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Effect of nano chitosan encapsulated spermine on growth, productivity and bioactive compounds of chili pepper (*Capsicum annuum* L.) under salinity stress

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Abstract

This study aimed to investigate the effects of chitosan nanoparticles (C-NPs), spermine (Spm) as well as chitosan nanoparticles encapsulated spermine (C-Spm NPs) on growth, yield, chlorophyll, bioactive compounds, and capsaicinoids in chili pepper cv. Omiga F1 under saline stress conditions in Ras Sudr region, Egypt, in years 2020-2021. Data indicated that the exogenous application of C-NPs, Spm, and C-Spm NPs alleviated the adverse effect of salinity on chili pepper plants and enhanced growth, yield, chlorophyll content, antioxidant enzymes activity superoxide dismutase (SOD), and catalase (CAT). The maximum values of these parameters were recorded when plants treated with C-Spm NPs at 1.5 mM. For GC/MS analysis, data revealed the presence of 22 active compounds whereas, the maximum area percent were, 1-Tetradecanol(CAS), Phenol,2,4-bis(1,1-dimethylethyl), 1-Heptadecene(CAS), 3-Eicosene,(E), (cis)2-nonadecene, and 5-Eicosene,(E). Regarding the capsaicinoids found in chili pepper, the main compounds identified among the capsaicinoids were capsaicin and dihydrocapsaicin. The maximum amount of capsaicin and dihydrocapsaicin were recorded when plants treated with C-Spm NPs at 0.5 mM. For the general taste of chili pepper, the Scoville heat unit (SHU) was calculated. Data indicated that plants treated with C-Spm NPs at 0.5 mM gave quite a high SHU related to higher contents of the capsaicinoids.

Keywords: Chili pepper, Chitosan nanoparticles, Spermine encapsulation, Salinity, Capsaicinoids, Bioactive compounds.

1. Introduction

Salinity is conditions that occur mainly in arid and semiarid regions; it is one of the major abiotic stresses that adversely affect the crop productivity and quality [1, 2, 3, 4]. Salinity affects almost all aspects of plant growth; it imposes ionic toxicity, osmotic stress, nutrient deficiency, and oxidative stress in plants, and therefore, limits the absorption of soil water [5]. Thus, improving salinity stress tolerance in crops is of paramount importance. To achieve this goal, it is necessary improve complex mechanism systems for adapting osmotic and ionic stresses caused by high salinity.

Chili pepper (*Capsicum annuum* L.) is an important vegetable crop which has a high economic value in Egypt, both for international export and national consumption. The pepper plant is not a salt-tolerant vegetable, and about 14% of fruit yield loss occurs as a result of each increase in salt level of 1.0 dS/m [6]. Numerous studies have been conducted to alleviate the harmful impact of salinity stress on chili pepper, but most have not been sufficient or broadly applicable. As a result, searching for cheap and ecologically-friendly strategies for salinity amelioration which enhance the growth and productivity of pepper has been very important to the agriculture sector [7]. Several researches have shown that nanotechnology has improved the application of exogenous nanoparticles on plants to fight

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environmental difficulties such as salt stress on wheat [8-10] and garlic [11].

Bio-stimulators as biological methods to overcome injurious impact of salinity in agriculture have received considerable attention, among it are chitosan nanoparticle and polyamines (spermine). Chitosan nanoparticle is a natural material with excellent physicochemical properties, making it a superior environmentally friendly material, and it possesses bioactivity that does not harm humans. Nanomaterials hold great promise regarding their application in agriculture in terms of plant protection and nutrition due to their size-dependent qualities, high surface-to-volume ratio and unique optical properties [12]. Chitosan nanoparticle is one of the most popular nanomaterials which have been used in this field as plant growth promoter [13]. It has proved to be effective in many crops to protect plants against oxidative stress and increased plant leaf area [14], chlorophyll content, photosynthesis rate, catalase (CAT), and superoxide dismutase (SOD) activities, yield [15]. Chitosan nanoparticle improved growth, enhancer of physiological and biochemical characters and yield under salinity stress in *Phaseolus vulgaris* plant [16]. Polyamines such as spermine (Spm) have been proposed as a new category of plant growth regulators that are purported to be involved in a large spectrum of physiological processes, such as embryogenesis, cell division, morphogenesis, and development [17,18]. Polyamines are beneficial for protein homeostasis, detoxification of reactive oxygen species (ROS), activation of the antioxidative machinery, and molecular chaperone activity under stress conditions, thereby providing broad-spectrum tolerance against a variety of stresses [19] and to increase crop yield and quality without any negative effect for crops or the environment [20]. Foliar application of polyamines (spermine) ameliorated the negative effect of salinity on plant growth, has increased SOD and CAT activities and scavenge ROS, to protect chlorophyll content, photosynthesis parameters, and improve photosystem functioning [21]. Spermine applications have led to improvements in the growth characteristics, the activities of antioxidant enzymes and enhanced yield of faba bean under saline conditions [22].

In this context, our group has developed chitosan spermine nanoparticles wherein spermine was encapsulated into chitosan-TPP (sodium tripolyphosphate) nano-matrix for its slow release. The developed chitosan nanoparticles encapsulated spermine was holistically evaluated in comparison with its bulk counterparts (chitosan nanoparticles and spermine) for effects on growth, productivity and bioactive compounds in chili pepper plant under saline conditions. The improved growth and yield could be implicated to unique physico-chemical properties of chitosan nanoparticles-spermine and physiological biochemical response of chili pepper plant towards it.

2. Material and Methods

2.1. Synthesis of chitosan NPs and chitosan encapsulated spermine NPs

Chitosan nanoparticles (C-NPs) and chitosan encapsulated spermine nanoparticles (C-Spm NPs) were prepared using ionic gelation method [23]. Chitosan powder 5g was dissolved in 700 ml distilled water containing 10 ml acetic acid, under stirring. After complete dissolution 0.5 g spermine (Spm) was dissolved in warm distilled water (20 ml) and added gradually to chitosan solution with stirring for about 0.5 h. Sodium tripolyphosphate (2.5 g) was dissolved in 300 ml distilled water and added dropwisely to chitosan solution under stirring for about half an hour. During the addition of TPP; the solution turned onto turbid indicating the formation of C-Spm NPs After complete addition process; the nanoparticles dispersion remain under stirring for further one hour, then removed and stored in refrigerator at 4^{o}C until use. Blank C-NPs was prepared using the same sequences but without spermine.

2.2. Growth conditions

Field experiments were performed at Experimental Ras Sudr Station, Desert Research Center, Egypt (30°34'N, 31°34'E), during the growing seasons of 2020 and 2021 to investigate the effects of chitosan nanoparticles (C-NPs), spermine (Spm) as well as chitosan nanoparticles encapsulated spermine (C-Spm NPs) on growth, yield, chlorophyll, bioactive compounds, and capsaicinoids in chili pepper cv. Omiga F1 under saline conditions. The seeds of chili pepper cv. Omega F1 were obtained from Namdeo Umaji Agritech (India) Pvt. Ltd. Seeds were sown in the nursery using foam trays in the first week of March under greenhouse conditions in both seasons. Forty-five days after sowing, seedlings were transplanted into open field (14-15th April) in first and second season, respectively. The plants were treated three times (20, 40 and 60 days after transplanting) with control (water spray), C-NPs (0.5 mM), Spm (0.5 mM), C-Spm NPs (0.5, 1, 1.5 mM). The experiment was done in a completely randomized design with three replicates. The experimental plot area was 10.0 m^2 containing 5 rows each with 4 m in length and 50 cm width. The distance between plants was 50 cm. The organic fertilizer (48 m^3/ha) was added at soil preparation, whereas the chemical fertilizers were added in three equal doses as recommended (NPK, 238:95:190 kg/ha), the first dose was added at soil preparation, the second was added at 30 days after transplanting and the third at the beginning of flowering stage [24]. Plants were irrigated from the well with saline water

at 3-days intervals. The irrigation water and soil were analyzed and presented in Table (1) according to Burt [25].

Table (1): Irrigation water and soil chemical analysis

pH	EC mS/cm	Cations (meq/l)				Anions (meq/l)			
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{-}	HCO_3^{-}	Cl^{-}	SO_4^{--}
Irrigation water analysis									
8.52	11.81	20.11	14.66	128.32	2.31	-	7.50	105.1	41.6
Soil analysis									
8.72	9.88	5.8	4.5	96.35	1.56	-	5.63	67.5	28.4

2.3. Measurement of plant growth

For plant growth parameters, five plants were randomly taken from each experimental plot after 90 days from transplanting to record number of leaves per plant, leaf area, fresh and dry weight per shoot. Leaf area was determined [26].

2.4. Fruit yield and its components

The chili pepper fruits were hand-picked five times, when the fruits reach to the full size characteristic of the cultivar and before ripening and turning red. At each harvest, the number of fruits per plant, the fruit fresh weight (g), the fresh weight of fruits per plant (g) was determined. The total fruit yield (ton ha^{-1}) was recorded at the end of harvest (120 days after transplanting).

2.5. Biochemical analysis

2.5.1. Photosynthetic pigments

Chlorophyll a, b and carotenoids were extracted from fresh leaves of chili pepper in 85% acetone under dark until the leaves became colorless. The chlorophyll contents were measured by a spectrophotometer (model Unico UV/VIS- SQ2800) by taking the readings at 663, 644 and 452 nm [27].

2.5.2. Antioxidant enzymes activity (SOD and CAT)

For extraction of antioxidant enzymes, samples of chili pepper leaves of each plot was harvested and frozen in liquid nitrogen immediately for the analysis of SOD, and CAT activities. Briefly, 1g fresh leaf tissue was homogenized in a precooled mortar in 1 mL of a 50 mmol/L phosphate buffer (pH 7.8) solution. The homogenate was centrifuged at 11000 g for 15 min at 5ºC. The supernatant was used to determine enzyme activities (SOD and CAT) content.

2.5.2.1. Superoxide dismutase (SOD) activity

The superoxide dismutase (SOD) activity was determined by measuring the inhibition in photoreduction of nitroblue tetrazolium (NBT) by SOD enzyme [28]. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.6), 0.1 mM EDTA, 50 mM sodium carbonate, 50 µM NBT, 10 µM riboflavin and 100 µL of crude extract in a final volume of 3.0 ml. A control reaction was performed without crude extract. The SOD reaction was carried out by exposing the reaction mixture to white light for 15 min at room temperature. After 15 min incubation, absorbance was recorded at 560 nm using a spectrophotometer. The amount of enzyme producing 50% inhibition of photochemical reduction of NBT was defined as one unit (U) of SOD activity.

2.5.2.2. Catalase (CAT) activity

Catalase (CAT) activity was measured spectrophotometrically according to Aebi [29] at room temperature by monitoring the decrease in absorbance at 240 nm resulting from the decomposition of H_2O_2. One unit (U) of catalase activity was defined as the amount of enzyme that caused an absorbance change of 0.001 per min under assay conditions. The reaction mixture contained 100 mM K- phosphate buffer (pH 7.0), 30 mM H_2O_2 and 100 µL of crude extract in a total volume of 3.0 ml.

2.5.3. Active compounds in chili pepper fruits by GC-MS analysis

2.5.3.1. Extraction of active constituents

The fresh fruits of chili pepper were taken separately and washed thoroughly using distilled water to remove dust or any other unwanted particles adhering to the surface of the fruits. 10 grams of the washed chili fruit was extracted using chloroform (anhydrous 99%, contains 0.5-1.0% ethanol as a stabilizer) as a solvent. Then the extract was evaporated to dryness [30].

2.5.3.2. GC-MS conditions

The GC/MS analysis was performed using a Thermo Scientific, Trace GC Ultra / IQS Single Quadrupole MS, TG-5MS fused silica capillary column (30 m, 0.251 mm, 0.1 mm film thickness).

For GC/MS detection, an electron ionization system with an ionization energy of 70 eV was used. Helium gas was used as the carrier gas at a constant flow rate of 1 ml/min. The injector and MS transfer line temperature was set at 280°C. The oven temperature was programmed at an initial temperature of 50°C (hold 2 min) to 150°C at an increasing rate of 7°C /min then to 270°C at an increasing rate of 5°C/min (hold 2 min) then to 310°C as a final temperature at an increasing rate of 3.5°C /min (hold 10 min). The quantification of all the identified components was investigated using a percent relative peak area. Tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the NIST, WILLY library data of the GC-MS system.

2.5.4. Capsaicinoids analysis by HPLC

2.5.4.1. Capsaicinoids extraction

The capsaicinoids were extracted from the dried fruits [31,32] slightly modified as follows: briefly, the dried fruits were powdered and 1 g was treated with 10 mL of acetonitrile at 65◦C along 20 min under sonication, with a working frequency of 35 kHz. The extracts were evaporated to dryness at 60◦C, resuspended in 0.5 mL of acetonitrile, and filtrated through 0.45 μm cellulose acetate membrane filters (GVS Filter Technology, Indianapolis, IN, USA). Samples were stored at −20◦C until they were analyzed.

2.5.4.2. HPLC conditions

Chromatography conditions were based on the validated method [33]. The analyses of capsaicinoids were performed by HPLC-DAD (Agilent 1200, Agilent Technologies Palo Alto, CA, USA) employing a reversed phase column Kromasil Eternity-5-C18 (4.6 × 150 mm) with precolumn (SUPELCO Analytical, Sigma-Aldrich) at 25 ◦C. Elution was performed with an isocratic mixture of water: acetonitrile 50: 50. Detection was set at 280 nm. Injection volume was 20 μL. All peaks related to capsaicinoids were eluted in about 15 min. Quantitative analysis was performed following the external standard method.

2.6. Statistical analysis

Data represent the mean SD (standard deviation). One-way analysis of variance was performed using SPSS ver. 19 (SPSS Inc., Chicago, IL, USA). A Tukey's test was also carried out to determine whether a significant difference ($p < 0.05$) existed between mean values according to Gomez and Gomez [34].

3. Results and discussion

3.1. Characterization of C- NPs and C-Spm NPs

The **C- NPs** and their encapsulated Spm analogue were successfully prepared via ionic gelation technique using TPP as crosslinking agent (REF). The prepared nanoparticles were characterized using transmission electron microscope (TEM) and dynamic light scattering (DLS) using Zeta Sizer instrument (Nano-ZS, Malvern Instruments Ltd., UK) to assess the particle size, size distribution and zeta potential (surface charge) of the prepared nanoparticles. The TEM of the prepared nanoparticles was displayed in (Figure 1). As can be shown in the figure the formed chitosan and the encapsulated nanoparticles showed relatively spherical shape with particle size around 20 to 40 nm for blank C-NPs and about 40-50 nm for C-Spm NPs. The C-Spm NPs showed relatively narrow size distribution than the blank chitosan. The amine groups of spermine may form ionic bond with sodium tripolyphosphate forming ionic gel like chitosan behavior.

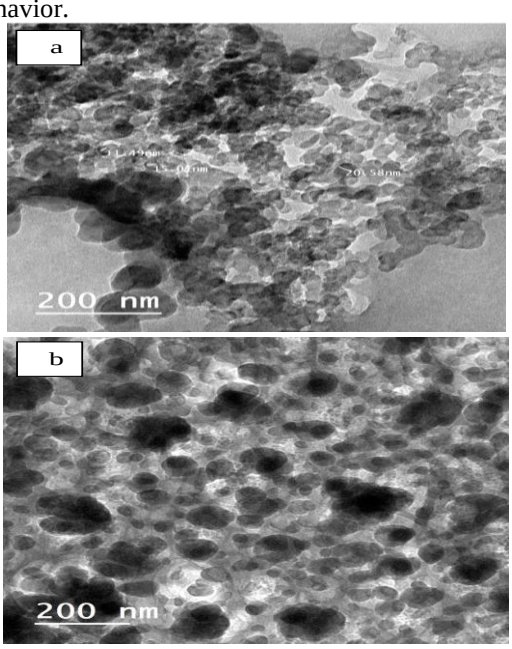


Fig. 1. TEM of (a) C-NPs and (b) C-Spm NPs

The DLS measurements for the prepared nanoparticles were shown in (Figure 2). The figure showed that the nanoparticles have narrow size distribution with average size of 97 nm for blank **C-NPs** and 170 nm for **C-Spm NPs**. The difference in the size of the prepared nanoparticles between DLS and TEM may be due to the swelling of **C-NPs** in aqueous solution and DLS provides a hydrodynamic radius of nanoparticles, whereas, TEM provides the dried diameter of nanoparticles (REF). In addition the TEM gives an image for selected area for measurement while DLS gives an overall image of the nanoparticles and their aggregations. Zeta potential for the blank **C-NPs** and Spm encapsulated one were +24 and + 41 mV, respectively. The positively charged nanoparticles were due to the cationic nature of amino groups of chitosan in acidic media formed ammonium acetate salt. The higher the value of zeta potential may be due to the more amine groups

present in both chitosan and spermine molecules. The higher the degree the zeta potential the higher repulsion forces between the particles the more stability of the nanoparticles and less aggregation and accumulation.

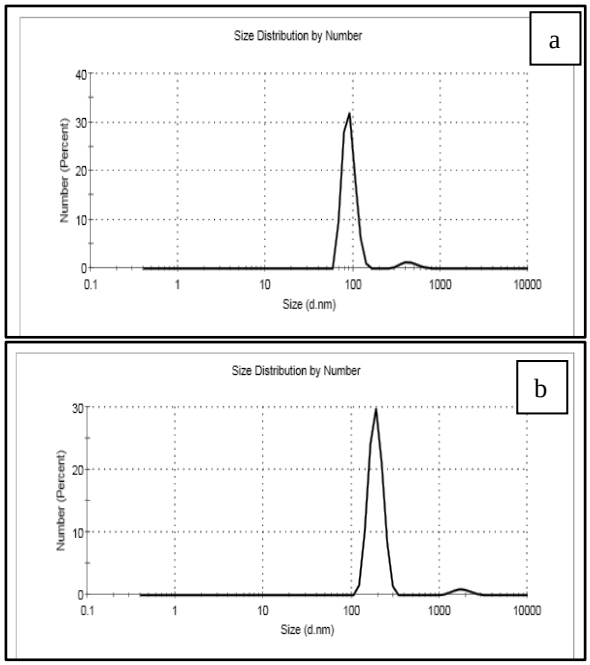


Fig.2. Particles size distribution of (a)) C-NPs and (b) C-Spm NPs

3.2. Plant growth parameters

The effect of C-NPs, Spm, and C-Spm NPs on plant growth parameters (number of leaves, leaf area; shoot fresh and dry weight) under salinity stress conditions in two seasons are shown in (Table 2). Data showed that the exogenous application of C-NPs, Spm and C-Spm NPs significantly improved the number of leaves per plant, leaf area, and shoot fresh and dry weight per plant compared with untreated plants.The C-NPs treatment was higher than the Spm treatment at plant growth parameters, however the highest growth parameters were recorded when plants were treated with C-Spm NPs. The application of C-Spm NPs at 1.5 mM showed the highest number of leaves per plant, leaf area, shoot fresh weight and shoot dry weight per plant in both seasons. This useful effect of the exogenous application of CS NPs, Spm NPs and CS-Spm NPs on plant growth parameters may be due to protect plants against oxidative stress [14], increased chlorophyll content, photosynthesis rate, antioxidant enzymes activities [15], detoxification of reactive oxygen species (ROS), activation of the antioxidative machinery, and molecular chaperone activity under stress conditions, thereby providing
broad-spectrum tolerance against a salinity stress [19], which consequently enhances the growth parameters of stressed plants. This result is similar to that obtained in [16, 22].

3.3. Fruit yield and its components

The data presented in (Table 3) show that number of fruits per plant, average fresh weight of fruit, weight of fruits per plant and total yield increased considerably in plants treated with C-NPs, Spm, and C-Spm NPs; the greatest increasing was recorded in plants treated with C-Spm NPs at 1.5 mM treatment compared with others plants and the increase was significant in both seasons. The increment of number of fruits per plant, average fresh weight of fruit, weight of fruits per plant and total yield under the exogenous application of C-NPs, Spm and C-Spm NPs are possibly due to the positive impacts of the exogenous application of C-NPs, Spm and C-Spm NPs on the plant growth parameters (Table 2), chlorophylls content (Fig. 3) and antioxidant enzymes activity (Fig. 4a and b), which led to increased number of fruits per plant, average fresh weight of fruit, weight of fruits per plant and total yield. These results are in harmony with previous findings of [16, 22, 35, 36].

Table2. Effect of chitosan nanoparticles, spermine, and chitosan nanoparticles encapsulated spermine on plant growth parameters of chili pepper at 90 days after planting in 2020 and 2021 seasons

Treatment (mM)		Plant growth parameters							
		Number of leaves		Leaf area (cm^2)		Shoot fresh weight (g)		Shoot dry weight (g)	
		2020	2021	2020	2021	2020	2021	2020	2021
Control	0	151.33^{d}	157.00^{e}	6.03^{e}	5.88^{e}	112.96^{d}	120.93^{e}	20.06^{d}	24.68^{f}
C-NPs	0.5	209.00^{c}	193.33^{d}	7.13^{d}	7.01^{d}	131.05^{d}	138.31^{d}	28.56^{c}	30.74^{d}
Spm	0.5	196.33^{c}	194.33^{d}	6.87^{d}	7.11^{d}	125.85^{d}	133.65^{d}	24.45^{cd}	28.44^{e}
C-Spm NPs	0.5	278.00^{b}	232.00^{c}	8.20^{c}	8.74^{c}	172.90^{c}	179.15^{c}	38.45^{b}	42.65^{c}
	1.0	297.67^{ab}	261.00^{b}	10.83^{b}	9.47^{b}	204.47^{b}	199.73^{b}	46.63^{a}	45.39^{b}
	1.5	320.33^{a}	283.33^{a}	11.77^{a}	10.00^{a}	226.65^{a}	214.40^{a}	53.17^{a}	49.86^{a}

Where: C-NPs=chitosan nanoparticles, Spm=Spermine, C-Spm NPs=chitosan nanoparticles encapsulated spermine

Table3. Effect of chitosan nanoparticles, spermine, and chitosan nanoparticles encapsulated spermine on fruit yield and its components of chili pepper in 2020 and 2021 seasons

Treatment (mM)		Fruit yield and its components							
		Number of fruits per plant		Average fresh weight of fruit (g)		Weight of fruits per plant (g)		Total yield (ton ha^{-1})	
		2020	2021	2020	2021	2020	2021	2020	2021
Control	0	22.86^{e}	26.98^{e}	5.91^{d}	6.21^{d}	134.54^{e}	167.00^{e}	1.94^{e}	2.42^{e}
C-NPs	0.5	36.88^{d}	42.87^{d}	6.31^{cd}	6.55^{c}	232.92^{d}	281.02^{d}	3.35^{d}	4.10^{d}
Spm	0.5	31.95^{d}	41.50^{d}	6.26^{cd}	6.51^{c}	200.09^{d}	270.33^{d}	3.04^{d}	3.92^{d}
C-Spm NPs	0.5	42.47^{c}	48.47^{c}	6.70^{bc}	6.88^{b}	284.19^{c}	333.99^{c}	4.22^{c}	4.82^{c}
	1.0	56.59^{b}	53.57^{b}	7.10^{b}	7.02^{b}	402.50^{b}	376.59^{b}	5.80^{b}	5.42^{b}
	1.5	68.41^{a}	60.16^{a}	7.63^{a}	7.34^{a}	521.55^{a}	451.25^{a}	7.06^{a}	6.50^{a}

Where: C-NPs=chitosan nanoparticles, Spm=Spermine, C-Spm NPs=chitosan nanoparticles encapsulated spermine

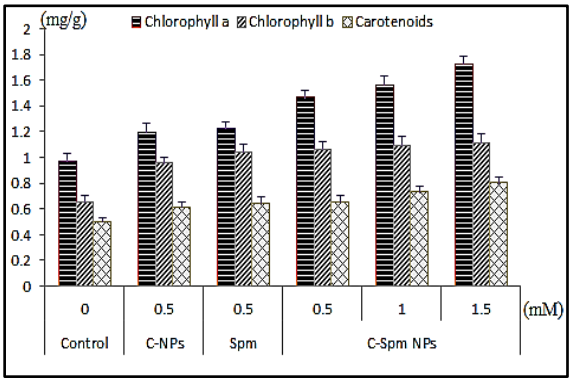
3.4. Biochemical and active compounds

3.4.1. Chlorophyll content

Data presented in (Figure 3) shows the effect of C-NPs, Spm, and C-Spm NPs on chlorophyll a, b and carotenoids content of chili pepper plants under saline stress conditions. The results declared that the use of C-NPs, Spm and C-Spm NPs alleviate the effect of salinity on photosynthetic pigments in the chili pepper plants. The Spm treatment gave higher photosynthetic pigments values than chitosan NPs. The plants treated with C-Spm NPs treatment showed increases in chlorophyll a, b and carotenoids content compared with the plants treated with others treatments. The maximum contents of chlorophyll a, b and carotenoids were recorded with C-Spm NPs at 1.5 mM. The foliar application of C-NPs, Spm, and C-Spm NPs under salinity conditions may minimize the destructive effect of salinity on chlorophyll content by their ability to scavenge ROS, to protect chlorophyll content, photosynthesis parameters, and improve photosystem functioning [21], or to enhance endogenous levels of cytokinins, which stimulated chlorophyll synthesis and growth or to the greater availability of amino compounds released from chitosan [37]. These results were supported by the findings reported in various plants [16, 21, 35].

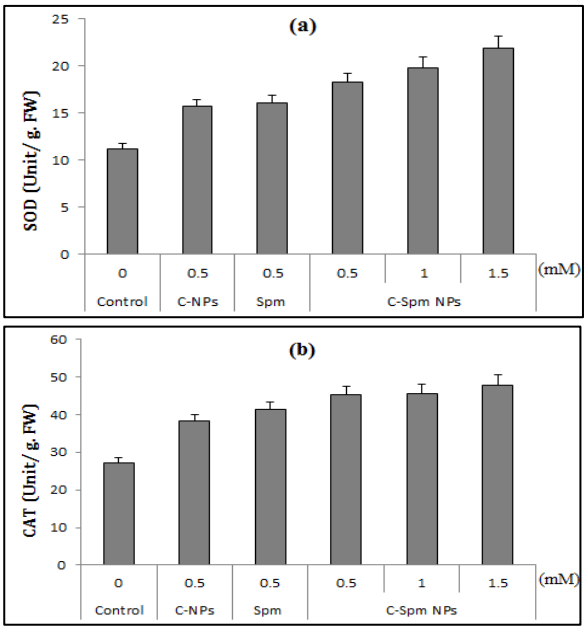
3.4.2. Antioxidant enzymes activity

Antioxidant enzyme activities (SOD and CAT) were assayed. The data presented in (Figure 4a and b) showed that a significant increase in the activities of antioxidant enzymes increased by application of C-NPs, Spm and C-Spm NPs treatments compared with the control treatment. Higher activities of SOD and CAT were recorded in the leaves of chili pepper treated with C-Spm NPs at 1.5 mM treatment. In this regard, chitosan chemical constitution includes uridine diphosphate N-acetyl-d-glucosamine (UDP-GlcNAc) as nucleotide sugars, which, when applied in plants recognized by cells throughout chitin synthase chitin deacetylase enzymes, caused the formation of chitosan oligomers that are involved in plant cell signals [38,39]. Chitosan oligomers enter the nucleus and act in cascade reactions as the production of hormones and the expression of antioxidant enzymes [38, 40-42]. Likewise, the role of spermine in SOD and CAT activity enhancement might be due to that Spm can regulate many enzyme activities by bonding with the enzyme protein or participation in the process of phosphorylation of the enzyme protein [43].Therefore, Spm may function not only as scavengers of ROS, but also as activators of the expressions of genes encoding antioxidant enzymes [44].



C-NPs: chitosan nanoparticles, Spm: spermine, C-Spm-NPs: chitosan nanoparticles encapsulated spermine

Fig.3. Effect of C-NPs, Spm and C-Spm NPs on pigments content of chili pepper under saline conditions



C-NPs: chitosan nanoparticles, Spm: spermine, C-Spm-NPs: chitosan nanoparticles encapsulated spermine

Fig.4. Effect of C-NPs, Spm and C-Spm NPs on antioxidant enzymes activity of chili pepper (a) SOD (b) CAT under saline stress conditions.

Increasing activities of SOD and CAT with the applications of **C-NPs**, Spm, and **C-Spm NPs** proved the role of these plant growth regulators in mitigating the adverse effects of salt stress in chili pepper. In the connection, plant cells contain a large number of antioxidants to prevent or repair the damage caused by ROS, as well as to regulate redoxsensitive signaling pathways. SOD convert superoxide radical into hydrogen peroxide and molecular oxygen [3, 45]. In addition, Catalase is one of the important enzymes that increase the antioxidant defense capability in plant cell under oxidative stress conditions, where it has an important role in the elimination of hydrogen peroxide in chloroplast, cytosol and mitochondria [3, 46]. Catalase enzyme is the main scavenger of strong oxidant H_2O_2 in peroxisomes and it converts hydrogen peroxide to water and molecular oxygen [47]. Moreover, C-NPS proved to be effective in many crops to protect plants against oxidative stress and increased catalase (CAT) [14], and superoxide dismutase (SOD) activities [15].

3.4.3. Active compounds by GC-MS analysis

GC-MS analysis of chili pepper cv. Omega F1 as affected by C- NPs, Spm and C-Spm NPs under saline stress conditions are described in (Tables 4 and 5). The GC/MS analysis revealed the presence of 22 active compounds which are, (E)-3-Iodo[22-H]but-2-enoic acid; 2,5-Diamino-2-methylpentanoic acid; 6,6,6-Trifluoro-5-hydroxy-2,2-dimethyl-3-hexanone; 1,5,9,13-Tetrathia-3,11-cyclohexadecaediol; Heptadecane, 2-methyl(CAS); 2-Undecene,9-methyl-E-; 1-Tetradecanol(CAS); Tetradecane (CAS); Phenol,2,4-bis(1,1-dimethylethyl); 1-Heptadecene (CAS); Heptadecane (CAS); Nonadecane (CAS); 3-Eicosene,(E); Hexadecane (CAS); (2-hydroxy-5,10,15,20-tetraphenylporphinato)zinc(II); Hexadecanoic acid,trimethylsilyl ester (CAS); Pentadecane (CAS); (cis)2-nonadecene; 5-Eicosene,(E); Silane, trichloroeicosyl; 2,2-Bis[4-[[4-chloro-6-(3-ethynylphenoxy)1,3,5-triazin2yl]oxy]phenyl]-propane; and 4-Trifluoro-acetoxyhexadecane (Table 4). Concerning the effect of C-NPs, Spm and C-Spm NPs on the area percent of these compounds data revealed that C-NPs, Spm and C-Spm NPs had an a positive effect in increasing the area percent of phytoconstituents compared with the control (Table 5). The maximum peak area is, 1-Tetradecanol (CAS), Phenol,2,4-bis(1,1-dimethylethyl), 1-Heptadecene (CAS), 3-Eicosene,(E), (cis)2-nonadecene, and 5-Eicosene,(E) which recorded the high area percent in all treatments compared with other compounds. As comparison the effect of C-NPs and Spm, it was noted that, C-NPs accumulated the active compounds more than Spm at all retention times except RT 20 (Silane, trichloroeicosyl). Regarding the effect of C-Spm NPs, it was observed that it led to an increase in the accumulation of active compounds compared to C-NPs and Spm, each separately. In this regard, the active compounds which recorded at retention times (8.45, 13.9, 14.11, 19.18, 22.39, 28.25, 28.38, 32.16, 33.21, 35.36, and 35.73) were high when applied C-Spm NPs at low concentration (0.5 mM) compared with other treatments and control. Likewise, C-Spm NPs (1.5 mM) recorded the maximum area percent at retention times (5.13, 5.18, 5.22, 23.95, and 40.76) as well as (24.11 and 32.26) at 1 mM. On the other hand, the active compounds identified at retention times (19.37, 26.31, and 42.07) were more in the control treatment than all C-NPs, Spm, and C-Spm NPs treatments. In the connection, Phytochemicals are part of a plant's internal defense mechanism against pests, parasites, and pathogens, and they usually give its distinctive color, taste, and flavor [48]. The active compounds identified by GC-MS analyses in chili pepper have a wide range of medical applications. Each of the compounds discovered has a unique feature that can be utilized to treat a wide range of ailments. To treat diseases effectively, more researches are needed to reveal their significance in a given field.

3.4.4. Capsaicinoids in fruits of chili pepper by HPLC

Data presented in (Table 6) showed the amounts of capsaicinoids found in chilli pepper cv. Omega F1 as affected by C-NPs, Spm and C-Spm NPs under saline stress conditions. From the chromatograms obtained, the main peaks of interest identified among the capsaicinoids were capsaicin and dihydrocapsaicin. The UV absorption spectra corresponding to capsaicin and dihydrocapsaicin peaks were obtained from the photo diode array detector (PDA). The ultraviolet detection wavelength was set at 280 nm for all the capsaicinoids, because it is the maximum absorbance for both capsaicinoids. The data declared that C-NPs, Spm, and C-Spm NPs had appositive effect on accumulation of capsaicin and dihydrocapsaicin in chili pepper compared with the control. Also, data showed that at all treatments capsaicin was higher than dihydrocapsaicin. As a comparison between the effect of C-NPs and Spm, C-NPs increased capsaicin and dihydrocapsaicin higher than Spm. On the other hand, C-Spm NPs with all its levels led to an increase in capsaicin and dihydrocapsaicin compared to each of the C- NPs and Spm separately. The maximum amount of capsaicin and dihydrocapsaicin were recorded when plants treated with C-Spm NPs at 0.5 mM. For the general taste of chili pepper, the correlation between the Scoville heat unit (SHU) and the two capsaicinoids obtained was calculated as shown in (Table 6) by using the relationship between this content (mg/g DW) and its SHU rating of approximately 15 SHU equivalent to 1 mg/g of capsaicinoids [49]. Therefore, their corresponding SHU was found in the range of 1597-2268. From these results, it is indicated that capsaicin and dihydrocapsaicin were primarily responsible for the SHU rating. Thus, plants treated with C-Spm NPs gave quite a high SHU related to higher contents of the capsaicinoids. In relation to the importance of phytochemicals for functional aspects of pepper, the capsaicinoids are a specific class of compounds that causes the spicy sensation (pungency) of chili pepper fruit [50].

Table 4. Active compounds identified by GC-MS of chili pepper fruit chloroform extract treated by chitosan nanoparticles, spermine, and chitosan nanoparticles encapsulated spermine under saline stress conditions

PN	RT	Probability	Name of the compound	MF	MW
1	5.13	83.74	(E)-3-Iodo[22-H]but-2-enoic acid	$C_4H_4DIO_2$	212
2	5.18	20.3	6,6,6-Trifluoro-5-hydroxy-2,2-dimethyl-3-hexanone	$C_8H_{13}F_3O_2$	198
3	5.22	38.45	1,5,9,13-Tetrathia-3,11-cyclohexadecaediol	$C_{12}H_{24}O_2S_4$	328
4	8.45	17.63	2,5-Diamino-2-methylpentanoic acid	$C_6H_{14}N_2O_2$	146
5	13.9	7.05	2-Undecene,9-methyl-E-	$C_{12}H_{24}$	168
6	14.11	17.77	Heptadecane, 2-methyl(CAS)	$C_{18}H_{38}$	254
7	19.18	10.11	1-Tetradecanol(CAS)	$C_{14}H_{30}O$	214
8	19.37	19.75	Tetradecane (CAS)	$C_{14}H_{30}$	198
9	22.39	44.85	Phenol,2,4-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	206
10	23.95	7.12	1-Heptadecene(CAS)	$C_{17}H_{34}$	238
11	24.11	12.25	Heptadecane (CAS)	$C_{17}H_{36}$	240
12	26.31	13.49	Nonadecane (CAS)	$C_{19}H_{40}$	268
13	28.25	4.71	3-Eicosene,(E)	$C_{20}H_{40}$	280
14	28.38	7.96	Hexadecane (CAS)	$C_{16}H_{34}$	226
15	32.16	11.13	(cis)2-nonadecene	$C_{19}H_{38}$	266
16	32.26	17.12	Pentadecane (CAS)	$C_{15}H_{32}$	212
17	33.21	78.76	Hexadecanoic acid,trimethylsilyl ester (CAS)	$C_{19}H_{40}O_2Si$	328
18	35.36	89.52	(2-hydroxy-5,10,15,20-tetraphenylporphinato)zinc(II)	$C_{44}H_{28}N_4OZn$	692
19	35.73	5.5	5-Eicosene,(E)	$C_{20}H_{40}$	280
20	39.02	9.34	Silane, trichloroeicosyl	$C_{20}H_{41}Cl_3Si$	414
21	40.76	28.66	2,2-Bis[4-[[4-chloro-6-(3-ethynylphenoxy)1,3,5-triazin2yl]oxy]phenyl]propane	$C_{37}H_{24}Cl_2N_6O_4$	686
22	42.07	5.15	4-Trifluoroacetoxyhexadecane	$C_{18}H_{33}F_3O_2$	338

PN: peak number, RT: retention time, MF: molecular formula, MW: molecular weight

Table 5. Effect of chitosan nanoparticles, spermine, and chitosan nanoparticles encapsulated spermine on area percent for active compounds of chili pepper fruit chloroform extract under saline stress conditions

Area percent (%) for active compounds in chili pepper chloroform extract							
PN	RT	Control	C- NPs (mM)	Spm (mM)	C-Spm NPs (mM)		
		0	0.5	0.5	0.5	1	1.5
1	5.13	1.08	1.32	1.26	1.39	1.42	2.11
2	5.18	2.69	3.27	3.25	3.35	3.81	4.15
3	5.22	2.60	2.92	2.79	3.14	3.52	3.87
4	8.45	1.38	1.71	1.69	1.90	1.81	1.76
5	13.90	3.29	3.59	3.54	3.65	3.60	3.60
6	14.11	2.29	2.39	2.33	2.52	2.45	2.44
7	19.18	8.28	8.40	8.37	8.51	8.48	8.42
8	19.37	6.98	2.50	2.45	2.15	2.22	2.57
9	22.39	10.00	10.47	10.40	10.87	10.82	10.70
10	23.95	11.69	11.94	11.85	12.14	12.18	12.25
11	24.11	2.38	2.44	2.44	2.46	2.55	2.49
12	26.31	5.38	4.53	3.68	1.34	1.64	1.03
13	28.25	13.91	14.29	14.28	14.69	14.41	14.34
14	28.38	2.24	2.40	2.39	2.60	2.44	2.37
15	32.16	11.66	12.11	12.09	12.23	12.19	12.13
16	32.26	1.45	2.29	2.21	2.33	2.38	2.24
17	33.21	1.59	1.72	1.65	2.37	2.29	1.76
18	35.36	0.49	0.62	0.61	0.89	0.70	0.65
19	35.73	6.98	7.43	7.41	7.89	7.78	7.46
20	39.02	2.49	2.62	4.31	2.66	2.71	2.79
21	40.76	0.31	0.39	0.36	0.42	0.42	0.49
22	42.07	0.89	0.66	0.65	0.52	0.51	0.40
Total		100.00	100.01	100.01	100.02	100.33	100.02

Where: C-NPs=chitosan nanoparticles, Spm=Spermine, C-Spm NPs=chitosan nanoparticles encapsulated spermine, PN=peak number, RT=retention time

Capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin, and homocapsaicin are the most common capsaicinoid compounds. Capsaicin (*N*-[(4-hydroxy-3-methoxyphenyl) methyl]-8-methyl-*E*-6-nonenamide) and dihydrocapsaicin (*N*-[(4-hydroxy-3-methoxyphenyl) methyl]-8-methyl-6-nonanamide), (Fig. 5) make up around 90% of the capsaicinoids found in chili peppers [50].These are the two most effective capsaicinoids; the only difference between their molecules is the saturation of an acyl group, and their amount constitutes a quality parameter for various items. Many types of research showed the important role of capsaicinoids in pharmaceutical properties. It has been used as an analgesic against arthritis pain, inflammation, anticancer, and to be active against neurogenic inflammation burning and stinging of hands, mouth, and eyes [32-33, 50-52].

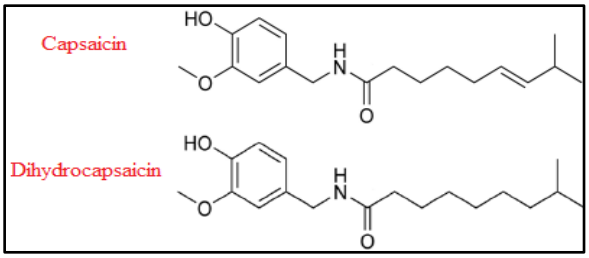


Fig. (5). Chemical structures of capsaicin and dihydrocapsaicin.

Table 6. Effect of chitosan nanoparticles encapsulated spermine on capsaicinoids of chili pepper fruit extract under saline stress conditions

Treatments (mM)		Capsaicin (mg/g DW)	Dihydrocapsaicin (mg/g DW)	Total capsaicinoids (mg/g DW)	SHU
Control	0	85.54	20.95	106.49	1597.35
C-NPs	0.5	96.79	28.41	125.2	1878.00
Spm	0.5	91.33	25.72	117.05	1755.75
C-Spm NPs	0.5	115.7	35.55	151.25	2268.75
	1	108.92	33.17	142.09	2131.35
	1.5	103.63	31.89	135.52	2032.80

Where: C-NPs=chitosan nanoparticles, Spm=Spermine, C-Spm NPs=chitosan NPs encapsulated spermine**,** SHU=Scoville heat unit (Total capsaicinoids *15)

Conclusion

We reported novel chitosan nanoparticles encapsulated spermine (C-Spm NPs) wherein chitosan-tripolyphosphate (TPP) nanomatrix has been used to encapsulate spermine (Spm) for its slow release. Besides, we studied its effect alongside with C-NPs and Spm separately on chili pepper cv. Omiga F1 under salinity conditions. The results showed that of C-NPs, Spm, and C-Spm NPs led to alleviate the adverse effect of salinity on plants and increased growth, yield, chlorophyll content, antioxidant enzymes activity (SOD and CAT), bioactive compounds and capsicinoids of plants. The application of C-Spm NPs at 1.5 mM level gave the maximum values of growth and yield of chili pepper under salinity stress conditions.

Conflict of Interests

Authors do not have any conflict of interest.

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