaporation of the solvent. The seed oil was extracted and drained under a nitrogen stream (N_2) before being stored at -20°C until analysis (Mejri et al., 2020).

The GC model 7890B from Agilent Technologies was equipped with flame ionization detector (FID) at Central Laboratories Network, National Research Centre, and Cairo, Egypt. Separation was achieved using a Zebron

ZB-FAME column (60 m x 0.25 mm internal diameter x 0.25 µm film thickness). Analyses were carried out using hydrogen as the carrier gas at a flow rate of 1.8 ml/min at a split-1:50 mode, injection volume of 1 µl and the following temperature program: 100°C for 3 min; rising at 2.5°C /min to 240°C and held for 10 min. The injector and detector (FID) were held at 250°C and 285°C, respectively.

3.8. ISSR "Inter Simple Sequence Repeat"

3.8.1. DNA extraction and amplification

Total DNA was extracted from fresh leaves using DNeasy Plant Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions.

3.8.2. SSR-PCR reactions

Eleven ISSR primers were used in the detection of polymorphism (Table 2). The amplification reaction was carried out in 25 µl reaction volume containing 12.5 µl Master Mix (sigma), 2.5 µl primer (10 pcmol), 3 µl template DNA (10 ng) and 7 µl dH_2O, according to Ibrahim et al. (2019).

Table (2). The primers and sequences.

Primer Name	Sequence
ISSR-4	5'-ACACACACACACACACYG-3'
ISSR-5	5'-GTGTGTGTGTGTGTGTYG-3'
ISSR-6	5'-CGCGATAGATAGATAGATA-3'
ISSR-7	5'-GACGATAGATAGATAGATA-3'
ISSR-8	5'-AGACAGACAGACAGACGC-3'
ISSR-9	5'-GATAGATAGATAGATAGC-3'
ISSR-10	5'-GACAGACAGACAGACAAT-3'
ISSR-14	5'-CTCCTCCTCCTCCTCTT-3'
ISSR-16	5'-TCTCTCTCTCTCTCTCA-3'

3.8.3. Thermocyling profile PCR

PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (*PE* Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94ºC. Each cycle consisted of a denaturation step at 94ºC for 1 min, an annealing step at 45ºC for 1 min, and an elongation step at 72ºC for 1.5 min. The primer extension segment was extended to 7 min at 72ºC in the final cycle.

3.8.4. Detection of the PCR products

The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts. PCR products were visualized on UV light and photographed using a Gel Documentation System (BIO-RAD 2000).

3.8.5. Data analysis

For ISSR analysis, only obvious and unambiguous bands were visually rated as present (1) or absent (0) for all samples, with polymorphic and monomorphic bands included in the final data sets. Following that, a

binary statistic matrix was created. The unweighted pair group approach with arithmetic averages was then used to generate Dice's similarity matrix coefficients between varieties (UPGMA). Using the PAST software Version 1.91, this matrix was used to create a phylogenetic tree (dendrogram) based on the Euclidean similarity index (Hammer et al., 2001).

4. Statistical Analysis

The data was subjected to two ways ANOVA and the differences between means at the 0.05 probability level were determined using Duncan's multiple range test. The SPSS software version 16 (Richmond, USA) was used as described by Dytham (1999).

RESULTS AND DISCUSSION

1. Characterization of Chitosan Encapsulated Ascorbic Acid and Coated with Glutathione

1.1. SEM micrographs of chitosan encapsulated ascorbic acid nanoparticles

Scanning electronic microscope of CH NPs encapsulated ascorbic acid in Fig. (2) proposed amorphous shaped CH NPs and small particles of ascorbic acid. In Fig. (3), the loading of glutathione has changed the CH NPs in shape which was clear in the graphs. Research results about *in situ* formulated CH NPs with biological substrates were compatible in some extent with Abo El-Fadl et al. (2022) and Ramadan et al. (2022).

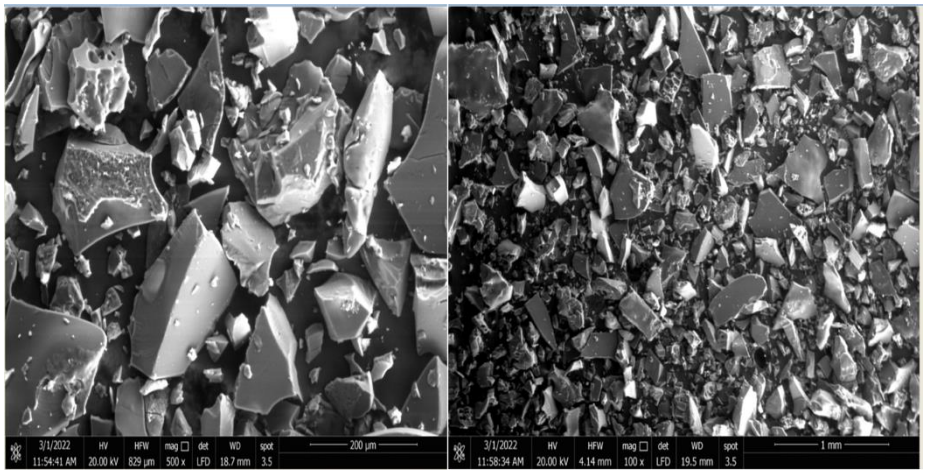


Fig. (2). SEM image of chitosan nanoparticles encapsulated ascorbic acid.

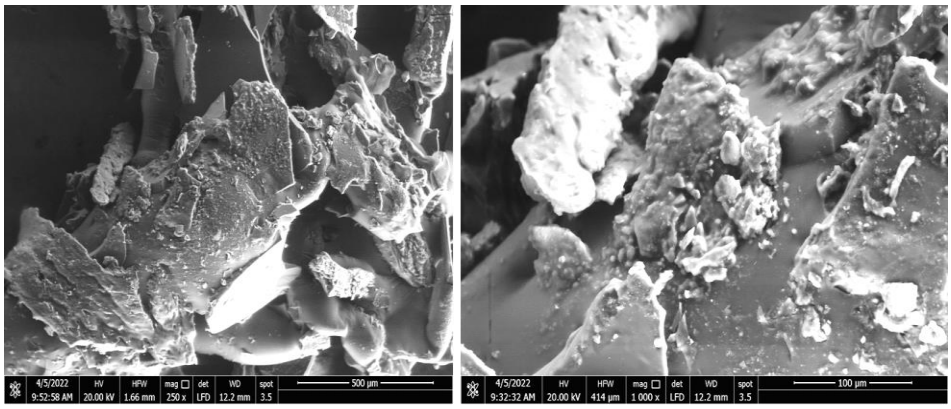


Fig. (3). SEM image of chitosan nanoparticles encapsulated ascorbic acid and coated with glutathione.

1.2. Particle size of chitosan nanoparticles encapsulated ascorbic acid and loaded with glutathione

According to Fig. (4), the dynamic light scattering was explored in a and b in the graphs below. The CH encapsulated ascorbic gave 25.7 nm but the loading of glutathione shifted the graph and changed particles size to 48.8 nm, which related to glutathione loading onto nano-CH. This finding resembled the *in situ* preparations of Abo El-Fadl et al. (2022), in their work on CH encapsulated substrates.

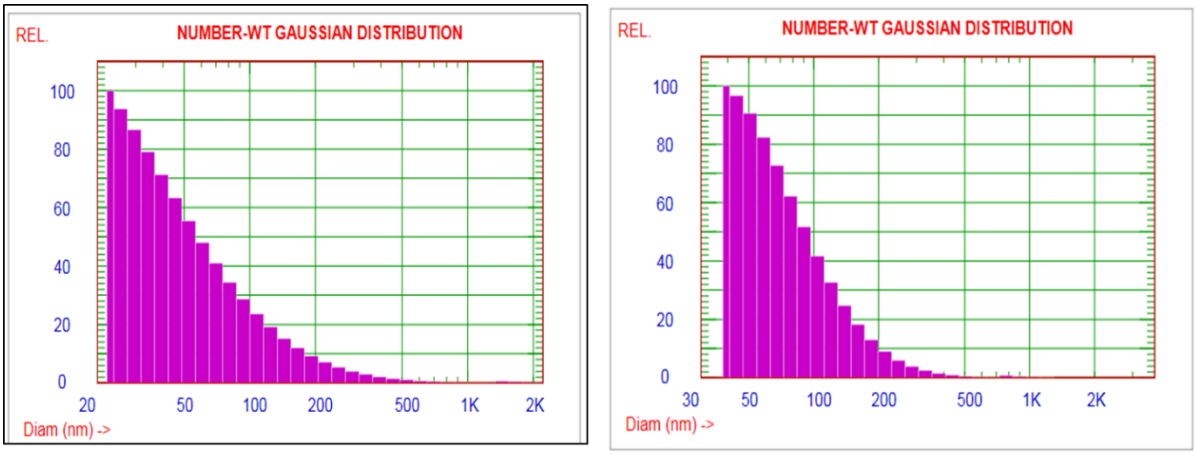
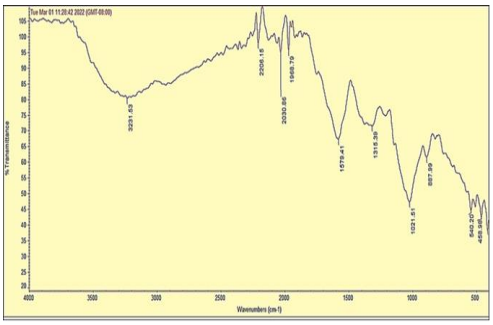


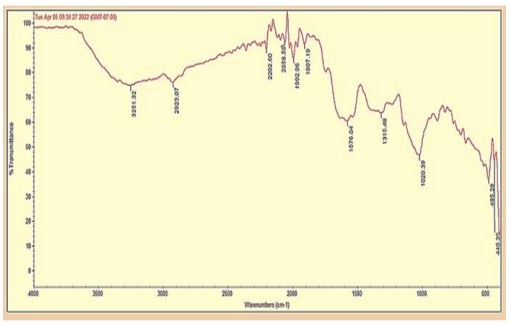
Fig. (4). Particle size and dynamic light scattering of chitosan nanoparticles encapsulated ascorbic acid.

1.3. FTIR of chitosan encapsulated ascorbic acid and loaded with glutathione

Regarding to Fig. (5), it clarified the function groups of CH NPs encapsulated ascorbic acid individually or loaded with glutathione. In the spectrum of CH NPs encapsulated ascorbic acid the function groups were assigned as represented in Table (3 and 4). According to Lustriane et al. (2018), It is clear from tables and graphs that there was a shifting in some peaks due to the loading of glutathione compound onto nano-CH.



FTIR spectrum of CH encapsulated ascorbic acid



FTIR spectrum of CH encapsulated ascorbic acid coated with Glu

Fig. (5). FTIR spectrum of chitosan nanoparticles encapsulated ascorbic acid**.**

Table (3). FTIR wave number and function groups of chitosan nanoparticles encapsulated ascorbic acid.

Wave number λ^{-1}	**Function group**
3231.53	OH/NH stretching (amine salt)
	O-H stretching (alcohol) intramolecular bonded
2206.15	C≡C stretching alkyne
2030.86	H_2C= C = CH_2 stretching allene
1968.79	C-H bending aromatic compounds
1579.41	N-O stretching
1315.39	O-H bending phenol
1021.51	C-O stretching in alcohol sulfoxide
887.99	C= C bending alkene
	N-H, glycoside bond (related to CH NPs)
540.20	
458.98	Alkyl halides

Table (4). FTIR wave number and function groups of chitosan nanoparticles encapsulated ascorbic acid loaded with glutathione.

Wave number λ^{-1}	Function group
3251.32	Strong broad, O-H stretching for alcohol, intramolecular bonded), O-H stretching, carboxylic acid
2923.07	C-H stretching alkane
2202.60	C≡C stretching alkyne
2058.50	Strong N=C=S stretching isothiocyanate (may be related to the glutathione loading)
1992.96	C-H bending, aromatic compound
1907.19	
1576.04	C=C stretching, cyclic alkene
1315.48	O-H bending, phenol
1020.39	C-N stretching, amine
	C-N stretching, amine
	C-O stretching, vinyl ether

2. Effect of Chitosan Encapsulated Ascorbic Acid on Growth Parameters and Biological Yield

Table (5) shows the mean values ± standard error and effects of chitosan encapsulated ascorbic acid (CH25 and CH50), as well as chitosan encapsulated ascorbic acid coated with glutathione (CH25@Glu and CH50@Glu), on the two sunflower varieties (Giza 102 and Sakha 53). All of the nano-applications described in the previous section had a significant effect on economic growth and yield characteristics. In terms of plant vegetative growth, CH50@Glu considerably achieved the highest mean value of plant height. The interaction between nano-treatments and varieties revealed that, Sakha 53 at CH50@Glu had the highest mean value of plant height. In respect to varieties, Sakha 53 variety outgrew Giza 102 in plant height. For fresh and dry weight characteristics, maximum mean record of them was determined by CH25. The interaction showed that, maximum mean value of fresh weight was detected by Sakha 53 at CH25, but for dry weight, the maximum mean value of it was detected in Giza 102 at the same CH concentration. The variety Giza 102 surpassed the dry weight of Sakha 53. Regarding the biological yield, the maximum mean value was detected by CH50@Glu, which related to the highest fresh weight. The interaction showed that, the highest mean value of yield was determined by Sakha 53 at CH50@Glu. Comparing the two varieties, the same variety was superior in yield parameters than Giza 102.

Salinity is a major stress that affects plant growth throughout the world. More than 20% of the world's agricultural fields are expected to have high salinity at a level that can significantly affect plant growth. The salt-

sensitive bean plant (*Phaseolus vulgaris*) showed better seed germination when treated with 0.1, 0.2 and 0.3% nano-CH at a salt concentration of 100 mM (Zayed et al. 2017). Because of its high surface to volume ratio, nano-CH may have a greater impact on these crops, resulting in increased penetrability and the ability to develop more contacts. Furthermore, the use of nano-CH on maize increased the amounts of organic molecules that act as stress tolerance regulators, such as phenols, aldehydes, and ketones. Plants also create additional antioxidants and antioxidative enzymes to counteract the greater levels of ROS produced during stress as a result of the disrupted electron transport chain, as well as express particular proteins with a defensive function, such as late embryogenesis abundant protein. During drought stress in Barley, nano-CH was found to increase the activity of antioxidative catalase (CAT) and superoxide dismutase (SOD) enzymes. As a result, nano-CH may work by activating antioxidant enzymes in plants and modulating osmo-protectant levels like proline. Chitosan is a linear polymer containing D-glucosamine and N-acetyl-D-glucosamine molecules linked by a 1,4-glycosidic bond and has been used in agriculture as a plant growth stimulant (Katiyar et al., 2014).

Furthermore, the stimulation of stomatal closure by bulk chitosan was demonstrated to have abscisic acid (ABA) dependent anti-transpirant effect. ABA is a well-known plant signaling molecule that builds up in response to diverse stressors, controlling stomatal closure and stress-responsive gene expression. As a result, it's probable that nano-CH has a role in modulating abiotic stress in plants via an ABA-dependent mechanism (Ma et al., 2012; Khati et al., 2017; and Behboudi et al., 2018). According to Fatemi (2014), the use of ascorbic acid increased sunflower productivity. Moreover, ascorbic acid (vitamin C) is an essential metabolite involved in many cellular processes, including cell division (De Gara et al., 2003).

Additionally, at the germination stage, sunflower seeds which primed in various concentrations of ascorbic acid showed superior saline tolerance than untreated seeds. In different study, Abou-Leila et al. (2012) found that ascorbic acid treatments under salinity circumstances have an increasing influence on some chosen ions, such as Mg^{++}, and a reducing effect on Na^{+} at the high salinity levels.

Table (5). Effect of chitosan nanoparticles on plant growth and yield parameters.

Treatment	Plant height (cm)		Mean	Fresh weight (g)		Mean	Dry weight (g)		Mean	Biological yield (kg/fad)		Mean
	Giza 102	Sakha 53		Giza 102	Sakha 53		Giza 102	Sakha 53		Giza 102	Sakha 53	
Control	122.00 ±	123.33 ±	122.67 ±	171.67 ±	148.33 ±	160.00 ±	75.53 ±	76.00 ±	75.77 ±	630.89 ±	704.97 ±	667.93 ±
	6.378 c	6.378 c	4.51 C	27.52 d	27.52 e	19.46 E	7.74 c	7.74 c	5.475 C	10.26 d	10.26 d	7.253 D
CH25	140.00 ±	142.33 ±	141.17 ±	476.67 ±	630.00 ±	553.33 ±	180.44 ±	171.90 ±	176.17 ±	852.87 ±	983.06 ±	917.96 ±
	6.378 b	6.378 b	4.51 B	27.52 b	27.52 a	19.46 A	7.74 a	7.74 a	5.475 A	10.26 d	10.26 c	7.253 C
CH50	143.00 ±	136.33 ±	139.67 ±	458.33 ±	494.00 ±	476.17 ±	112.28 ±	112.34 ±	112.31 ±	1029.00 ±	1096 ±	1062.0 ±
	6.378 a	6.378 b	4.51 C	27.52 b	27.52 a	19.46 B	7.74 b	7.74 b	5.475 B	10.26 b	10.26 b	7.253 B
CH25@Glu	115.33 ±	125.67 ±	120.50 ±	276.67 ±	143.33 ±	210.00 ±	64.34 ±	47.84 ±	56.088 ±	1010.00 ±	1129.00 ±	1069.0 ±
	6.378 d	6.378 c	4.51 C	27.52 c	27.52 d	19.46 CD	7.74 d	7.74 d	5.475 D	10.26 c	10.26 b	7.253 B
CH50@Glu	138.33 ±	173.33 ±	155.83 ±	279.33 ±	220.00 ±	249.67 ±	76.89 ±	83.11 ±	80.01 ±	932.50 ±	1270.00 ±	1101.0 ±
	6.378 b	6.387 a	4.51 A	27.52 c	27.52 cd	19.46 C	7.74 c	7.74 c	5.475 C	10.26 c	10.26 a	7.253 A
Mean	131.73 ±	140.20 ±		332.53 ±	327.13 ±		101.898 ±	98.24 ±		890.86 ±	1037.00 ±	
	2.85 B	2.85 A		12.31 A	12.31 A		3.46 A	3.46 B		4.587 B	4.587 A	

3. Effect of Chitosan Encapsulated Ascorbic Acid on Some Biochemical Parameters

Table (6) reveals the effect of encapsulated nano-CH on some biochemical parameters related to oxidative stress. Salinity is a major problem from abiotic stress and common with water deficit (Mahdi et al., 2020), so, using modified nano-CH significantly enhanced antioxidant scavenging system, lowering malondialdehyde content (MDA) (Etesami et al., 2021), glutathione content and increase antioxidant capacity.

Concerning the MDA content, the lowest record was determined by CH25. The interaction showed that, lowest value was detected by Sakha53 variety at CH25 which was bound to the higher fresh weight. The results were similar to those obtained by Mahdi et al. (2021a) on *Silybium marianum* by application of CH NPs. In the same table, glutathione level was also determined where, the maximum level of glutathione was recorded at CH50@Glu. The comparison between varieties reported the superiority of Giza102 and the interaction showed that, Giza102 recorded the maximum value of GSH was determined by CH50@Glu. In the same table (6), represented the antioxidant capacity, where the highest mean value was detected by CH25@Glu. The variety Giza102 exceeded Sakha53 in the antioxidant capacity. The interaction showed that, the highest mean value of antioxidant capacity was recorded by Giza102 in CH25@Glu.

Peroxidase activity was also elucidated in the same table, the maximum activity was determined by application of CH25@Glu which was linked with highest antioxidant capacity. Regarding to varieties, Giza102 surpassed Sakha53 in the peroxidase activity. The interaction between nano-CH and varieties revealed that, highest mean value of peroxidase was determined by Giza102 at CH25@Glu.

The boosting effect on sunflower biochemical parameters of the applied glutathione may be due to, it provides as a reservoir and transporter for reduced sulphur (Tausz et al., 2004). The researchers paid much more attention to GSH's role as an antioxidant. The central cysteine residue's nucleophilic nature gives GSH its strong reductive potential. It neutralizes

Table (6). Effect of encapsulated chitosan nanoparticles on some biochemical parameters.

Treatment	Biochemical parameters											
	Malondialdehyde (nmol/g fr.wt)		Mean	Glutathione µmol/g fr.wt		Mean	Antioxidant capacity		Mean	Peroxidase Δ abs/ fresh wt /3 min		Mean
	Giza 102	Sakha 53		Giza 102	Sakha 53		Giza 102	Sakha 53		Giza 102	Sakha 53	
Control	62.23 ±	50.01 ±	56.12 ±	11.30 ±	10.01 ±	10.66 ±	81.26 ±	75.05 ±	78.15 ±	25.22±	17.26 ±	21.52 ±
	0.273 a	0.273 a	0.193 A	0.132 d	0.132 d	0.093 D	0.249 b	0.249 d	0.176 D	0.189 b	0.189 c	0.134 E
CH25	47.07 ±	7.96 ±	27.52 ±	15.26 ±	13.07 ±	14.16 ±	77.37 ±	82.87 ±	80.12 ±	30.17 ±	14.03 ±	22.09 ±
	0.273 b	0.273 f	0.193 E	0.132 b	0.132 c	0.093 C	0.249 d	0.249 a	0.176 B	0.189 a	0.189 d	0.134 D
CH50	32.50 ±	33.48 ±	32.99 ±	12.06 ±	9.58 ±	10.82 ±	77.30 ±	80.673 ±	78.99 ±	25.94 ±	19.39 ±	22.66 ±
	0.273 c	0.273 c	0.193 C	0.132 c	0.132 e	0.093 D	0.249 d	0.249 b	0.176 C	0.189 b	0.189 c	0.134 C
CH25@Glu	11.28 ±	63.33 ±	37.31 ±	12.60 ±	18.30 ±	15.45 ±	86.78 ±	74.68 ±	80.73 ±	34.64 ±	23.83 ±	29.23 ±
	0.273 e	0.273 a	0.193 B	0.132 c	0.132 a	0.093 B	0.249 a	0.249 e	0.176 A	0.189 a	0.189 b	0.134 A
CH50@Glu	27.03 ±	30.87 ±	28.97 ±	25.61 ±	17.32 ±	21.47 ±	85.15 ±	73.62 ±	79.38 ±	25.64 ±	26.36 ±	26.00 ±
	0.273 d	0.273 d	0.193 D	0.132 a	0.132 a	0.093 A	0.249 a	0.249 e	0.176 C	0.189 b	0.189 b	0.134 B
Mean	36.03 ±	37.13 ±		15.37 ±	13.66 ±		81.57 ±	77.38 ±		28.32 ±	20.17 ±	
	0.122 A	0.122 A		0.059A	0.059 B		0.111 A	0.111 B		0.085 A	0.085 B	

harmful H_2O_2 and engages in non-enzymatic reactions with other ROS, such as hydroxyl, singlet oxygen, and superoxide radicals (Larson, 1988) Ascorbic acid has biochemical function, it is considered to be an important antioxidant to environmental stress, such as salinity, electron transport, and an enzymatic cofactor (Prasad and Upadhyay, 2011). It also relieves ROS (Dolatabadian et al., 2009).

4. Effect of Chitosan Encapsulated Ascorbic Acid on Chlorophyll Content

Salinity stress is one of the most damaging non-biological stresses in plants, ravaging on many agricultural regions throughout the world. By raising salt and chlorine ions in plant cells, salinity stress produces several morphological, physiological, epigenetic, and genetic alterations. Plants may ease this disorder to some extent through various mechanisms and restore the cell to its former state, but if the salt dose is too high, the plants may be unable to respond appropriately and withstand salt stress. Nowadays, scientists have proposed various answers to this problem. Nanotechnology is one of the most snipping and effective technologies to have emerged in this sector, with outstanding achievements.

Chitosan has a variety of unique features due to its amine and –OH groups, making it useful in a variety of applications and readily available for chemical reactions. Chitosan is a non-toxic substance that forms complexes and gels when it reacts with poly anions (Se and Niranjan, 2005). In table (7), chitosan encapsulated ascorbic acid led to significant increase in chlorophyll content especially applied CH25 for Cha and CH50 for Chb. The interaction showed that, maximum value of Cha and Chb achieved by Giza102 in treatment with CH50 and there was no significant difference between both varieties in Cha. However, Giza102 exceeded Sakha53 in Chb. The results were compatible with Van et al. (2013), Chitosan also can increase chlorophyll content and nutrient uptake of plants. CH NP synthesized via ionic gelation has been found to have a considerable impact on the biophysical characterization of coffee seedlings, with CH NP-treated seedlings exhibiting a large increase in pigment content, nutrient absorption, net photosynthetic rate, and CO_2 concentration. When compared to chitosan-treated and untreated seedlings, these benefits result in better growth (Van et al. 2013).

Since ascorbic acid has antioxidant properties, it is crucial in controlling many metabolic changes in plants that emerge from salt toxicity, such as stomatal regulation (Chen and Gallie, 2004).

Table (7). Effect of chitosan encapsulated ascorbic acid on chlorophyll content.

Chlorophyll content µg/g fresh weight						
Treatment	Chlorophyll a		Mean	Chlorophyll b		Mean
	Giza 102	Sakha 53		Giza 102	Sakha 53	
Control	46.04 ± 0.189 b	45.18 ± 0.189 c	45.61 ± 0.134 C	23.03 ± 0.137 c	22.27 ± 0.137 d	22.65 ± 0.097 D
CH25	46.01 ± 0.189 b	48.12 ± 0.189 a	47.06 ± 0.134 A	23.97 ± 0.137 c	22.38 ± 0.137 d	23.17 ± 0.097 C
CH50	49.79 ± 0.189 a	41.34 ± 0.189 d	45.57 ± 0.134 C	25.21 ± 0.137 a	23.89 ± 0.137 c	24.55 ± 0.097 A
CH25@Glu	46.49 ± 0.189 b	46.12 ± 0.189 b	46.30 ± 0.134 B	24.27 ± 0.137 b	23.79 ± 0.137 b	24.03 ± 0.097 B
CH50@Glu	41.53 ± 0.189 d	46.35 ± 0.189 b	43.94 ± 0.134 D	23.01 ± 0.137 c	22.36 ± 0.137 d	23.69 ± 0.097 C
Mean	45.97 ± 0.084 A	45.42 ± 0.084 A		23.89 ± 0.061 A	22.94 ± 0.061 B	

5. Effect of Chitosan Encapsulated Ascorbic Acid on HPLC Separated Phenolic Content

The presence of phenolic compounds in the major morphological portions of sunflowers, as well as the biological activity of some of them, leads to the conclusion that green sunflower plants can be used not only as an useful feed component, but also as a natural antioxidant source. Because the profile of secondary metabolites might change as a plant grows, it's important to keep track of them (Karamać et al., 2019).

Under salinity stress, a change of phenolic metabolites concentration may occur as a response to saline stress and varieties difference. From this direction, a significant enhancing effect was determined on some secondary metabolites represented by table (8) in dry leaves of sunflower plants. Clearly applied treatments enhanced most of the phenolic and flavonoids content which reflect the antioxidant importance of green part of sunflower plant. In table (8), the separated phenolics were quinic acid, apigenin, gallic acid, chlorogenic acid, cinnamic acid, resorcinol, vanillic acid, hesperidin, P-coumaric acid, ferulic acid, quercitin, kaempferol, ellagic acid**.** The response of the two varieties was differed to the chitosan encapsulated NPs. In Giza102 variety, the highest value in most indices was detected by CH50@Glu. In variety Sakha53, the loading with glutathione in CH25@Glu resulted in the maximum content of quinic acid but cinnamic acid and P-coumaric acid were recorded higher values by CH25 which may be related to higher chlorophyll a. the results went in the same line

with Shaker et al. (2020) where, treatment with different concentrations of chitosan raised IAA contents, which led to greater growth and generated more rises in phenol content in sunflower plants. The 50 mg/L chitosan level concentration gave the highest level of IAA and phenol. Also, the same was introduced by Ramadan et al. (2022), who used nano-CH encapsulated spermine which enhanced bioactive compounds in (*Capsicum annuum* L.) under salinity stress.

Table (8). Effect of chitosan encapsulated ascorbic acid on phenolic compounds.

Giza 102					
Phenolic compounds (mg/g)	**Control (mg/g dry wt.)**	**CH25**	**CH50**	**CH25@Glu**	**CH50@Glu**
Quinic acid	7.324	1.719	6.834	3.871	8.358
Apigenin	8.994	0.205	1.520	0.867	2.747
Gallic acid	0.656	N.D.	0.038	0.183	0.731
Chlorogenic acid	N.D.	0.014	0.158	0.263	0.530
Cinnamic acid	N.D.	N.D.	0.948	0.720	1.336
Resorcinol	N.D.	0.002	N.D.	N.D.	0.674
Vanillic acid	N.D.	N.D.	N.D.	N.D.	0.330
Hesperidin	N.D.	N.D.	N.D.	N.D.	N.D.
P-Coumaric acid	N.D.	0.004	0.206	0.629	0.741
Ferulic acid	N.D.	0.007	N.D.	N.D.	0.899
Quercetin	N.D.	0.153	0.469	N.D.	0.332
Kaempferol	N.D.	N.D.	N.D.	0.009	0.019
Ellagic acid	N.D.	N.D.	N.D.	N.D.	N.D.
Sakha 53					
Quinic acid	7.483	5.461	2.989	9.031	7.974
Apigenin	1.284	1.187	0.160	0.868	0.780
Gallic acid	N.D.	0.311	N.D.	N.D.	N.D.
Chlorogenic acid	0.029	0.111	0.049	0.029	N.D.
Cinnamic acid	0.213	0.623	0.560	0.275	0.168
Resorcinol	N.D.	0.238	0.129	0.148	N.D.
Vanillic acid	N.D.	N.D.	N.D.	N.D.	N.D.
Hesperidin	54.72	N.D.	N.D.	N.D.	N.D.
P-Coumaric acid	N.D.	1.454	0.629	N.D.	N.D.
Ferulic acid	0.008	N.D.	N.D.	0.035	0.068
Quercetin	0.014	N.D	0.573	0.105	0.301
Kaempferol	N.D.	0.016	0.011	N.D.	N.D.
Ellagic acid	N.D.	N.D.	N.D.	0.113	0.122

N.D.: not detected; below the detection limit of the device

In the same direction, Hassanen et al. (2021) applied coated chitosan NPs on *Silybium marianum* callus and induced accumulation of phenolic compounds. Additionally, the research results about in-Situ formulated Chitosan NPs were compatible in some extent with Abo El-Fadl et al. (2022), where encapsulation technique gave the maximum accumulation of polyphenolic components in avocado callus culture.

6. Effect of Chitosan Encapsulated Ascorbic Acid on Fatty Acids Content by GLC

There are three types of fatty acids in lipids, saturated fatty acids (SFAs—without double bonds), monounsaturated fatty acids (MUFAs—with one double bond), and polyunsaturated fatty acids (PUFAs—with two (FAs), o or up to six double bonds). SFAs are bad for your health, and eating too much of them can lead to heart disease, obesity, hypertension, and colon cancer. Unsaturated FAs, on the other hand, have health benefits such as lowering cholesterol and preventing cardiovascular disease (MUFAs), lowering insulin levels in diabetes, and stabilizing protein and mineral shortage in the human body [PUFAs with more than two double bonds] (Béligon et al., 2016).

Saturated fatty acids are fatty acids that have a single covalent connection between carbon-carbon atoms and are solid at normal temperature. The most important saturated fatty acids found in vegetable oils are lauric acid (C12:0), palmitic acid (C16:0), myristic acid (C14:0), stearic acid (C18:0), behenic acid (C22:0), and arachidic acid (C20:0). Unsaturated fatty acids are those that possess one or more covalent double bonds between carbon-carbon atoms at various points along the carbon chain. Saturated fatty acids can be generated in the human body, and they can be synthesized from molecules formed during carbohydrate metabolism even if no fat is consumed. Unsaturated fats, as a result, are vital fatty acids that the body need. They're liquid at room temperature, and the majority of them come from plants (Karaca and Aytac, 2007).

The fatty acid composition of sunflower seeds, which have a high importance in national production, should be determined. The separated sunflower fatty acids by GLC were, myristic acid, palmitic acid, palmitoleic acid, margaric acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid, *cis*-11-eicosenoic acid, behenic acid, tricosanoic acid, and lignoceric acid.

The profile of FAs of sunflower oil can be classified as low-oleic acid, medium-oleic acid, and high-oleic acid. Most of the oilseed crops contain polyphenols as endogenous antioxidants that prevent lipid oxidation. Natural antioxidants from the bioactive components of foods and the predominant phenolic compound in sunflower seeds are chlorogenic acid (Li et al., 2018).

Some plants contain natural antioxidants which help in scavenging free radicals against toxic molecules, reducing the risk of chronic diseases,

and cellular damage. Natural antioxidants from plants could be categorized as enzymes (catalase, glutathione dehydrogenase, and guaiacol peroxidase), peptides (reduced glutathione), carotenoids, and phenolic compounds (tocopherols, flavonoids, and phenolic acids).

Cerebrosides are neutral compounds that consist of ceramide (sphingosine and FA) and a monosaccharide bound by a β-glycosidic bond to the C1 of esfingol.

In table (9), figures (6 and 7), the application of nano-CH encapsulated ascorbic acid with or without glutathione under salinity circumstances makes a variation in determined fatty acids, and between the two sunflower varieties which may be bound with chlorophyll content as a source of acetyl CoA. In Giza102 variety, a marked decrease in myristic, palmitic, palmitoleic, margaric acid and stearic was detectable especially at CH50@Glu. Behenic acid highest content also was detected by CH50@Glu.

The monounsaturated fatty acid ῳ-9 oleic acid increased due to salinity and NPs application especially CH25. Moreover, *cis*-11-Eicosenoic acid also called gondoic acid as a monosaturated omega-9 maximum content achieved by CH25 and as a FA it is found in variety of plants oils and it is one of number of eicosenoic acids.

ῳ-6 highest content of linoleic acid was detected by CH50@Glu and highest content of arachidic acid by CH25@Glu.

As an ῳ-3 polyunsaturated fatty acid, linolenic acid which cannot synthesized by humans and very important for heart health, it remained unchanged when compared to control.

In same table (9), the application of CH encapsulated ascorbic acid NPs with or without glutathione with salinity makes a variation in determined fatty acids and between the two sunflower varieties. In Sakha53 variety, a noticeable increase in myristic, palmitic, palmitoleic, margaric acid and stearic especially at CH25@Glu and CH50@Glu. Lignoceric and Behenic acid highest content was detected by CH25

The monounsaturated fatty acid ῳ-9 oleic acid decreased due to NPs application especially CH50@Glu. In addition to that, *cis*-11-Eicosenoic acid also omega-9 maximum content decreased at most of treatments.

ῳ-6 highest content of linoleic acid was detected by CH25@Glu and highest content of arachidic acid by the same treatment. As an ῳ-3 polyunsaturated fatty acid, linolenic acid which can't be synthesized by humans and very important for heart health, it increased by CH50@Glu when compared to control.

It is noteworthy that, considering the absence of prior literature about chitosan encapsulated with ascorbic and coated with glutathione effect on saline stressed sunflower, it has been found that salinity has a massive effect on fatty acid composition: in saline conditions, oleic acid increased and linoleic acid decreased gradually with increasing salinity level due to salt-induced inhibition of the oleate desaturase enzyme, according to (Flagella et al., 2004).

Table (9). Effect of chitosan encapsulated ascorbic acid on fatty acids of seed oil.

Fatty acids %	Control area sum %	CH25	CH50	CH25@Glu	CH50@Glu
Giza 102					
Myristic acid C14:0	0.08	0.06	0.07	0.07	0.07
Palmitic acid C16:0	8.20	6.98	8.54	8.01	8.99
Palmitoleic acid C16:1	0.08	0.08	0.07	0.07	0.06
Margaric acid C17:0	0.03	0.02	0.03	0.03	0.02
Stearic acid C18:0	3.47	3.69	2.87	2.86	2.64
Oleic acid C18:1 cis	25.93	32.54	27.69	27.88	25.24
Linoleic acid C18:2 cis	61.59	55.86	60.19	60.43	62.30
Linolenic acid C18:3	0.06	0.06	0.05	0.06	0.05
Arachidic acid C20:0	0.15	0.18	0.14	0.18	0.16
***cis*-11-Eicosenoic acidC20:1 cis**	0.05	0.09	0.05	0.05	0.04
Behenic acid C22:0	0.13	0.20	0.18	0.19	0.29
Tricosanoic acid C23:0	0.06	0.02	0.04	0.04	0.03
Lignoceric acid C24:0	0.18	0.22	0.10	0.13	0.12
Sakha53					
Myristic acid C14:0	0.03	0.03	0.03	0.04	0.03
Palmitic acid C16:0	5.78	5.40	6.57	6.73	5.78
Palmitoleic acid C16:1	0.01	0.07	0.05	0.05	0.05
Margaric acid C17:0	0.04	0.03	0.03	0.02	0.03
Stearic acid C18:0	2.78	3.21	2.55	2.32	3.05
Oleic acid C18:1 cis	26.87	30.81	21.31	18.73	19.88
Linoleic acid C18:2 cis	63.67	59.02	68.90	71.61	70.40
Linolenic acid C18:3	0.08	0.08	0.08	0.08	0.09
Arachidic acid C20:0	0.17	0.25	0.11	0.13	0.17
***cis*-11-Eicosenoic acid C20:1 cis**	0.11	0.15	0.06	0.05	0.08
Behenic acid C22:0	0.28	0.66	0.16	0.16	0.26
Tricosanoic acid C23:0	0.04	0.03	0.03	0.01	0.01
Lignoceric acid C24:0	0.15	0.25	0.11	0.07	0.15

Unsaturated fatty acids (UFAs), which are alphatic carboxylic acids with one or more double bonds that are typically in the *cis* configuration, are essential for higher organisms. The most common 18-carbon (C18) species in plants are 18:1 (oleate), 18:2 (linoleate), and 18:3 (-linolenate), where m:n denotes an FA with m carbon atoms and n double bonds. These basic molecules play a variety of important activities and are intimately linked to both abiotic and biotic stressors. C18 UFAs serve as intrinsic antioxidants,

precursors of various bioactive molecules [typically the stress hormone jasmonic acid (JA)], and stocks of extracellular barrier constituents such as cutin and suberin, in addition to membrane ingredients and modulators in glycerolipids and carbon and energy reserve in triacylglycerols (TAGs) (He et al., 2018).

Tetracosanoic acid, often known as lignoceric acid, is a saturated fatty acid having the formula $C_{23}H_{47}COOH$. It can be found in minor levels in most natural fats, as well as in hardwood tar and certain cerebrosides. This fatty acid is also produced as a byproduct of the lignin manufacturing process. (https://en.wikipedia.org/wiki/Lignoceric_acid).

Behenic acid (docosanoic acid) is a carboxylic acid, the saturated fatty acid with formula $C_{21}H_{43}COOH$ is commonly incorporated in commercial hair conditioners and moisturisers to give them their smoothing effects. It's also utilized in lubricating oils and paint removers as a solvent evaporation retarder. Its amide is used in detergents, floor polishes, and dripless candles as an anti-foaming ingredient.
(https://en.wikipedia.org/wiki/Behenic_acid#cite_note-lee-3)

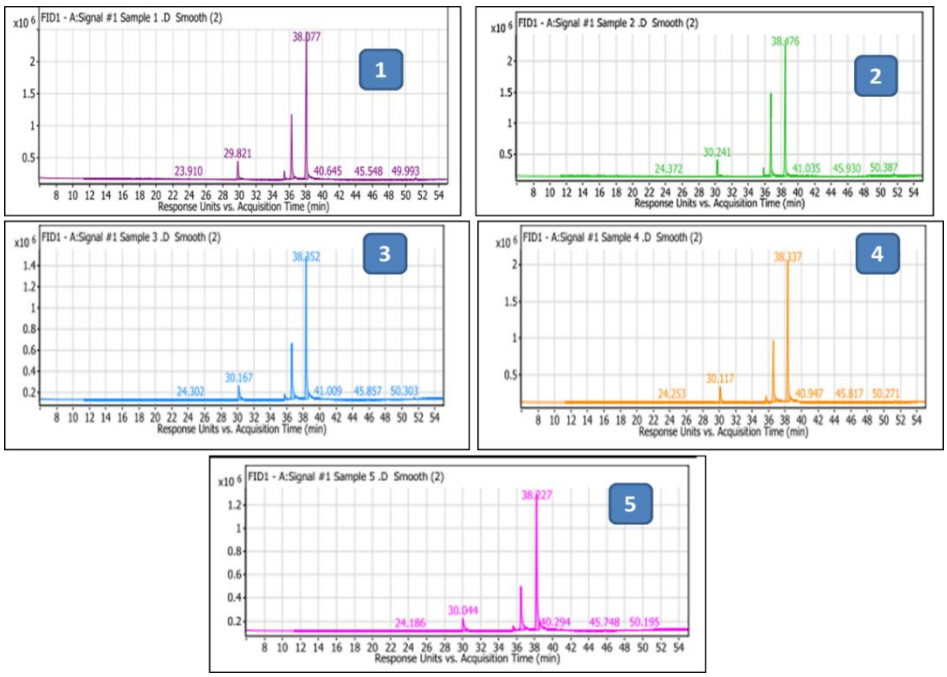


Fig. (6). Fatty acids in Giza102 separated by GLC as influenced by chitosan encapsulated nanoparticles.

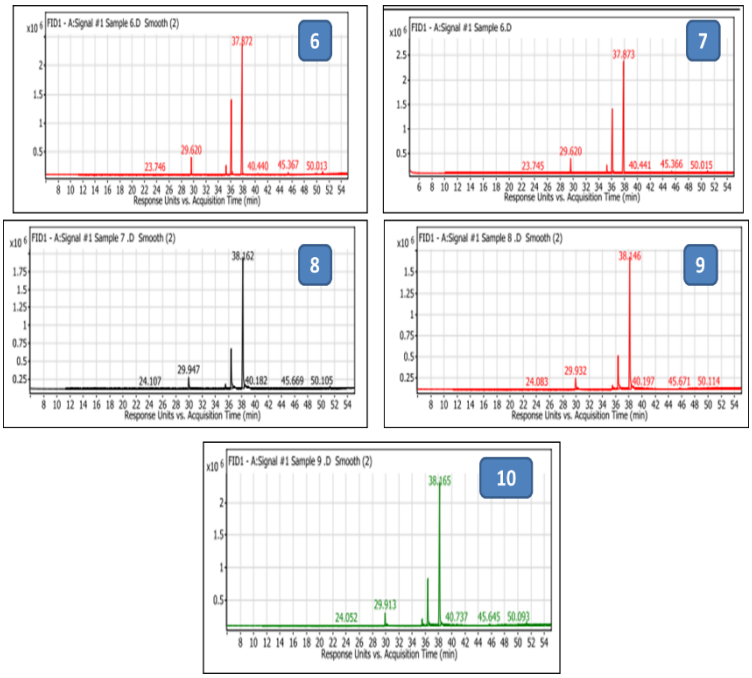


Fig. (7). Fatty acids in Sakha53 separated by GLC as influenced by chitosan encapsulated nanoparticles.

7. Effect of Chitosan Encapsulated Ascorbic Acid on Genetic Stability of Varieties

Many publications have used the inter-simple sequence (ISSR-PCR) approach to confirm genetic similarities, including Mahdi et al. (2021a and b). Figure (8,9,10,11) and table (10) display the results of nine ISSR-DNA primers for the two sunflower varieties.

The nine AFs were ISSR-4 (690 – 190 bp), ISSR-5 (430 – 230 bp), ISSR-6 (840 – 280 bp), ISSR-7 (1000 – 240 bp), ISSR-8 (450 – 180 bp), ISSR-9 (1200 – 230 bp), ISSR-10 (550 – 280 bp), ISSR-14 (750 – 200 bp) and ISSR-16 (1448 – 216 bp). The presence of unique bands may be due to the reaction of CH NPs with DNA strands or the impact of salinity stress.

Regarding to Giza102 variety, there were 25 monomorphic bands, 27 polymorphic with unique AFs, 36 polymorphic with unique, 9 unique bands and these unique fragments were resulted from, ISSR-5 which gave 2 unique amplicons at (770, 1000 bp) when plant treated with formulated CH25@Glu, ISSR-8 (350 bp) by treatment with CH25@Glu, one amplicon at 1300 bp produced from ISSR-10 when Giza102 treated with CH50@Glu. ISSR-6 gave (280bp), by application of CH50 and ISSR-7 gave unique amplicons at (950, 460 and 340bp) when plant treated by CH25@Glu and AF 440bp by

Table (10). polymorphism of two sunflower varieties subjected to chitosan encapsulated nanoparticles.

Primers	Bands range (kbp)	Number of monomorphic bands	Polymorphic without unique bands	Unique bands	Polymorphic with unique bands	Total number of bands	Polymorphism %	Means of band frequency
Giza 102								
ISSR-4	690 - 190	3	4	0	4	7	57	0.8
ISSR-5	430 - 230	4	8	2	10	14	71	0.6
ISSR-6	840 - 280	3	2	1	3	6	50	0.7
ISSR-7	1000 - 240	6	5	4	9	15	60	0.7
ISSR-8	450 - 180	4	3	1	4	8	50	0.7
ISSR-10	550 - 280	5	5	1	6	11	55	0.7
Sakha 53								
ISSR-4	690 - 190	6	1	1	2	8	25	0.9
ISSR-5	430 - 230	5	1	1	2	7	29	0.8
ISSR-8	450 - 180	5	1	0	1	6	17	0.9
ISSR-9	1200 - 230	7	6	0	6	13	46	0.8
ISSR-14	750 - 200	2	5	1	6	8	75	0.7
ISSR-16	1448 – 216	2	8	3	11	13	85	0.5

CH50@Glu. The polymorphism was changeable from primer to another as elucidated in table (8), by ISSR-5 where the highest polymorphism was 71% and least one 50% by ISSR-8 and the average polymorphism for all tested primers was 57.16%. In Sakha53, there were about 25 monomorphic bands resulted from all used primers, 22 polymorphic without unique bands, 28 polymorphic with unique, 6 unique bands resulted from ISSR-5 gave about 1 unique AF (170 bp) when treated with CH50@Glu. ISSR-5 gave the unique band at 220 bp when applied CH50@Glu, ISSR-14 gave 1 unique band at 750 bp in CH25. The ISSR-16 gave 3 unique bands at 1448 bp (CH50@Glu), 1087 bp (control), 614 bp (CH50@Glu).

Table (10) shows that the polymorphism may be switched from one primer to another. The highest polymorphism was 85% achieved by ISSR-16 in Sakha53 and least percent of polymorphism was detected by ISSR-8 (17%) in same variety. In this molecular study, the average polymorphism in Giza102 was 57.16% however, in Sakha53 is 46.16% so that, Sakha53 is stable than Giza102 under salinity conditions and applied CH NPs. The cluster analysis was also determined in figure (8 and 10) for both varieties.

An effective biomarker assay for determining the mutagenicity NPs application on plants is DNA fingerprinting (Cenkci et al., 2009). It has been effectively employed to determine changes in DNA fingerprints that represent DNA variations in the genome using molecular markers including RAPD, ISSR, and SRAP (Salama et al. 2019). Because they are simpler to use, less expensive, quicker, and do not require knowledge of genomic sequences, ISSRs are commonly employed (Semagn et al. 2006). Using DNA-based markers called ISSRs, polymorphisms in inter-microsatellite loci can be detected, and the resulting DNA profiles can vary as a result of the appearance of new bands, the absence of bands, or changes in band intensities (Izzatullayeva et al., 2014).

Despite exposure time and concentration have a direct correlation, it has been demonstrated that NP size generally has an inverse relationship with genotoxicity as in this investigation on sunflower plants. Fouda et al. (2021) worked on *Salvadora persica,* and the changes in the ISSR profile revealed that the NPs administered doses created genetic variation that was not beneficial.

Giza 102	Control	CH25	CH50	CH25 @Glu	CH50 @Glu
Control	100				
CH25	88	100			
CH50	84	93	100		
CH25@Glu	72	80	83	100	
CH50@Glu	70	70	75	80	100

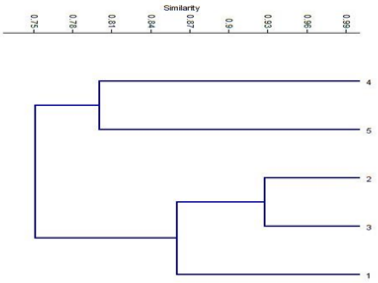


Fig. (8). Cluster analysis of treatments application on Giza 102 variety.

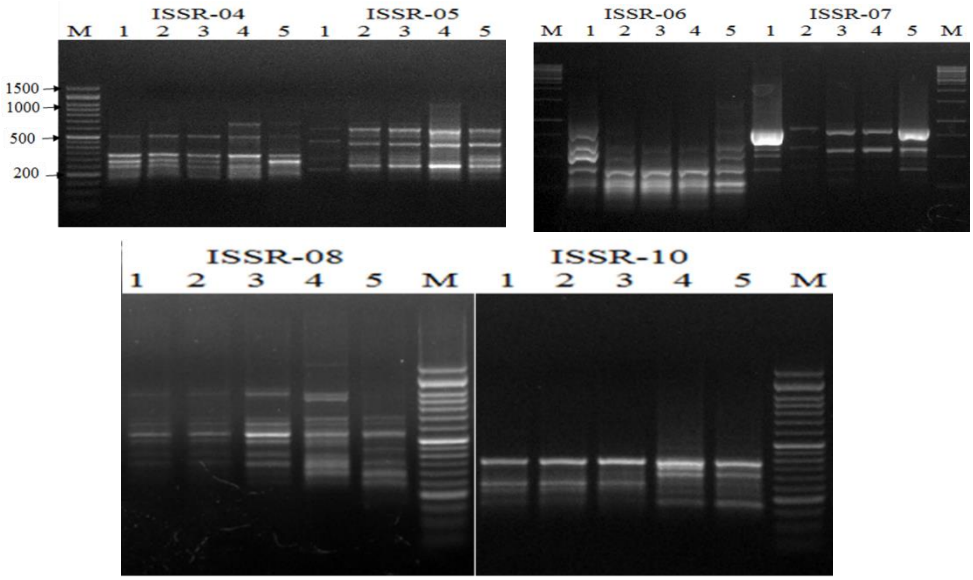


Fig. (9). ISSR PCR for Giza 102 variety as affected by applied CH NPs treatments, where M: marker, 1: control, 2: CH25, 3: CH50, 4: CH25@Glu, 5: CH50@Glu.

Sakha 53	Control	CH25	CH50	CH25 @Glu	CH50 @Glu
Control	**100**				
CH25	**84**	**100**			
CH50	**83**	**89**	**100**		
CH25@Glu	**79**	**86**	**87**	**100**	
CH50@Glu	**76**	**75**	**79**	**80**	**100**

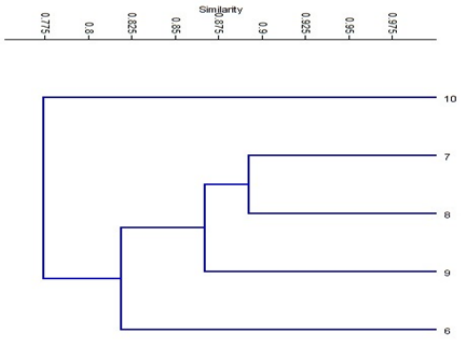


Fig. (10). Cluster analysis of treatments application on Sakha 53 variety.

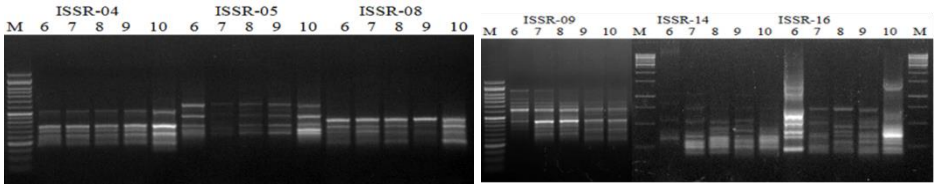


Fig. (11). ISSR PCR for Sakha 53 variety as affected by applied CH NPs treatments, where M: marker, 6: control, 7: CH25, 8: CH50, 9: CH25@Glu, 10: CH50@Glu.

CONCLUSION

In conclusion, the goal of the research was succeeded where the addition of glutathione to the prepared nano-encapsulated CH NPs with ascorbic acid changed the SEM graphs, FTIR spectrum and dynamic light scattering related numbers. It is clear that coating with glutathione as a tripeptide had a beneficial effect than individual encapsulated nano-CH. Where, it gave higher biological economical yield of variety Sakha 53 under salinity stress so, this may be a way of decreasing the gap between consuming

and production of oil crops, and higher omega-3 fatty acid linolenic with lower omega-6 fatty oleic acid. Also, the DNA polymorphism was lesser in Sakha 53 variety (46.16%).

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تأثير الشيتوزان النانوي المغلف بالأسكوربيك والجلوتاثيون على بعض الجزيئات الحيوية لدوار الشمس تحت ظروف الإجهاد الملحي

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أجريت تجربة حقلية فى الفترة من ٢٠٢٠/٢٠٢١ و٢٠٢١/٢٠٢٢ بمحافظة شمال سيناء بمحطة بالوظة التجريبية بمصر. تم إجراء هذا البحث على صنفين من دوار الشمس (جيزة ١٠٢ وسخا ٥٣) حيث تم زراعتهما وتعرضوا لإجهاد الملوحة والجسيمات النانوية من الشيتوزان المكبسل بحمض الأسكوربيك (CH) بحجم ٢٥.٧ نانومتر ونفس المعاملات ولكن محمل عليها الجلوتاثيون بحجم ٤٨.٨ نانومتر (٢٥ مجم/لتر CH NPs، ٥٠ مجم/لتر CH NPs، ٢٥ مجم/لتر CH NPs محملة ب ١ ملي مولار الجلوتاثيون، ٥٠ مجم/لتر CH NPs محملة ب ١ ملي مولار الجلوتاثيون) مقارنة بالكونترول. تم عمل توصيف للنواتج بتقنية DLS وSEM وFTIR لجسيمان الشيتوزان النانوية الناتجة بعد التحضير. من أفضل النتائج، الحصول على أعلى زيادات معنوية في المحصول الإقتصادي/الفدان بواسطة ٥٠ مجم/لتر CH NPs محملة ب ١ ملي مولار الجلوتاثيون للصنف سخا ٥٣ وزيادة الحمض الدهني لينولينيك المصنف ضمن أوميجا٣ ومنخفض في الأوليك متبوعًا بـ ٢٥ مجم/لتر CH NPs محملة ب ١ ملي مولار الجلوتاثيون لنفس الصنف. في المقابل ، كانت أفضل الصفات البيوكيميائية مثل نشاط مضادات الأكسدة في جيزة ١٠٢ نحو ٢٥ مجم/لتر CH NPs محملة ب ١ ملي مولار الجلوتاثيون متبوعًا بـ ٥٠ مجم/لتر CH NPs محملة ب ١ ملي مولار الجلوتاثيون ، وكان أعلى محتوى من الجلوتاثيون لنفس الجرعة السابقة لنفس الصنف. تم الحصول على أقل منتج سمية malondialdehyde بواسطة الصنف سخا ٥٣53 المعالج بـ ٢٥ مجم/لتر CH NPs عند مقارنته بالكونترول والمعاملات الأخرى. على المستوى الجزيئي، لا توجد تغيرات ملحوظة في المادة الجينية النباتية متعددة الأشكال بمتوسط تعدد الأشكال ٥٧.١٦٪ في جيزة ١٠٢ و٤٧.١٦٪ في سخا ٥٣ لجميع المعاملات، بحيث تكون سخا ٥٣ أكثر استقرارًا وراثيًا من جيزة ١٠٢ تحت تطبيق المعاملات النانوية سالفة الذكر. إن إستخدام الشيتوزان النانوي المغلف بالأسكوربيك ومحمل بالجلوتاثيون يعظم الإستفادة من محصول دوار الشمس (سخا ٥٣) وإرتباطه ببعض المؤشرات الكيميائية الحيوية وجودة الزيت. كما أن تطبيق هذه التوصيات في المناطق الصحراوية يمكن أن يلعب دورًا هامًا في تقليص الفجوة الغذائية لزيت الطعام في مصر.