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ORIGINAL RESEARCH PAPER

Evaluation of Chitosan Nanoparticles Effects on Yield and Yield Components of Barley (*Hordeum vulgare* L.) under Late Season Drought Stress

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ABSTRACT
As a step towards the profitable employment of nanoparticles (NPs) in agriculture, effects of chitosan NPs was probed on barley plants under late season drought stress. A factorial experiment was performed based on a randomized complete block design with three replications. The experimental factors included the chitosan NPs concentrations (0 (control), 30, 60 and 90 ppm), application methods (foliar and soil application) and irrigation regimes (well-watered and withholding of irrigation for 15 days after pollination). The barley seeds were separately planted in pots. Then, the NPs were added to them through the soil and foliar application at three stages. The results indicated that using the chitosan NPs, especially 60 and 90 ppm, significantly increased the leaf area (LA), the leaf color (SPAD), the number of grain per spike, the grain yield and the harvest index compared to the control. Also, drought stress significantly decreased the yield and yield components compared to the well-watered plants. In contrast, using the chitosan NPs in plants under drought stress significantly increased the relative water content (RWC), the 1000-grain weight, the grain protein, the proline content, the catalase (CAT) and the superoxide dismutase (SOD) compared to the control. There was no a significant difference between two methods of using NPs in most studied traits. The results highlighted that using the chitosan NPs, especially 60 and 90 ppm, in both irrigation regimes can significantly improve the majority of the studied traits compared to the control and mitigate the harmful effects of drought stress.

Keywords: *Chitosan, Enzyme Activity, Nanoparticles, Protein, Yield*

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INTRODUCTION

Nanotechnology is a process to generate, manipulate and deploy nanomaterials into a system [1]. This technology employs NPs having at least one dimension in the order of 100 nm or less [2]. So, 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 [3].

Barely (*Hordeum vulgare* L.) is one of the five important crops that is commonly used as human and animal food and also in malt production [4]. Barley is often subjected to extreme drought stress

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that significantly affects production [5]. Underwater stress, plants can avoid drought harm through several ways such as stomatal closure, leaf rolling, osmotic adjustments, reductions and consequently decreases in the cellular expansion, and alterations of various essential physiological and biochemical processes [6]. In this respect, Bittelli *et al.* [7] reported that occasional or episodic drought events can be counteracted through the use of compounds such as chitosan.

Chitosan is a natural polysaccharide derived by *N*-deacetylation of chitin, and a major component of the shells of a crustacean such as a crab, shrimp, and crawfish [8]. Chitosan and their derivatives are non-toxic, biodegradable, and friendly to the environment and have a great potential for agricultural application and enhancing crop production [9-10]. Also, due to its cationic character, chitosan presents a wide variety of physicochemical and biological properties, including antimicrobial, antioxidant and antihypertensive properties [11]. It has proved to be effective in many crops to protect plants against oxidative stress [12] and to stimulate plant growth [13]. Some researchers reported that chitosan has been widely used as growth stimulator, germination acceleration, and yield enhancement in many crop species such as orchid [14], faba bean [15] and corn [16]. Finally, Saharan *et al.* [17] stated that Cu-chitosan NPs improved growth, seed germination, seedling length, fresh and dry weight of tomato at 0.08, 0.10 and 0.12% levels.

Generally, research work of chitosan NPs on growth and yield of barley under late-season drought stress is almost rare. So, the aim of present work was studying the effect of chitosan NPs with two application methods on the barley plants under late season drought stress.

MATERIALS AND METHODS

Growth Condition

A pot test was carried out to assess the effects of chitosan NPs on barley plants under late-season drought stress in a randomized complete block design arrangement in a factorial experiment with three replications. The experimental factors included the NPs concentrations (0 (control), 30, 60 and 90 ppm), application methods (foliar and soil application) and irrigation regimes (well-watered and withholding of irrigation for 15 days after pollination). The experiment was conducted at the College of Agriculture, Tarbiat Modares University (35 43′ N; 51 8′ E; 1215 m sea level), Tehran, Iran.

Soil Characteristics

The soil characteristics were as follow: sandy loam in texture (12.5% clay, 17.25% silt and 72.2% sand), total nitrogen 0.11%, organic matter 1.08%, available phosphorus 69.46 ppm, available potassium 616.08 ppm, iron 7.76 ppm, pH, 7.7 and EC, 0.4 $ds.m^{-1}$.

Plant Materials

The seeds of Barley (*Hordeum vulgare* cv. Reyhan) were purchased from the Plant, Breeding and Seed Institute of Karaj, Iran.

Synthesis of Chitosan NPs

5.0 g of the chitosan powder with a size of 10 to 20 µm and 99% purity was passed through ultrasonic vibration (100 W, 40 KHz) and then electric arc. Next, the powder was transferred to a grinding machine. The grinding machine in our study had five containers and particles were collected in each container with sizes smaller than 900 nm, 600 nm, 300 nm, 200 nm, and 100 nm, respectively. A continuous cycle of high rotation was performed which included four steps as follows:

1. Repeated rotation cycle with 18000 rpm for 5 consecutive cycles, 120 seconds per each cycle
2. Repeated rotation cycle with 22000 rpm for 5 consecutive cycles, 120 seconds per each cycle
3. Opening the exhaust vent while when applying 22000 rpm for 3 consecutive cycles, 180 seconds per each cycle
4. Collecting the fine powder in the dishes embedded along the exhaust pipe of the grinding machine

It should be noted that the fifth container was equipped with a thin multi-layer filter that only the air was able to get out of it but the NPs accumulated behind the filter. A summary of the chitosan NPs production process by a grinding machine is showed in Fig. 1.

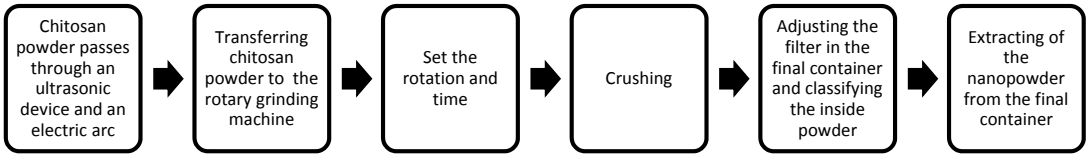

Fig 1. Summary of the chitosan NPs production process by a grinding machine

Chitosan NPs Solution Preparation

1 g of the chitosan NPs was solubilized in 1% acetic acid. Then, 100 mL distilled water was added to the above solution under constant stirring until it was completely dissolved. Next, the solution was alkalized to pH 6 with 1 M NaOH solution [18]. Finally, different doses of the chitosan NPs (30, 60 and 90 ppm) were prepared for the pottest .

Treatments

The ten seeds of barley were surface sterilized and sown in a plastic pot (27 cm in height and 26 cm in diameter) containing 10 kg of field soil. Fertilizers were applied to the pots according to the soil analysis. Urea fertilizer was added at the amount of 1.03 g N/pot in two equal portions; the first during the seedling stage and the second at the stem elongation stage.

The seedling was thinned out to allow four plants per pot for data recording. Four concentrations of chitosan NPs (0 (control), 30, 60, and 90 ppm), were applied three times at the stage of tillering, stem elongation and heading in the soil and through foliar application. Only distilled water was used in the control treatment. For the well-watered group, pots were regularly watered by watering with tap water every 7 days and for the drought-stressed group, the imposition of water stress started at 15 days after pollination to maturity. During rainy days, a mobile rain shelter was used in the drought stress treatments to prevent infiltration of the rain.

Measurements

At the end of a week stress period, three flag leaves of four plants were labeled and some traits were determined *i.e.* leaf color (SPAD), leaf area (LA), proline content, catalase (CAT), superoxide dismutase (SOD) activities and relative water content (RWC). Also, a number of tillers, plant height, biomass, harvest index, yield and yield components were recorded at the harvest time. The harvest index (HI) was accounted for following:

$$HI = (Grain\ yield / Biomass)*100$$

RWC

The RWC was calculated using the method devised by Mata and Lamattina [19] using the following equation:

$$RWC\ (\%) = (FW - DW)/ (TW-DW) \times 100 \qquad (1)$$

The fresh weight (FW) was measured immediately after excision, the full turgid weight (TW) was determined after rehydration of the leaves placing them in a test tube containing distilled water for 24 hours at 4°C in darkness, and the dry weight (DW) was determined after oven drying at 80°C for 48 hours.

LA and SPAD

The LA was estimated using portable area meter model Li-3000A LI-COR. Also, the SPAD was measured by chlorophyll meter (SPAD-502, Minolta, Japan).

Proline, CAT, and SOD

On the 7th day after drought stress, three flag leaves four plants for each pot were harvested and frozen in liquid nitrogen immediately for the analysis of CAT, SOD, and proline. Fresh leaves were ground to a fine powder in liquid N_2 and then homogenized in 5 mL of 50 mM sodium phosphate buffer (pH 7.8). The homogenate was centrifuged at 12,000 g for 15 min at 4 °C, and the supernatant was used to determine the following enzyme activities. SOD activity was determined following inhibition of the photochemical reduction by nitro blue tetrazolium (NBT) by the method proposed by Ali *et al.* [20]. One SOD unit was defined as the amount of enzyme needed to produce a 50% inhibition of NBT at 560 nm using a spectrophotometer (Specord 200, Analytical Jena, Germany). The CAT activity was determined as the consumption of H_2O_2 (extinction coefficient 39.4 mM cm^{-1}) measured at 240 nm for 3 min at 25 °C [21]. CAT activity was expressed by H_2O_2 reduced min^{-1} mg^{-1} of protein. For measurement of proline, 0.2 g of fresh samples were homogenized in 3% sulphosalicylic acid and then the extract was centrifuged at 10000 g for 10 min. Next, the supernatant was mixed with ninhydrin and glacial acetic and phosphoric acids, incubated at 90 °C for 30 min and later cooled on ice. The reaction mixture was extracted with toluene and was read at 520 nm [22].

Grain Protein

The protein content of grain was determined in the dry seeds after harvesting using near-infrared reflectance (NIR).

Statistical Analysis

Data were statistically analyzed using two-way analysis of variance (SAS Institute, 9.1.3). The significance of differences among treatment means was compared by Duncan's multiple range tests at $P<0.05$.

RESULT AND DISCUSSION

Characterization of Chitosan NPs

Specific surface area of chitosan NPs was > 80 $m^2 g^{-1}$, average primary particle size was > 100 nm and purity was >99%. The size of chitosan NPs was determined through Field Emission-Scanning Electron Microscope (FE-SEM) of the Holland Philips EM3200 microscope with an accelerating voltage of 26 kV (Fig. 2.a). Also, energy dispersive X-ray spectroscopy (EDS) analysis showed that the chitosan NPs was primarily composed of elements, such as C, K, and O (Fig. 2.b).

Analysis of Variance

Analysis of variance showed that the three-way interaction among NPs concentration, application methods, and irrigation regimes was significant for RWC, SOD, CAT, proline content, 1000-grain weight, and grain protein (Table 1). There was a significant two-way interaction between NPs concentration and application methods as well as NPs concentration and irrigation regimes interaction on a number of tiller per plant and plant height (Table 2). Also, effects of NPs concentration and irrigation regimes were significant for SPAD, grain yield, and harvest index. Finally, the main effect of NPs concentration for a number of grain per spike and LA as well as the interaction between application method and irrigation regime were significant for biomass.

LA, SPAD, and RWC

Use of 60 and 90 ppm the chitosan NPs significantly increased LA and SPAD of leaves compared to the control (Table 3). Also, drought stress significantly decreased SPAD compared to the well-watered plants (Fig. 3). In well-watered plants, foliar application of 90 ppm chitosan NPs led to the highest RWC (89.94 %) (Table 4). Use of the NPs in plants under drought stress significantly increased RWC of leaves compared to the control. In plants under drought stress, there was no significant difference between two methods of using the NPs on the mean of RWC; whereas, in well-watered plants, the foliar application was better than soil application of the NPs.

Photosynthetic pigments and LA were significantly affected by applying chitosan NPs and

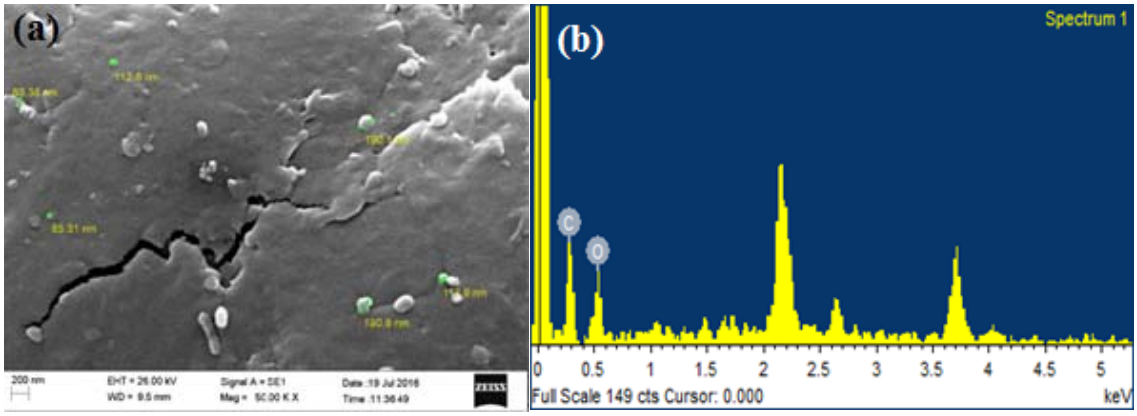

Fig 2. FE-SEM (a) and EDS spectrum (b) images of chitosan NPs.

Table 1: Analysis of variance on some measured traits at barley affected by the treatments

		Mean Square						
S.O.V	df	LA	SPAD	RWC	SOD	Catalase	Proline	Grain protein
Repeat	2	4.68	4.45	28.37	111.25	826.58	22.80	16.17
NPs (A)	3	100.27 **	82.40 **	279.95 **	42.18 **	1520.30 **	571.74**	9.67 **
Methods (B)	1	1.63 ns	1.32 ns	0.35 ns	2.07 **	2.41 ns	0.92 *	0.01 ns
Irrigation regimes (C)	1	6.11 ns	267.62 **	26853.15 **	237.22 **	131387.03 **	24630.06 **	18.50 **
A×B	3	22.51 ns	6.01 ns	51.95 **	0.59 **	211.07 **	41.81 **	14.32 **
A×C	3	31.70 ns	7.88 ns	658.82 **	47.16 **	1278.84 **	429.86 **	5.43 **
B×C	1	17.34 ns	1.43 ns	38.30 *	31.50 **	205.63 **	1.97 **	5.29 **
A×B×C	3	22.59 ns	5.58 ns	852.69 **	4.06 **	171.47 **	27.70 **	4.36 **
Error	30	21.64	3.25	6.02	0.01	5.61	0.18	0.44
CV (%)	-	18.15	3.44	5.19	7.57	16.31	8.67	6.62

*, ** and ns: significant at 0.05, 0.01 probability level and no significant, respectively

Table 2: Analysis of variance on some measured traits at barley affected by the treatments

		Mean Square						
S.O.V	df	1000-grain weight	Number of grain per spike	Number of tiller per plant	Grain yield	Biomass	Harvest index	Plant height
Repeat	2	3.11	49.94	5.14	18.66	9.36	113.18	5.34
NPs (A)	3	14.11 *	132.92 **	37.80 **	46.78 **	25.13 *	82.25 *	4.80 ns
Methods (B)	1	51.04 **	1.68 ns	6.75 ns	0.66 ns	1.43 ns	7.79 ns	5.63 ns
Irrigation regimes (C)	1	3694.27 **	27.28 ns	161.33 **	784.16 **	653.94 **	895.96 **	97.44 **
A×B	3	30.40 **	9.49 ns	20.02 **	2.28 ns	4.19 ns	6.32 ns	21.97 *
A×C	3	12.30 *	8.89 ns	18.05 **	10.73 ns	6.35 ns	54.90 ns	17.57 *
B×C	1	61.42 **	3.85 ns	1.33 ns	11.22 ns	297.95 **	81.38 ns	1.64 ns
A×B×C	3	4.17 ns	10.52 ns	8.05 ns	2.50 ns	4.24 ns	6.33 ns	6.53 ns
Error	30	3.90	21.53	3.41	4.22	7.97	24.47	5.13
CV (%)	-	6.57	10.41	10.53	8.19	5.18	10.77	9.72

*, ** and ns: significant at 0.05, 0.01 probability level and no significant, respectively

Table 3: Means comparison the effects of NPs concentration on some measured traits at barley

NPs (ppm)	LA (cm^2)	SPAD	Number of grain per spike	Grain yield ($g.pot^{-1}$)	Harvest index (%)
0	21.87 b	48.91 c	39.85 b	22.71 b	43.07 b
30	25.42 ab	52.01 b	44.54 a	24.31 b	44.44 b
60	26.43 a	54.52 a	46.4، a	27.18 a	48.82 a
90	28.84 a	54.34 a	47.33 a	26.11 a	47.28 ab

Means by the uncommon letter in each row and column are significantly different according to Duncan tests ($p<0.05$).

Table 4: Means comparison the effects of irrigation regimes, application methods and NPs concentration on some measured traits at barley

Treatment	RWC (%)	Proline ($\mu mol.g^{-1}$ F.W)	SOD ($unit.mg^{-1}.pr.min^{-1}$)	Catalase (ΔA mg $pro.^{-1} min^{-1}$)	1000-Grain Weight (g)	Protein (%)
CSN1*F*N	71.17 c	38.64 f	20.21 fg	120.75 g	37.53 b	7.40 i
CSN2*F*N	77.34 b	39.36 f	20.97 efg	122.42 g	36.83 b	8.28 hi
CSN3*F*N	78.40 b	39.92 f	20.70 efg	123.63 g	39.13 ab	11.53 abc
CSN4*F*N	85.94 a	40.27 f	21.10 efg	125.17 g	42.13 a	11.67 ab
CSN1*F*D	32.16 h	73.80 e	24.11 d	214.76 ef	17.40 f	8.39 hi
CSN2*F*D	35.98 gh	75.34 e	25.17 c	232.85 c	18.36 f	10.34 cdef
CSN3*F*D	45.84 f	90.73 c	28.86 ab	220.10 de	18.10 f	11.08 bcd
CSN4*F*D	56.50 e	105.76 a	29.71 a	272.69 a	22.53 de	11.38 bc
CSN1*S*N	70.51 c	39.04 f	21.08 efg	122.86 g	37.33 b	7.27 i
CSN2*S*N	70.23 c	39.41 f	20.57 fg	124.16 g	39.76 ab	9.95 def
CSN3*S*N	71.25 c	41.75 f	20.25fgj	125.63 g	39.86 ab	9.68 efg
CSN4*S*N	68.40 c	40.32 f	19.66 g	124.42 g	37.84 b	10.78 bcde
CSN1*S*D	32.87 h	75.81 e	24.38 d	212.33 f	20.03 ef	8.63 gh
CSN2*S*D	37.62 g	78.84 d	26.26 bc	222.76 d	23.90 cd	11.89 ab
CSN3*S*D	45.54 f	94.30 b	25.69 c	238.42 c	23.00 de	9.46 fgh
CSN4*S*D	59.17 d	96.16 b	27.54 b	248.54 b	26.76 c	12.67 a

CSN represents chitosan NPs. 1, 2, 3, 4 represents 0, 30, 60, 90 ppm of NPs, respectively. Also, F, S show foliar and soil a pplication, respectively. The N, S represents Well-watered and Drought stress, respectively. Means by the uncommon letter in each column are significantly different according to Duncan test ($p<0.05$).

drought stress. The decrease in SPAD and RWC of leaves under drought stress is a commonly observed phenomenon [23]. The decrease in chlorophyll content might be due to reduced synthesis of the main chlorophyll pigment complexes encoded by the *cab* gene family [24], or to oxidative damage of chloroplast lipids, pigments and proteins [25]. Also, a decrease of RWC in plants under drought stress, suggests less relative water absorption or water maintenance in barley plants when they were faced with drought stress. These results are supported by a reduction of chlorophyll content under drought stress in barley [26]. Also, Kirnak *et al.* [27] found that water stress resulted in significant decreases in chlorophyll content, leaf area, electrolyte leakage, RWC and vegetative growth.

Furthermore, our results showed that chitosan NPs may increase photosynthetic pigments and leaf area by enhancing endogenous levels of cytokinins, which stimulated chlorophyll synthesis and growth or to the greater availability of amino compounds released from chitosan [28]. In 2012, Farouk and Amany [6] reported that the total content of chlorophylls and carbohydrates were significantly decreased under water stress compared to the

control cowpea plants; whereas, foliar application of chitosan, especially at 250 mg.l^{-1}, significantly increased these parameters compared to the untreated plants under stress. In another report, soil addition of chitosan increased height, canopy diameter, and leaf area of *Capsicum annuum* L. [29].

Proline, CAT, and SOD

In both methods of using chitosan NPs, drought stress significantly increased the proline content, CAT, and SOD activities compared to the well-watered plants (Table 4). Use of chitosan NPs in well-watered plants had no significant effect on proline content, CAT and SOD activity compared to the control. In contrast, in plants under drought stress, application of NPs significantly increased proline content, CAT, and SOD activities compared to the control. There was no significant difference between methods of using NPs on the mean of proline content, CAT and SOD activities.

Amino acid proline has been described as an osmoprotectant and is accumulated under several stresses such as drought [30], as seen in the present study (Table 3). Proline accumulation may be due to the increase of proline synthesis or reduction of proline degradation in response to drought stress. It is responsible for the hydration of biopolymers surviving as a readily utilizable energy source and nitrogen source compound during periods of inhibited growth [31]. Some researchers stated that water deficit in castor bean plants increased free proline and foliar application of 5 g.L^{-1} chitosan had no significant effect on it [32].

Also, exposure of plants to drought stress lead to deregulation or disruption of electric transport chain and consequently give rise to the generation of reactive oxygen species (ROS), which are considered as strong oxidizing and potentially harmful agents for cells [33]. Thus, plants protect cell systems from the cytotoxic effects of drought-accumulated active oxygen species using anti-oxidative enzymes such as SOD, and CAT [34]. The SOD detoxifies superoxide anion free radicals (O_2^-) by forming H_2O_2, and then the H_2O_2 can be eliminated by CAT and peroxidase [35]. Our results have been widely studied [36-37-38].

Moreover, higher CAT and SOD activities via chitosan NPs seems to indicate the effectiveness of this compound as an antioxidant system inductor of the plant. Ortega-Ortíz *et al.* [39] stated that enzymes activity increased due to the treatment of chitosan or chitosan NPs in tomato fruits under oxidative stress.

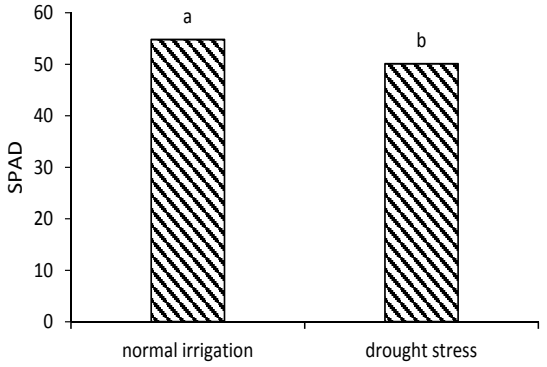

Fig 3. Effect of irrigation regimes on SPAD. Means by the uncommon letter in each column are significantly different according to Duncan test (p<0.05).

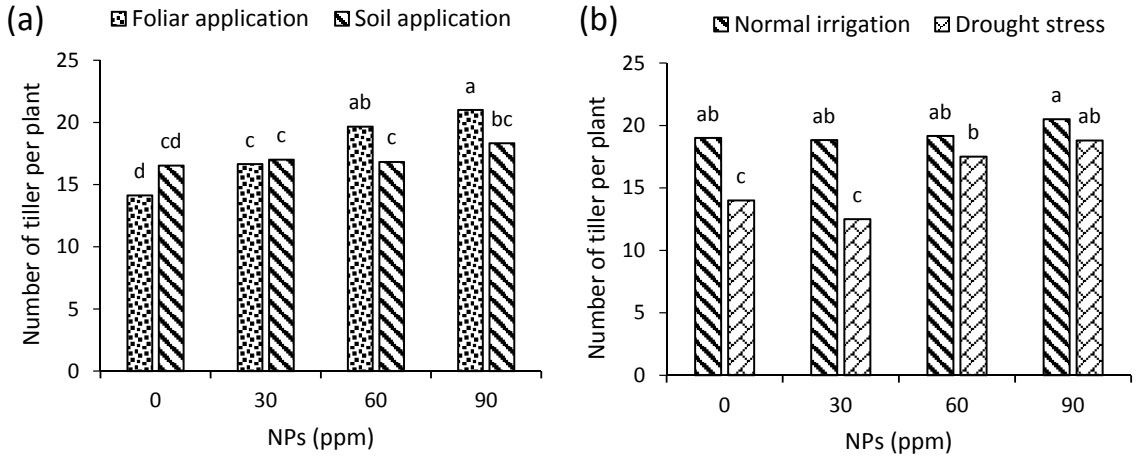

Fig 4. The effect of NPs concentration, application method and irrigation regime on number of tiller. Means by the uncommon letter in each column are significantly different according to Duncan test (p<0.05).

Grain Protein

The results in Table 4 demonstrated that the highest grain protein (12.67%) found when 90 ppm chitosan NPs used as soil application in plants under drought stress. In contrast, the lowest grain protein found in the control plants. Use of the NPs in both irrigation regimes significantly increased the percent of grain protein. Drought stress had no significant effect on the mean of grain protein compared to the control. There are many different reports of drought stress effects on grain protein. For example, highly induced rice protein has been observed under abscisic acid, salt and drought stresses [40]; but Jansen [41] recorded the insignificant effect of water stress on protein content in *Lupinus angustifolius* cultivars.

On the other hand, the role of chitosan NPs in increasing grain protein in both irrigation regimes may be due to the N content of chitosan that plays important role in the synthesis of protein. Xianling *et al.* [42] observed that mulberry grains were coated with chitosan solution increased the respiration rate of germination seeds, chlorophyll, protein content and peroxidase in seedlings. Also, Lizarraga-Paulin *et al.* [43] stated that chitosan sprinkling increased protein content in maize varieties.

Yield and Yield Components

The results of this study showed that use of chitosan NPs significantly increased the number of grain per spike and grain yield compared to the control (Table 3). The highest 1000-grain weight (42.13 g) obtained with foliar application of 90 ppm NPs in well-watered plants (Table 4). Using chitosan NPs, especially soil application of them, significantly increased the 1000-grain weight in plants under drought stress compared to the control. The interaction between concentration and application methods of NPs showed that the highest number of tiller per plant encountered with foliar application of 60 and 90 ppm NPs (Fig. 4a). Also, the interaction between NPs concentration and irrigation regime showed that drought stress decreased the number of tiller per plant; whereas, use of 60 and 90 ppm NPs decreased negative effect of drought stress compared to the control (Fig. 4b). The mean of grain yield reductions due to drought stress was 28% compared to the well-watered plants (Fig. 5).

Closure of stomata and decrease in CO_2 concentration as an initial response to drought stress that inhibits dry mater production due to the limitation of photosynthesis [44] and so that decrease yield and its components. Drought stress also reduces the uptake of essential elements and the excessive accumulation of intermediate compounds such as reactive oxygen species which cause oxidative damage to DNA, lipid, and proteins and consequently decreased plant growth and yield [45-46]. Reduction in yield and grain weight of wheat under drought stress was reported by various researchers [47-48].

Furthermore, foliar and soil application of the chitosan NPs, especially 60 and 90 ppm, tended to reverse negative effect of drought stress on grain yield and yield components compared to the control. The role of chitosan NPs in alleviating the harmful effect of drought stress might be due to an increase in stomatal conductance and net photosynthetic CO_2-fixation activity under drought stress [49].Also, this compound is able to increase leaf resistance to water vapor loss, thus improving plant water use and increasing biomass or yield [50]. Moreover, in plants under

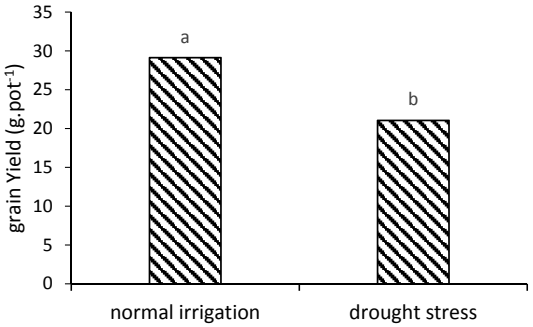

Fig 5. Effect of irrigation regime on grain yield. Means by the uncommon letter in each column are significantly different according to Duncan test ($p<0.05$).

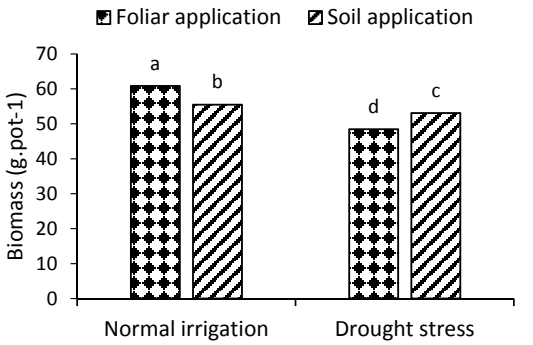

Fig 6. The effect of irrigation regime and application method on biomass. Means by the uncommon letter in each column are significantly different according to Duncan test ($p<0.05$).

drought stress, soil application of NPs increased mean of 1000-grain weight more than the foliar application of NPs. This result could explain that the NPs availability periods in soil were longer than those of foliar spraying. Also, chitosan NPs had a positive ionic charge which chemically binds to plant nutrients that showed a negative ionic charge resulting in a slowly released action in plants which closely contributed to 1000-grain weight increase. Utsunomiya and Kinai [51] recorded precocious flowering and increased flower numbers when chitosan was applied to passion fruit (*Passiflora edulis*) as a soil drench. Some researchers stated that chitosan NPs at 10, 25 or 100 ppm increased spike length, plant height, grain yield, and harvest index of wheat compared to the control [52].

Biomass, Harvest Index, and Plant Height

The results of Fig 6 indicated that in well-watered plants, the foliar application of NPs increased the biomass more than the soil application of NPs. In contrast, in plants under drought stress, the soil application of NPs increased the biomass more than the other application method. Also, using 60 and 90 ppm NPs increased harvest index compared to the control (Table 3). Drought stress significantly decreased harvest index by 18% compared to the well-watered plants (Fig. 7). Furthermore, drought stress significantly decreased plant height compared to the well-watered plants (Fig. 8a). Usage chitosan NPs in well-watered plants increased plant height compared to the control plants. In the same level of chitosan NPs, there was no significant difference between methods of using NPs on plant height (Fig. 8b). Foliar application of 30 and 60 ppm NPs significantly increased plant height compared to the control.

It is well known that drought stress affects plant growth and development by a multitude of molecular, biochemical and physiological changes [53]. For example, the depressive effect of water stress on growth parameters may be attributed to a drop in leaf water content and reduction in the assimilation of nitrogen compounds [54], affecting the rate of cell division and enlargement. The inhibiting effects of water stress on plant growth have been previously reported for soybean [55] and white lupins [56].

Therewith, our results showed that using chitosan NPs increased biomass, harvest index, and plant height. These findings could be linked to the previous reports of chitosan that it increases availability and uptake of water and essential

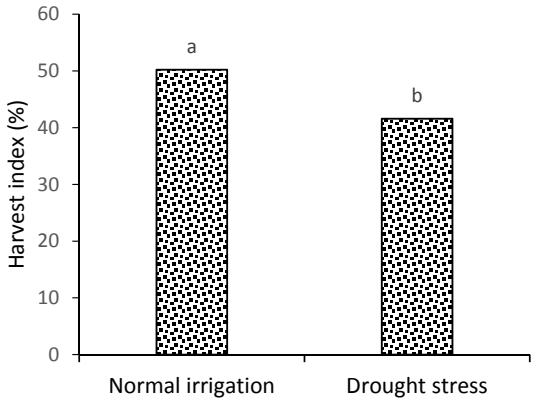

Fig 7. The effect of irrigation regime on harvest index. Means by the uncommon letter in each column are significantly different according to Duncan test ($p<0.05$).

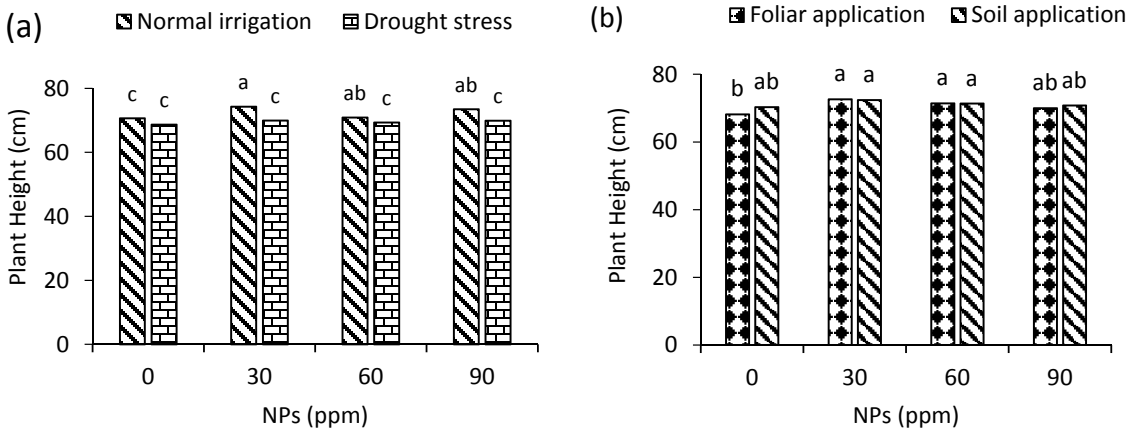

Fig 8. The effect of NPs concentration, irrigation regime and application method on plant height. Means by the uncommon letter in each column are significantly different according to Duncan test ($p<0.05$).

nutrients through adjusting cell osmotic pressure, and reducing the accumulation of harmful free radicals (ORS) by increasing antioxidants and enzyme activities [57] so that chitosan could increase growth. Chamnanmanoontham *et al.* [58] found that 40 ppm chitosan solution significantly enhanced rice growth compared to the control.

Also, chitosan-silver (Ag-CS) NPs (0.1%, w/v) increased seed germination, fresh and dry weight as well as peroxidase (POD) and catalase (CAT) activity compared to the control [59]. Finally, Boonlertnirun *et al.* [60] observed that soil application of chitosan before drought caused the highest growth or yield of rice plants compared to the control, soil application after drought and foliar application of chitosan. Also, seed soaking and foliar application, four times tended to show an ability on disease control.

CONCLUSIONS

Results of this study showed that the drought stress affected the biomass, enzyme activity, leaf color, RWC, harvest index, yield and yield components of barley plants; whereas, using chitosan NPs, especially 60 and 90 ppm, decreased the harmful effects of drought stress. The mechanisms of chitosan NPs in counteracting the harmful effects of drought stress are not well understood and there are few reports in the literature. Although, it can be concluded that chitosan NPs may produce various metabolites which more water become available to plants for better growth and production. Furthermore, improvement in biomass, 1000-grain weight and grain yield from the NPs could be due to the N content of chitosan that plays important role in photosynthesis. So, the results suggested that chitosan NPs can be applied to barley plants either through soil or foliar application with different doses in both irrigation regimes to get the desired results.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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