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Research paper

Alleviation of salt stress damages by chalcone-based nanocomposite in lettuce plants

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Abstract
This study aimed to investigate the effect of chalcone-based nanocomposite in various concentrations on lettuce plants under salinity treatment. The composite was synthesized by chemical methods with a mean particle size of 89.4 ± 4.2 nm. The factors consisted of salinity concentrations of 100, 125, and 150 mmol/l and nanocomposite concentrations of 0.1 and 0.01 ppm. After harvest, different physiological and biochemical characteristics were measured. The results indicated that the nanocomposite significantly increased the fresh and dry weight of roots and glycine betaine in the lettuce plants at higher salinity stress conditions (150 mmol/l). Contrarily, the nanocomposite significantly reduced malondialdehyde at the salinity stress conditions. Also, the nanocomposite caused a significant increase in total flavonoid content under the salinity stress conditions of 150 mmol/l and activity of ascorbate peroxidase, an antioxidant enzyme, at the salt concentration of 150 mmol/l. It can be concluded that chalcone-based nanocomposite may ameliorate the negative effects of the salinity stress on plants.

Keywords: chalcone; lettuce; nanocomposite; salinity

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Introduction

Abiotic stresses as salinity, drought, cold, and heat are of significant concern to the scientific community because they negatively impact survival, biomass production, and yield of crops (Parida *et al.* 2004).

Salty soil is one of the most serious threats to agriculture and an important environmental factor. Salinity found in water and soil limits the quality, efficiency, and growth of crops in the world. Plants are affected by salinity in almost every physiological and biochemical aspect, as well as crop productivity (Al-Maskari *et al.* 2010).

Plants that are stressed by salt, allocate the majority of their energy to coping with the adverse effects of salinity, leaving low energy for forming or filling seeds. Also, due to lower energy, leaves age prematurely and therefore, the photosynthesis efficiency of the plant declines (Kafi *et al.* 2011).

Lettuce (*Lactuca sativa* L.) is an annual plant of the daisy family. Lettuce is known as a plant with medium tolerance to salinity. A salinity level exceeding 2 dS m^{-1} may reduce fresh lettuce growth and yield (Al-Maskari *et al.* 2010). Also, lettuce is rich in vitamin C, carotenoids, antioxidants, caffeic acid, and flavonoids (Aguero *et al.* 2014).

Secondary metabolites have various ecological advantages, including the protection of plants against vegetarian animals or contamination by pathogens. Additionally, secondary metabolites such as carotene have adsorbent properties (in terms of color, smell, and taste), attract pollinators, and draw them to the plant. These compounds can

also promote symbiosis between plants and living organisms such as bacteria (Taiz and Zaiger 2006).

Chalcones and their related compounds, known as chalconoids, are aromatic ketones and belong to the flavonoid group. This compound is made up of 1,3-diphenyl-2-propen-1-ol and is one of the first isolatable compounds from biosynthetic flavonoids found in many different fruits and vegetable sources. Also, these compounds have therapeutic, antibacterial, anti-inflammatory, antifungal, and antitumor properties, including scavenging free radicals (Opletalova *et.al.* 2000). Some earlier reports indicated the role of chalcone in increasing plant defense and tolerance against biotic or abiotic stresses by stimulating flavonoid biosynthesis (Dao *et al.* 2011). It was pointed out that chalcone biosynthesis is intensified under stress conditions to moderate the harmful effect of stress. It can be a precursor of the biosynthesis of anthocyanins and various flavonoids as other potent antioxidant compounds (Javaraman *et al*. 2021). However, chaconne insolubility in water creates some difficulties to be applied externally as a spray on plants or supplement in nutritional solutions. Integration of chalcone into polymeric nanocomposites may be the best solution for its water insolubility problem. Polymeric nanoparticles can be used as agrochemical or pharmaceutical carriers, and can be made from both biodegradable and non-biodegradable polymers. Due to their ability to improve surface quality, polymeric nanocomposites can increase agrochemicals and drug absorption efficiency (Faraji *et al*. 2009).

Up to now, there are no reports in the literature about the application of chalcone-based nanocomposites on plants against environmental stresses. This research aimed to study the effect of chalcone-based nanocomposite on lettuce plants under salinity stress.

Materials and Methods

Synthesis and characterization of nanocomposites

An amount of 2 mg chalcone (Sigma, 614-47-1) was dissolved in 1 mL of distilled water using vortex and sonication at 80 °C. In another container, 1 mg carboxy methyl cellulose (CMC) was slowly dissolved in 1 ml of distilled water, and placed in an ultrasonic bath at 60 ° C for 15 min. Then, the two solutions were mixed and stirred at 35 °C. This solution was centrifuged at 8000 rpm for 20 min, and the supernatant was poured into a petri dish and dried. Then, various tests including SEM, FTIR, TGA, and DLS were performed on the dried material to identify the size, morphology, and nature of the synthesized nanocomposites (Upadhyaya *et al.* 2014; Joshi *et al*. 2015).

Sowing and treatments

Lettuce seeds were disinfected and transferred to pots for sowing. The pots with a size of 21 × 20 cm were filled with a substrate consisting of vermiculite: perlite (1:1) and were placed in a plastic greenhouse. At the seven-leaf stage, salinity treatments of 150, 125, and 100 mmol/l were applied along with 0.1 and 0.01 ppm of the nanocomposite. The salt and nanocomposite were dissolved in the Hoagland nutrient solution (50%) and the mixed solution was poured into the pots every two days three times. After 4 weeks, physiological characteristics including

chlorophyll, and chlorophyll fluorescence were measured.

Measurement of growth traits

After harvest, the fresh weight of roots and leaves was measured. Then, the plant leaves were dried in an oven at 75 °C for 20 min and the dry weight was determined.

Measurement of physiological and biochemical characteristics

To measure the proline content of the lettuce plants, the aerial parts were homogenized with sulfosalicylic acid. After centrifugation, ninhydrin and acetic acid were added to this mixture, and after putting in Bain-Marie at 100°C, ice was added and absorbance was measured at 520 nm.

To measure glycine-betaine, the fresh tissue of the plants was crushed in liquid nitrogen and homogenized with distilled water. Then, it was blended with sulfuric acid 2N after two hours of incubation. Within one hour of the ice bath, KI3 reagent was added. Then, the mixture was incubated after a refrigerated centrifuge for 24 hours at 4 °C. The ice crystals formed were dissolved in 14 ml of 2,1- dichloroethane and were stirred using a shaker for 24 hours. Ultimately, the absorption of the solution was measured at 360 nm and compared with the standard glycine-betaine curve.

To assay the hydrogen peroxide, the plant's fresh tissue was homogenized in 1% trichloroacetic acid solution in the ice bath and centrifuged in a refrigerated centrifuge. Then, the supernatant was blended with 10 mmol/l phosphate buffer and 1 mmol/l KI, and its absorption was measured at 390 nm wavelength.

For the measurement of malondialdehyde, the plant's fresh tissue was crushed in liquid nitrogen and mixed with trichloroacetic acid (TCA), and centrifuged at 15000 rpm for 14 min. Then, the supernatant was mixed with TCA containing thiobarbituric acid (TBA) and kept at 95 °C for 15 minutes. After being cooled, the absorption of the resulting solution was measured at the wavelengths of 532 (red-complex absorption) and 600 nm (absorbing the remaining nanoparticles).

To measure the activity of enzymes and total protein, the aerial organs of the plant were powdered in the presence of a liquid bath, and then, were homogenized with the phosphate buffer, and the supernatant was used for assays.

For the catalase assay, 0.05 M phosphate buffer and oxygenated water were mixed, and immediately the extract was added to it. The absorption change was recorded by a spectrophotometer at 290 nm. The enzyme activity was calculated in mmol/l of the substrate and converted to mg of total protein per minute.

To assay for the ascorbate peroxidase enzyme, 0.05 M buffer phosphate was mixed with the oxygenated water and ascorbic acid, and then, the extract was added to it immediately and absorption change was recorded at 290 nm.

To measure the protease enzyme activity, the casein solution and enzyme extract were mixed and kept at 45 °C. To stop the reaction, TCA was added after one hour and the absorption change was recorded at 280 nm.

To assay the polyphenol oxidase enzyme, 0.2 M buffer phosphate was blended with 0.2 M pyrogallol and kept at 40 °C. Then, 0.2 ml of the

enzyme extract was added and the absorption change at 430 nm wavelength was recorded. The activity of enzymes was evaluated by the Beer-Lambert law.

To measure the total soluble protein, the extract of the plant's aerial organs using buffer phosphate was mixed with the Bradford reagent. The protein concentration was calculated using the bovine albumin serum standard curve and its absorption at 595 nm wavelength after 15 minutes.

For the free amino acids assay, the plant tissue was homogenized in 5% M phosphate buffer and after centrifuging, the supernatant was mixed with the ninhydrin reagent and exposed to 70 °C. After cooling, the absorption of the solution was measured at 570 nm. The glycine standard curve was used to determine the total amino acid.

To measure the soluble sugars, the fresh tissue of the plant leaves was homogenized in ethanol-80 and kept at 95 °C for one hour to extract the soluble carbohydrates. The final extract was dissolved in 2.5 ml distilled water after evaporation of the alcohol. Then, 0.5 liters of the anthrone reagent was added and kept at 90 °C for 11 min. After cooling, the absorption was measured at 625 nm wavelength and assayed using the standard anthrone curve.

Measurement of phytochemical characteristics

To measure the amount of anthocyanin, plant samples were homogenized in acidic methanol and then, measured at 550 nm after centrifuging the supernatant.

The aluminum chloride calorimetry method was used to determine the amount of total flavonoids (Toor and Savage 2005).

Tannin was assayed as follows: 1 mL methanol extract was mixed with 100 ml of polyvinyl poly pyrrolidone (PVPP) and centrifuged in the refrigerated centrifuge. The supernatant absorption was measured at a 760 nm wavelength. Since tannins in the sample are precipitated by PVPP, therefore, to measure the total tannin content, the total phenol content should be deducted from the total tannin.

To assay for phenols, 0.1 g of each sample was crushed in 10 ml of ethanol 96%. After 24 hours of exposure to darkness, 1 ml ethanol 95%, 0.5 ml folin 50%, and 1 ml sodium carbonate 5% were added to the solution. After one hour of maintenance in the dark, absorption was measured at 725 nm wavelength. The gallic acid curve was used as the standard curve for calculating the concentration of total phenolic compounds.

Statistical analyses

The experiment was designed as factorial based on a completely randomized design with three replications. Statistical analyses were performed using SPSS 22.0 statistical software (IBM, Chicago, IL, USA). Data were analyzed by the two-way analysis of variance and the means were compared with the Duncan Multiple Range Test at the 5% probability level.

Results

Characterization of nanocomposites

Morphological characteristics of the synthesized nanocomposite particles were studied by electron microscopy (Figure 1). The nanocomposite particles had irregular polyhedron shapes and sizes of less than 100 nm.

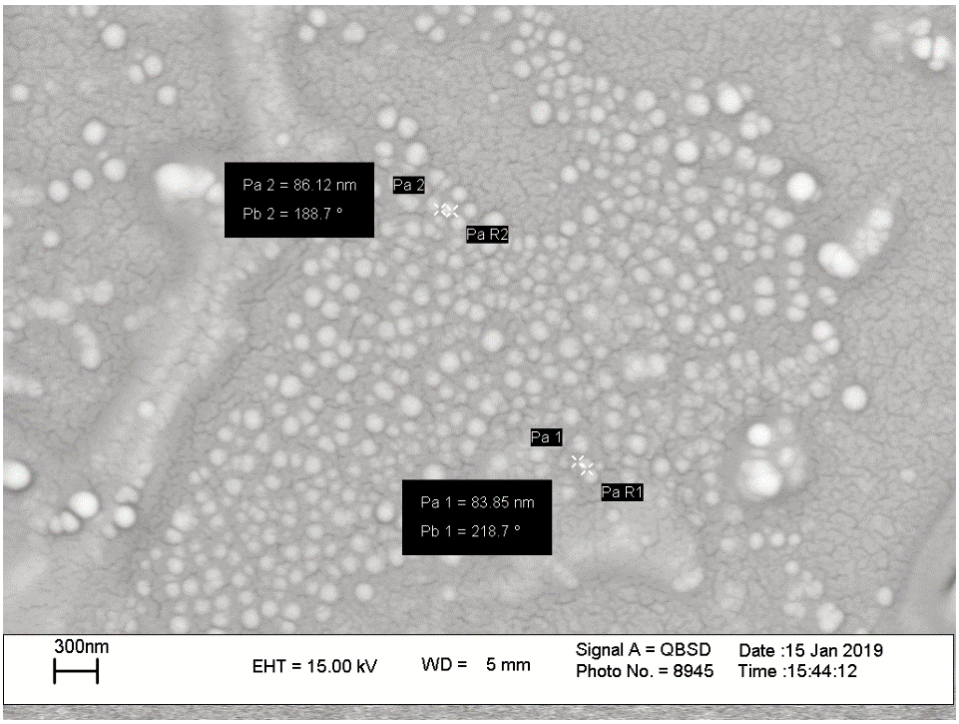


Figure 1. The electron microscopy image of the chalcone-based nanocomposites

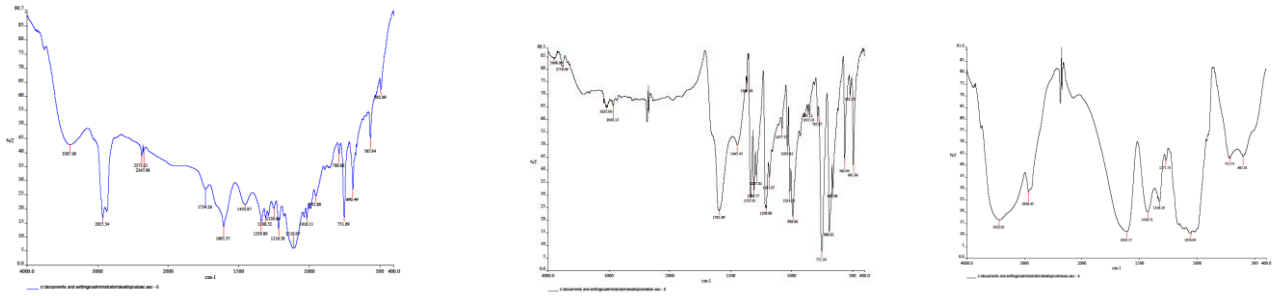
One of the important features of nanostructures is their particle size. The average size of nanocomposite units was 89.4 ± 4.2 nm. The number of particles with a size of 88.6 nm was more than other particles.

The FTIR spectra for CMC, chalcone, and nanocomposite are shown in Figure 2. The FTIR spectra of the nanocomposite showed some characteristic peaks at 3387 (-OH stretching vibration) and 1605 cm^{-1} (C=O absorption) of chalcone. The frequency of the carbonyl group (C=O) of the chalcone shifted from 1591 cm^{-1} to 1605 cm^{-1} in the nanocomposites, and this is because of a hydrogen bond formation between the chalcone and CMC to form the nanocomposite.

In the TGA graph for chalcone-based nanocomposite, a weight loss of about 5% at 104 °C (first stage) was observed, which was related to the moisture removal (Figure 3). From 225 °C to 250 °C and from 250 °C to 350 °C, 56 and 20.9% of the nanocomposite degradation was observed, respectively. Degradation of the composite in the range of 225 °C to 500 °C causes severe weight loss (about 90%) of the nanocomposites. It was revealed that the nanocomposite pattern reflects a combined degradation pattern of chalcone and CM. These results indicate that chalcone was combined with CMC to form nanocomposites.

Growth traits

The results for growth traits indicated that the nanocomposite treatment without any salt significantly reduced the fresh weight of the lettuce roots compared to the control. However, the nanocomposite increased the root fresh and dry weight of the lettuce plants at 150 mmol/l of salt.



(a) (b) (c)

Figure 2. FT-IR spectrum of a) nanocomposite b) Chalcone, and c) carboxy methyl cellulose.

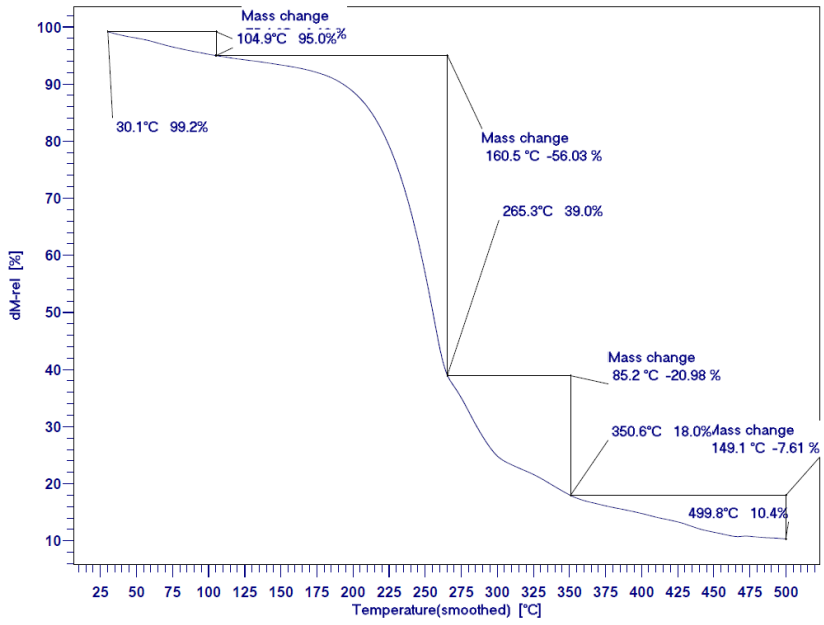


Figure 3. TGA curves for chalcne-based nanocomposites.

Also, the fresh and dry leaf weight of the lettuce plants significantly increased by applying the nanocomposite (Table 1). However, the dry weight of lettuce increased in the nanocomposite-treated plants only at the low concentration of salt.

Physiological and biochemical characteristics

Application of the nanocomposite on lettuce plants, either alone or in combination with different salt concentrations, significantly increased the glycine betaine content compared to the related controls (Table 2). Nanocomposite alone also significantly increased the proline content, however, when combined with the higher salt concentrations (125 and 150 mmol/l), the proline content decreased significantly in the lettuce plants (Table 2).

Although the salt stress at higher levels (125 and 150 mmol/l) increased the hydrogen peroxide and malondialdehyde content, these compounds decreased significantly when accompanied with

Table1. The effects of chalcone nanostructure on growth traits of lettuce plants under salinity stress.

Salt mmol/l	Nano ppm	Fresh root weight (g)	Fresh leaf weight (g)	Dry root weight (g)	Dry leaf weight (g)
0	0	1408.67±221.49^{a}	257.00±6.80^{b}	55.33±8.84^{a}	18.70±0.85^{b}
	0.1	801.67±120.49^{b}	319.33±21.94^{a}	60.67±13.32^{a}	25.97±0.76^{ab}
	0.01	764.00±63.35^{b}	349.00±20.07^{a}	48.00±2.89^{a}	27.87±0.90^{a}
100	0	687.00±91.88^{c}	321.00±55.60^{a}	37.67±3.18^{b}	23.40±3.62^{b}
	0.1	508.67±40.05^{c}	381.67±23.39^{a}	21.33±1.86^{b}	39.10±2.80^{a}
	0.01	610.67±155.33^{c}	303.67±43.97^{a}	30.67±9.02^{b}	29.77±3.95^{a}
125	0	608.00±58.94^{c}	305.67±21.98^{a}	34.00±5.03^{b}	28.80±6.22^{a}
	0.1	591.67±32.91^{c}	262.67±22.04^{b}	24.67±2.03^{b}	20.33±2.61^{b}
	0.01	626.33±95.78^{c}	209.67±14.31^{b}	32.33±2.96^{b}	19.53±3.39^{b}
150	0	285.00±107.87^{d}	268.67±26.16^{b}	26.00±8.14^{b}	29.87±2.60^{a}
	0.1	616.67±185.56^{c}	288.33±51.96^{b}	43.00±3.06^{a}	31.33±3.67^{a}
	0.01	911.33±14.90^{b}	279.67±11.86^{b}	41.00±4.04^{a}	25.17±5.74^{ab}

The means with the same letters in each column are not significantly different based on Duncan Multiple Range Test at the 5% probability level.

Table 2**.** The effects of chalcone nanostructure on proline and glycine betaine of lettuce plants at salinity stress.

Treatments		Proline	Glycine betaine
Salt (mmol/l)	Nano (ppm)		
0	0	18.25±0.27^{g}	5.32±0.013^{c}
	0.1	37.83±1.26^{e}	6.30±0.013^{b}
	0.01	25.75±2.97^{f}	7.43±0.034^{b}
100	0	72.26±3.92^{c}	4.16±0.031^{d}
	0.1	69.91±16.12^{c}	3.52±0.023^{d}
	0.01	65.07±12.96^{c}	5.75±0.027^{c}
125	0	89.86±0.31^{b}	5.17±0.033^{c}
	0.1	96.53±18.24^{b}	10.52±0.016^{a}
	0.01	60.15±0.63^{c}	9.42±0.022^{a}
150	0	117.57±4.16^{a}	1.89±0.016^{e}
	0.1	86.81±1.72^{b}	6.00±0.025^{b}
	0.01	42.36±0.91^{d}	8.80±0.117^{a}

The means with the same letters in each column are not significantly different based on Duncan Multiple Range Test at the 5% probability level.

the nanocomposite (Table 3). This is attributed to the nanocomposite's role in alleviation of the salinity damages in plants.

The results indicated that salinity did not significantly affect the total protein content of lettuce plants. However, nanocomposite along with the salinity of 100 mmol/l led to a significant increase in the total proteins compared to the related control (Table 4). The soluble sugars were significantly increased at the salinity of 100 mmol/l but decreased at higher salt concentration of 150 mmol/l compared to the control. Nanocomposite alone decreased the amount of soluble sugars significantly, however, 0.1 ppm nanocomposite in the presence of all salt concentrations increased the soluble sugars. In contrast, 0.01 ppm nanocomposite along with higher salt concentrations (125 and 150 mmol/l) decrease the soluble sugars considerably compared to the control. Higher salt concentrations (125 and 150

Table 3. The effects of chalcone nanostructure on hydrogen peroxide and malondialdehyde content of lettuce plants under salinity stress

Treatments		Hydrogen peroxide	Malondialdehyde
Salt (mmol/l)	Nano (ppm)		
0	0	0.306±0.039^{c}	0.0025±0.0002^{b}
	0.1	0.110±0.053^{d}	0.0015±0.0013^{d}
	0.01	0.156±0.032^{d}	0.0018±0.0007^{d}
100	0	0.355±0.078^{c}	0.0205±0.0007^{a}
	0.1	0.448±0.115^{b}	0.0101±0.0015^{b}
	0.01	0.547±0.02^{b}	0.0214±0.0012^{a}
125	0	0.407±0.056^{b}	0.0234±0.0001^{a}
	0.1	0.0943±0.041^{e}	0.0118±0.0006^{b}
	0.01	0.105±0.063^{d}	0.0115±0.0001^{b}
150	0	0.701±0.052^{a}	0.0277±0.0017^{a}
	0.1	0.208±0.068^{cd}	0.0014±0.0010^{d}
	0.01	0.569±0.161^{b}	0.0062±0.0009^{c}

The means with the same letters in each column are not significantly different based on Duncan Multiple Range Test at the 5% probability level.

Table 4. The effects of chalcone nanostructure on some biochemical characteristics of lettuce plants under salinity stress

Treatments		Total protein	Soluble sugars	Amino acids
Salt (mmol/l)	Nano (ppm)			
0	0	0.918±0.024^{b}	0.0314±0.0003^{c}	2.364±0.002^{b}
	0.1	0.973±0.006^{b}	0.0069±0.0002^{e}	2.568±0.001^{a}
	0.01	0.957±0.012^{b}	0.0261±0.0002^{d}	2.579±0.0003^{a}
100	0	0.9173±0.021^{b}	0.0557±0.0009^{b}	2.146±0.003^{b}
	0.1	1.002±0.010^{a}	0.0617±0.0002^{a}	2.569±0.001^{a}
	0.01	0.970±0.010^{a}	0.0652±0.00004^{a}	2.572±0.001^{a}
125	0	0.919±0.027^{b}	0.0362±0.0005^{c}	2.627±0.003^{a}
	0.1	0.988±0.002^{a}	0.0685±0.0002^{a}	2.368±0.002^{b}
	0.01	0.942±0.023^{b}	0.0191±0.0004^{d}	2.620±0.004^{a}
150	0	0.921±0.023^{b}	0.0237±0.0003^{d}	2.675±0.002^{a}
	0.1	0.884±0.011^{c}	0.0541±0.0005^{b}	2.453±0.004^{b}
	0.01	0.883±0.014^{c}	0.0029±0.0003^{f}	2.676±0.001^{a}

The means with the same letters in each column are not significantly different based on Duncan Multiple Range Test at the 5% probability level.

mmol/l) increased the amount of total amino acids in the lettuce. Nanocomposite alone or together with 100 mmol/l salt also elevated the total amino acids content compared to the related control. However, 0.1 ppm nanocomposite combined with 125 and 150 mmol/l salt reduced the amount of total amino acids compared to the related control.

It was revealed that salinity caused a significant rise in ascorbate peroxidase. Also, the activity of ascorbic peroxidase in the lettuce plants treated with 0.01 ppm of the nanocomposite + 125 or 150 mmol/l salinity significantly increased compared to the related controls (Table 5). However, combining the nanocomposite with 100 mmol/l salinity, decreased the ascorbic peroxidase activity. Salinity alone didn't affect the activity of catalase. However, nanocomposite alone or nanocomposite + 100 mmol/l salinity, decreased the catalase activity. At 150 mmol/l salinity, the protease activity increased, however, there was no specific trend in the effect of nanocomposite or its combination with salinity on the protease activity

Table 5. The effects of chalcone nanostructure on antioxidant and protease enzymes of lettuce plants under salinity

Treatments		Enzymes			
Salt (mmol/) l	Nano (ppm)	Ascorbate peroxidase	Catalase	Protease	Polyphenol oxidase
0	0	3.793±0.817^{d}	0.942±0.029^{a}	0.3626±0.0151^{c}	7.61±0.69^{d}
	0.1	4.790±0.154^{d}	0.812±0.038^{b}	0.1666±0.0035^{d}	10.27±0.32^{d}
	0.01	7.567±0.443^{a}	0.805±0.046^{b}	0.4680±0.0053^{b}	10.81±0.15^{d}
100	0	6.810±0.251^{b}	0.974±0.055^{a}	0.4015±0.0076^{b}	16.18±0.36^{a}
	0.1	4.143±0.483^{d}	1.145±0.082^{a}	0.6609±0.0038^{a}	11.68±0.71^{c}
	0.01	5.290±0.660^{c}	1.119±0.007^{a}	0.3845±0.0020 ^{c}	13.04±0.22^{b}
125	0	6.017±0.394^{b}	1.040±0.046^{a}	0.37196±0.0059^{c}	11.36±1.17^{c}
	0.1	6.830±0.078^{b}	0.889±0.010^{b}	0.1600±0.0040^{d}	6.52±1.57^{e}
	0.01	8.073±0.471^{a}	0.817±0.041^{b}	0.1542±0.0020^{d}	12.21±0.81^{b}
150	0	7.860±0.547^{b}	1.100±0.060^{a}	0.6271±0.0049^{a}	12.24±1.25^{b}
	0.1	6.963±0.120^{b}	1.075±0.025^{a}	0.4657±0.0079^{b}	10.78±0.02^{d}
	0.01	8.837±0.686^{a}	0.955±0.068^{a}	0.6024±0.0101^{a}	12.63±0.37^{b}

The means with the same letters in each column are not significantly different based on Duncan Multiple Range Test at the 5% probability level.

of the lettuce plants. Salinity or nanocomposite alone increased the activity of polyphenol oxidase considerably. However, when salinity was combined with 0.1 ppm nanocomposite, the polyphenol oxidase activity at all salinity concentrations decreased significantly.

Phytochemical characteristics

At the salinity concentrations of 100 and 125 mmol/l, total tannins increased significantly, however, at 150 mmol/l, the amount of total tannins was not significantly different from that of the related control. Higher salinity concentrations (125 and 150 mmol/l) decreased the total phenols content of lettuce plants significantly. Nanocomposite alone or together with 100 mmol/l salt also decreased the content of total phenols significantly compared to the related controls. Salinity or nanocomposite alone enhanced the amount of anthocyanin significantly, however, the 0.01 ppm concentration of nanocomposite along with 100 mmol/l salt and also both concentrations of nanocomposite at higher doses of the salt, decreased the anthocyanin content significantly compared to the related controls. Salinity reduced the flavonoids, however, at the highest concentration (150 mmol/l), when combined with the nanocomposite, the anthocyanin content declined significantly compared to the related control (Table 6).

Discussion

It is well known that salinity is one of the major stresses damaging plants and cause a considerable decrease in crops and horticultural products. Our results revealed that the chalcone-based nanocomposite considerably alleviated the harmful effect of higher salinity conditions (150 mmol/l) on the fresh and dry weight of roots in lettuce plants. This strategy can be regarded as a tolerance mechanism to increase the root hydraulic capacity of water absorption in saline stress. Also, it might be attributed to the nanostructure function to reduce harmful effects of reactive oxygen species

Table 6. The effects of chalcone nanostructure on phytochemicals of lettuce plants under salinity stress.

Treatments					
Salt (mmol/l)	Nano (ppm)	Total tannins	Total phenols	Anthocyanin	Flavonoids
0	0	0.09615±0.00066^{b}	0.07265±0.00064^{b}	1.190±0.012^{de}	4.157±0.019^{a}
	0.1	0.09969±0.00089^{b}	0.065927±0.00081^{c}	1.563±0.003^{c}	4.157±0.019^{a}
	0.01	0.0989±0.00056^{b}	0.06003±0.00060^{c}	1.337±0.009^{d}	3.579±0.049^{b}
100	0	0.12185±0.00046^{a}	0.07811±0.00048^{b}	1.677±0.012^{c}	2.805±0.048^{c}
	0.1	0.07781±0.00073^{c}	0.03992±0.00081^{e}	1.863±0.007 ^{b}	2.292±0.040^{c}
	0.01	0.08145±0.00075^{c}	0.03104±0.00070^{e}	0.950±0.010^{e}	1.571±0.120^{d}
125	0	0.10113±0.00065^{a}	0.06902±0.00070^{c}	1.943±0.003^{a}	2.458±0.051^{c}
	0.1	0.09694±0.00062^{b}	0.06249±0.00060^{c}	1.830±0.010^{b}	2.431±0.029^{c}
	0.01	0.07557±0.00078^{c}	0.04056±0.00074^{d}	1.733±0.009^{bc}	2.447±0.016^{c}
150	0	0.09487±0.00023^{b}	0.04474±0.00019^{d}	2.040±0.016^{a}	1.611±0.322^{d}
	0.1	0.07232±0.00027^{c}	0.03532±0.00028^{e}	1.560±0.006^{c}	3.544±0.064^{b}
	0.01	0.13609±0.00028^{a}	0.08945±0.00028^{a}	1.823±0.003^{b}	3.621±0.018^{b}

The means with the same letters in each column are not significantly different based on Duncan Multiple Range Test at the 5% probability lev

(ROS), such as hydrogen peroxide, which damages photosynthesis membranes in the chloroplasts with the lipid peroxidation process (Vandenabeele *et al.* 2003). Hydrogen peroxide production increases due to abiotic and biotic stresses, resulting in tissue damage and oxidative stress. Although, there has been some evidence that this compound may function as a messenger molecule in plants to increase plant tolerance to salinity, however, at high salt concentrations it may activate cell programmed death.

Malondialdehyde accumulation under salinity stress is a characteristic sign of lipid peroxidation caused by ROS. Our results indicated that the nanocomposite decreased the malondialdehyde content of salt-treated plants demonstrating its inhibitory effects on lipid peroxidation. Moreover, results showed that the nanocomposite stimulated the lettuce plants' enzymatic system (i.e. ascorbate peroxidase) under higher salinity concentrations (125 and 150 mmol/l). It has been shown that ascorbate peroxidase enzyme activity enhances to the elimination of ROS to prevent its harmful effects on plants (Kim *et al*. 2008; Feng *et al.* 2011). The nanostructure also enhanced the biosynthesis of secondary metabolites such as flavonoids, phenols, and tannins at higher salinity conditions (150 mmol/l). Chalcone is regarded as the precursor of the flavonoid biosynthetic pathway (Rudrapal *et al.* 2021). The nanostructure form of chalcone is dissolvable in water (Faghihi *et al.* 2012) and facilitates its entrance to the plant organs.

Salinity stress may cause water loss and deficiency in plants, which results in the malfunction of enzymes in the plant cells. At the higher salt concentration (150 mmol/l) the glycine betaine declined drastically, however, the nanocomposite managed to enhance its biosynthesis at this salinity conditions. Also, 0.1 ppm nanocomposite alleviated the reduction of soluble sugars at higher salinity conditions (125 and 150 mmol/l) in the lettuce plant to maintain the water balance of the cells and protect them from desiccation damage (Ahmad *et al.* 2008).

Similar to our results, it has been shown that

The biosynthesis of some plant secondary metabolites such as anthocyanins is an important strategy in plants to overcome environmental abiotic stresses such as salinity. These metabolites donate free radicle scavenging capacity to plant cells and protect the cell from the harmful effects of peroxide anion, hydroxyl radicle, hydrogen peroxide, and other ROS (Shah and Smith 2020).

Conclusions

It was concluded that the chalcone-based nanocomposite can be considered as a candidate for alleviation of salt stress effects in plants. It may improve the plants' detoxification systems to eliminate harmful ROS produced at the salinity conditions. The compound may stimulate some compatible solutes to maintain the plant water balance and play a chaperone role for enzymes and other bio-macromolecules.

Conflict of interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

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کاهش آسیب‌های ناشی تنش از تنش شوری با استفاده از نانوکامپوزیت حاوی شالکون در گیاه کاهو

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چکیده

هدف این تحقیق، بررسی اثرات نانوساختار شالکون در غلظت‌های مختلف بر گیاه کاهو در شرایط تنش شوری بود. تیمار شوری شامل غلظت‌های ۱۰۰، ۱۲۰ و ۱۵۰ میلی مول بر لیتر نمک و غلظت‌های ۰/۱ و ۰/۰۱ از نانوکامپوزیت بود. نانوکمپوزیت با روش‌های شیمیایی و به اندازه ۸۹/۴ نانومتر ساخته شد. بعد از برداشت گیاهان، صفات مختلف فیزیولوژیکی و بیوشیمیایی مورد سنجش قرار گرفتند. نتایج نشان داد که نانوکامپوزیت حاوی شالکون به طور معنی‌داری موجب افزایش برخی صفات مانند وزن تر و خشک وزن تر و خشک ریشه و میزان گلیسین بتا بتائین را در گیاه کاهو در شرایط تنش شوری بالاتر (۱۵۰ میلی مول در لیتر) افزایش داد. برعکس، نانوکمپوزیت شالکون به طور معنی داری نشانگر تنش یعنی مالون دی آلدئید را در شرایط شوری کاهش داد. از طرف دیگر، نانوکمپوزیت منجر به افزایش معنی‌دار فلاونوئید کل در شرایط تنش شوری ۱۵۰ میلی مول در لیتر و فعالیت آنزیم آنتی اکسیدان آسکوربات پراکسیداز در شوری ۱۵۰ میلی مول در لیتر شد. می‌توان نتیجه گرفت که نانوکمپوزیت بر پایه شالکون قادر است اثرات منفی تنش شوری در گیاهان را تعدیل نماید.

واژه‌های کلیدی: شالکون؛ شوری؛ کاهو؛ نانوکمپوزیت