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Potential impacts of chitosan on growth, yield, endogenous phytohormones, and antioxidants of wheat plant grown under sandy soil conditions

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Abstract. A field experiment was carried out in sandy soil, during two winter successive seasons to study the impacts of different concentrations of chitosan (50, 100 & 150 mg L^{-1}) on several growth parameters and biochemical changes as well as quantitative and qualitative grain yield. Foliar treatment of chitosan significantly increased the growth parameters concurrently with an increment in the photosynthetic pigments, total soluble sugar, proline, free amino acid total carbohydrates, antioxidant activities, phenol, flavonoids, and some minerals nutrition of wheat plant. Wheat plants treated with chitosan at different concentrations significantly increased different endogenous phytohormones auxins (IAA), abscisic acid (ABA), gibberellins (GAs), and cytokinins (Cyt), as compared with the untreated plants. Moreover, chitosan concentrations induced significantly increments in grains yield, nutritive values, carbohydrates %, proteins %, antioxidant compounds and macronutrients of the grain yield. Cultivation of wheat plants under sandy soil conditions and treated with foliar application of 100 mg^{-1} chitosan gave the higher values of the grain yield as well as the nutritional values contents.

Key words: chitosan, endogenous phytohormones, antioxidants, nutritional values, wheat, yield.

INTRODUCTION

In recent years, the forever-growing request for food worldwide, the ongoing climate change, the severe consumption of farmlands and the rising consideration of consumers to high-quality, secure and environmental-friendly food production collectively have encouraged the search for other biological methods that could encounter this request. Moreover, the changeable environmental conditions form a challenge to agriculture production, thus improving any agricultural system of the country is a necessity to encounter its people's requirements. Scientists paid their interest to compensate for this reduction by reclaiming and cultivating new sandy soils. So, due to the bad properties of sandy soils, the experiment was carried out in the reclaimed sandy soil in Egypt. The chosen district, is a portion of the Sahara Desert of Northern

Africa, and it is insecure due to the to the presence of different environmental stress conditions such as low water availability, high irradiances, temperature variations, and nutrient deficiency (Ramadan et al., 2020).

Thus, it is significant to increase wheat production by utilizing several planning, like extending wheat cultivation in sandy soil and conditions utilizing either high crop varieties or natural compounds as bio-stimulants and elicitors in agriculture to enhance its growth and yield in such type of soil. Elicitor is one of the important efficient strategies for promoting the plant defense mechanism. Moreover, elicitor is promoting transportation via a signal transduction system leading to alteration in the various aspects of cellular processes and finally enhancing the biosynthesis of secondary metabolites (Delaunois et al., 2014 and Suarez-Fernandez et al., 2020). Elicitorshave an important influence on various metabolic processes which promote plant growth and development through rising photosynthesis, endogenous bioregulator, nucleic acid,protein synthesis and ion uptake (Abbas, 2013). The discernment of sustainable crop production and rising agricultural yield with inexpensive inputs are the requirements for farmers. Accordingly, various strategies for the exogenous application of various natural compounds must be used to achieve this goal. One of those natural substances which could be provided safely to crops is the chitosan shell of shrimp extract. Chitosan is a natural compound and is commercially proceeded from seafood shells (Boonlertnirum et al., 2010). Chitosan is a low toxic and cheap substance beside being biodegradable and environmentally friendly with different applications in agriculture. Chitosan is a biopolymer, from the carbohydrate family primly created from a glucose ring and has a free amino group at carbon number - 2, however, the employment of chitosan increments with rising amino groups (Badawy & Rabea, 2011). Moreover, because of its cationic properties, chitosan submits a broad set of physicochemical and biological characteristics, inclusiveof both antimicrobial, and antioxidant characteristics (Aranaz et al., 2009). Chitosan is considered by individual characteristics, like bioactivity and biocompatibility (Dias et al., 2013). Chitosan application could increase the plant's yield (Mondal et al., 2012), decrease transpiration and induce a range of metabolic changes (Dzung et al., 2011). Also, the usage of chitosan improved key enzyme activities of nitrogen metabolism (nitrate reductase, protease, and glutamine synthetase) and increased the transmission of nitrogen in the workable leaves which increased plant growth and development. Chitosan stimulates plant hormones, lipid signaling and protection compounds in tomato root exudates, such asphenolic compounds (Suarez-Fernandez et al., 2020). Chitosan also propitiates the accumulation of auxin in the apex of plant roots (Lopez-Moya et al., 2017), and promotersmetabolic pathways (e.g., phenylpropanoid) participated in the biosynthesis of phenolic compounds (Singh et al., 2020).

Wheat (*Triticum aestivum* L) is the utmost substantial cereal crop grown in the world.At least one-third of the world's people consists of wheat as its major staple (Abdallah et al., 2015). The grains of wheat contain great amounts of proteins, carbohydrates, several minerals and vitamins.For these purposes, efforts must be directed toward the incrementwheat yield to fill the gap between production and consumption; so, the cultivated area could be increased by reclaiming new lands, supplying them with water from wells and devoting them to wheat production.

This study was conducted to investigate the performances of different doses of chitosan on grain yield and some biochemical aspects of wheat plants (Gimmeza 9) under sandy soil conditions. The specific objectives of the current study were to (i) Keep

up the growing attention to high-quality, secure, and environmentally friendly grain production. (ii) Eliminate pollutants and use environmentally friendly materials by using the remaining shrimp shell by recycling action (iii) Utilizing chitosan as a natural compound (bio-stimulant) in agriculture toevaluate the effects of three different doses of foliar spray of chitosan on growth and grain yield of wheat (iv) Assess the effects of foliar spray of chitosan on the endogenous phytohormones, photosynthetic pigments and total antioxidant activities. induction of total carotenoids, lycopene, flavonoid and phenolic compounds and (vi) determine the effect of chitosan spray on the nutritional values of the yield grains.

MATERIALS AND METHODS

Materials

Wheat grains variety (Gimmeza 9) with 98 germination percentage by certified seed was obtained from Agricultural Research Center, Giza, Egypt.

Experiment location

The experimental farm of the National Research Centre was located in Nubaria region, Egypt, (30_86'67" N 31_16'67" E, with a mean altitude 21 m above sea level), two field trials were sown in two consecutive winter seasons in 2019/2020 & 2020/2021. The soil Farm is considered to be either an arid or semi-arid zone. The temperature at night was 8.7 to 16.23 °C, with an average of 11.8 and 10.12 to 16.54 with an average of 12.81, while daytime temperatures varied from 18.82 to 28.15 °C with an average of 22.52 °C and 17.05 to 28.91 °C with an average of 22.43 °C. During the years 2019–2020 and 2020–2021, respectively, the relative humidity ranged from 58.0 to 69.38% with an average of 64.4% and 58.22 to 70.9% with an average of 65.52%. According to Chapman & Pratt (1978), the soil at the experimental site was reclaimed sandy soil, and mechanical and chemical analyses are described in (Table 1).

Table 1. Analysis of the experimental soil's mechanics, chemistry, and nutrition

Sand			Silt	Clay	Soil texture
Course 2000-200µ	Fine 200-20µ		20-0µ	< 2µ	
47.44%	36.21%		12.88%	4.26%	Sandy

Chemical analysis:

pH	EC	$CaCo_3$	OM	Soluble Cations meq/l				Soluble anions meq/l			
1:2.5	dSm^{-1}		%	Na^+	K^+	Mg^+	Ca^{++}	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}
7.60	0.14	5.30	0.07	0.56	0.14	0.93	1.10	0.0	1.27	0.46	0.87

Nutritional analysis:

Available nutrients

Macro element ppm		Micro element ppm				
N	P	K^+	Zn^+	Fe^{++}	Mn^{++}	Cu^{++}
53.0	12.0	75.0	0.14	1.40	0.30	0.00

Experiment design

Four replications were used in the randomized complete block design of the experiment. In both seasons, wheat grains were sown on November 20th in rows that were each 3.5 meters long and spaced 20 cm apart on a 10.5 m^2 plot (3.0 m in width and

3.5 m in Length). When wheat was sown in sandy soil, agricultural operations were carried out as recommended, and the seeding rate was (140 kg ha^{-1}). The soil was amended with 360 kg ha^{-1} of calcium super-phosphate (15.5% P_2O_5) before sowing. Following plant emergence, ammonium nitrate 33.5% at a rate of 180 kg ha^{-1} was used as a nitrogen fertilizer, it was divided into five equal amounts before the first, second, third, fourth, and fifth irrigation water. Before the first and third irrigation water, two equal dosages of 120 kg ha^{-1} of potassium sulphate (48.52% K_2O) were applied. The new sprinkler irrigation system, which added water every five days, was used for irrigation. Chitosan at concentrations (0.0, 50, 100, & 150 mg L^{-1}) were applied twice to wheat plants in the tillering and elongation stage (about 4 to 5 leaves) at 45 & 60 days after sowing.

Irrigation water requirements:

Irrigation water requirements calculated using Penman Monteith equation and crop coefficient according to Allen et al. (1989). The average amount of irrigation water applied with sprinkler irrigation system was 5,950 m^3 ha^{-1} $season^{-1}$ for both seasons of the experimental work (Table 2).

The amounts of irrigation water were calculated according to the following equation:

$$IWR = \left[\frac{ET_0 \times K_c \times K_r \times I}{E_a} + LR\right] \times 4.2 \quad (1)$$

where IWR = Irrigation water requirement m^3 ha^{-1}/ irrigation; ET_0 = Reference evaporation - transpiration (mm day^{-1}); K_c = Crop coefficient; K_r = Reduction factor (Keller & Karmeli, 1975); I = Irrigation interval, day; E_a = Irrigation efficiency, 90%; LR = Leaching requirement = 10% of the total water amount delivered to the treatment.

Preparation of Shrimp shells

In the current study, we described the low-cost chitosan's extraction from shrimp shells via chitin extraction, followed by alkaline deacetylation of chitin with a strong alkaline solution at various times. Chitin can be taken from a variety of sources and transformed to chitosan using variable levels of deacetylation and varying NaOH concentrations. The technique used to extract the chitosan was essentially the same as that used by Saleh et al. (2016).

Table 2. Water for growth stages of the wheat crop at Nubaria station during two successive winter seasons 2019/2020–2020/2021

Growth stages	No. of days	The applied water to the growth stages (m^3 ha^{-1})
Initial	20	326.04
Develop.	30	1,086.76
Mid	65	3,260.27
Late	40	1,141.08
Harvest	27	135.85
Total	182	5,950

Materials

Shells of shrimp was obtained from Suez Shrimp (Egypt). Sodium hydroxide (NaOH), hydrochloric acid (HCl), and acetic acid gotten from Aldrich, Egypt. They were then diluted to the concentration required for the methodology with distilled water.

Methods (Extraction of chitosan)

The extraction of chitosan can be carried out after removing the loose tissue from the shrimp shells. The shells were washed for 6 h. For chitin and chitosan productions

drying, thoroughly with water to remove impurities in a hot-air oven at 90 °C and grinding to obtain a dry powder. The major procedure for obtaining chitosan is based on the alkaline deacetylation of chitin with a strongly alkaline solution at different periods of time.

Demineralization process

The demineralization was carried out by weighing 50 gm of powdered shrimp shells and using 4% HCl (1.3 N) at a ratio of 14 mL: 1 g (w/v) for 24 h at room temperature. The product was washed to neutrality under running tap water. Electricoven at 70 °C was used to dry the solid after it had been collected and rinsed with distilled water.

Deproteinization process

5% NaOH (1.25 N), 12 mL: 1 g (w/v), 90 °C, and 24 hours of deproteinization were performed. The deproteinized material was gathered and cleaned with distilled water.

Deacetylation

The product was deacetylated with 70% NaOH (17.5 N) with a ratio of 14 mL: 1 g (w/v) at room temperature for 75 h. with stirring. The solid was collected and washed with distilled water. The deacetylated product was then dried in an oven at 70 °C.

Plant sample

After 75 days from seeding, plant samples were taken in order to determine the growth characteristics (shoot height (cm), number of leaves per tiller, tiller fresh and dry weight per plant (g), and water content %). Photosynthetic pigments, endogenous phytohormones, organic solutes (proline, total free amino acids, and total soluble sugar), total carbohydrates, total proteins, flavonoids, total phenol contents, lipid peroxidation total antioxidant activities, and a few minerals (nitrogen, phosphorus, potassium, and calcium) were all measured in wheat leaves as part of the biochemical analysis.

The following characteristics were measured at harvest on randomly selected groups of 10 guarded tillers in every plot to estimate the following characteristics: plant height (cm), spikelets no/spike, 1,000-grains weight (g), grain yield/spike (g), straw yield, biological yield, and grain yield (ton ha^{-1}). In wheat grains, the percentages of carbohydrates, proteins, flavonoids, lycopene, and minerals (nitrogen, phosphorus, potassium, and calcium) were measured.

Water Content

Water content was determined according to (Jin et al., 2017). The fresh leaves of each sample were weighed and then dried at 80 °C for 72 hourswas record as dry weight. The leaf water content was calculated as the following:

Water content(%) = (Wf − Wd)/Wf × 100 Where, Wf, fresh weight and Wd, dry weight. Each sample was measured in biological triplicate.

Water-productivity (WP):

WP was determined by (Howell et al., 1990). The relationship between grain yield and irrigation water quantity is known as water productivity (WP). WP in (kg/mm ha^{-1}) was computed using the following formula:

Water productivity = wheat grain yield (kg ha^{-1}) / total utilized of irrigation water, m^3ha^{-1} /season.

Physiological and biochemical studies

Photosynthetic pigments: The method previously described (Li & Chen, 2015) was used to quantify the amounts of both chlorophyll a and b and carotenoids in fresh leaves using spectrophotometer (Shimadzu UV-1700, Tokyo, Japan). In terms of g per 100 g of fresh weight, photosynthetic pigment levels were expressed.

Extraction, isolation, and identification of endogenous phytohormones: The methylation process was carried out in accordance with the previously stated approach (Urbanová et al., 2013), and the hormone extraction procedure was carried out in accordance with the previous method employed by Zhang et al. (2015). Hewlett Packard carried out the identification and quantification of auxins, gibberellins, and abscisic acid, gas-liquid chromatography (5890) with a flame ionization detector (Tarkowská et al., 2014). In accordance with Tarkowski et al. (2009) approach, cytokinin fractions were identified by HPLC isocratic UV analyzer.

Organic solute: The phenol sulphuric acid methodwas used to determine total soluble sugar (TSS) concentrations Mecozzi (2005). Proline (Pro) and free amino acids (FAA) were extracted using the technique outlined by Vartanian et al. (1992). Using Yemm et al. (1955) ninhydrin reagent technique for determination free amino acids. According to Bates et al. (1973) approach, proline was measured.

Total soluble protein (TSP) was appointed using the technique of(Maehreet al., 2018). Total carbohydrate was measured according to DuBois et al. (1956).

Antioxidant compounds: The total phenolic compound was measured using the spectrophotometer as previously determined (Siddiqui et al., 2017).With the use of the technique described by Dewanto et al. (2002) total flavonoids were estimated. Lycopene levels were measured using the method of Nagata & Yamashita (1992) approach.

Lipid peroxidation, through estimating the quantity of malondialdehyde (MDA) produced via the previously published thiobarbituric acid (TBA) reaction, lipid peroxidation was ascertained (Wang et al., 2013).

Antioxidant activity: in order to measure the antioxidant activity (DPPH radical scavenging) method of Liyana-Pathiranan & Shahidi (2005).

Mineral contents: The grains samples were powdered after being oven-dried at 70 °C for 72 hours. N, P, and K were measured. According to Paul et al. (2017) the Kjeldahl procedure was used to determine the nitrogen content of plant leaves. Paul et al. (2017) state that the spectrophotometer approach was used to estimate the phosphorus content. According to Paul et al. (2017) the potassium content of the plants was assessed using a flame photometer method.

Statistical analysis

Complete randomized block design statistical analysis was performed on the data. Given that the trend was consistent across two seasons, the homogeneity test using Bartlet's equation was used to combine the analyses of the two seasons. Using SAS software, the Duncan's multiple range test was measured to compare the means at $P < 0.05$ (SAS Institute Inc. 2002; Steel & Torrie, 1980). Correlation coefficient was calculated to determine the relationship between grain yield and each of the physiological and chemical traits.

RESULTS AND DISCUSSION

Growth parameters

The influence of various concentrations of chitosan (50,100 and 150 mg L^{-1}) on growth parameters of wheat plants are presented in (Table 3). The results revealed that, using chitosan as foliar treatment at different concentrations significantly increased plant height, leaves number / tillers, tiller fresh and dry weight and relative water contents as compared with untreated plants. While, the maximum significant ($P < 0.05$) increment was achieved in number of leaves/tiller, fresh and dry weight and relative water content were reported at 100 mg L^{-1} chitosan. The percentage of increases in response to 100 mg L^{-1} chitosan extended to 20% & 9% in fresh and dry weights of shoots as compared with the untreated plants.

Table 3. Effect of different concentrations of chitosan (50, 100 and 150 mg L^{-1}) on morphological, criteria of wheat plant (Gimmeza 9) variety at 75 days from sowing, (mean of two seasons)

Chitosan (mg L^{-1})	Plant height (cm)	Number of leaves /tiller	Tiller Fresh wt. (gm)	Tiller Dry wt. (gm)	RWC %
control	70.0c	4.52d	7.12d	1.92c	72.97c
50	73.0b	6.11b	8.36b	2.05ab	75.45b
100	76.5a	6.60a	8.87a	2.13a	76.00a
150	78.87a	5.63c	7.82c	2.01bc	74.19bc

The obtained value came in line with Zayed et al. (2017) who stated that the application of chitosan improved the morphological characters of common beans. The beneficial effects of chitosan may be attributed to its role in various physiological processes. Sheikha & AL-Malki (2011) reported that chitosan improved bean growth parameters may be due to improving photosynthetic machinery. Moreover, Ke et al. (2001) stated that the application of carboxymethyl chitosanimproved enzyme activities of nitrogen metabolism which in turn enhanced photosynthesis and that followed byimproved plant growth.Chitosan increases plant growth might be because of an increment in the availability ofwater uptake and necessary nutrients led to adjusting cell osmotic pressure, cell division and elongation, increased protein biosynthesis and induction of the antioxidant defense system (Ma et al., 2014). Moreover, Chitosan also induces endogenous plant hormone synthesis (Uthairatanakij et al., 2007) or encourages closure of stomata, which decreases transpiration (Karimi et al., 2012).

Photosynthetic pigments

The effect of different concentrations of chitosan (50, 100 & 150 mg L^{-1}) on photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids and total pigments) of wheat plants are illustrated in (Fig. 1, A, B, C, D). Chitosan applications significantly ($P < 0.05$) enhanced photosynthetic pigments. Chitosan at 100 mg L^{-1} induced the highest values by 37.5%, 29.3%, 53.3% & 38.5% at chlorophyll a, chlorophyll b, carotenoid and total pigments respectively a contrast to the corresponding untreated plant.

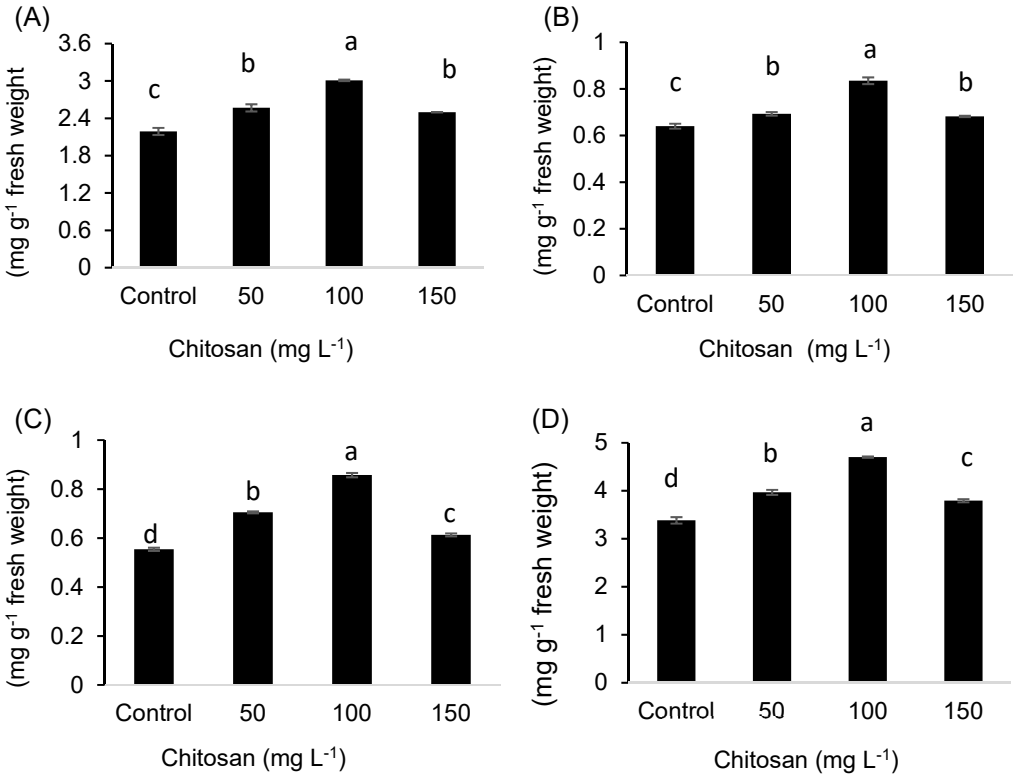

Figure 1. Effect of different concentrations of chitosan (50, 100 and 150 mg L^{-1}) on photosynthetic pigments as (mg g^{-1} fresh wt.) (A) chlorophyll a (B) chlorophyll b (C) carotenoids (D) total chlorophyll on wheat plant (Gimmeza 9) variety at 75 days from sowing. The different letters (a–d) show statistical significance at $p < 0.05$; vertical bars indicate ± SE.

The significant increase in photosynthetic pigments of wheat plants due to chitosan treatment under sandy soil conditions (Fig. 1)might be due to improving cytokinin contents (Fig. 2, D) that encourage chlorophylls synthesis or preventing the reduction in the light-harvesting pigment protein complexes or raise the availability of amino compounds liberated from chitosan (Chibu & Shibayama, 2001). Chitosan could progress the plant defense mechanism via activating photochemistry and enzymes associated with photosynthesis (Faqir et al., 2021). It is obvious that chitosan enhances the photosynthesis performance and the accumulation of organic matter in wheat plants. This may be due to an increase in total carbohydrate contents (Fig. 3, E), N % and K % in plant leaves (Fig. 5, A, C) which assists in raising the numbering of chloroplasts per cell, so participating in the improved synthesis of chlorophyll. Farouk & Ramadan (2012) found that chitosan improved chlorophylls and total carbohydrates in cowpea (*Vigna unguiculata* L.) after foliar treatment with chitosan at 250 mg L^{-1}. Moreover, the application of chitosan induces carbon assimilation which leads to an increase in the photosynthetic pigment (Bistgani et al., 2017). The application of chitosan to coffee seedlings led to increase in chlorophyll contentswhich of attributed to enhance uptake of nutrients (Nguyen Van et al., 2013). Moreover, the application of chitosan-enhanced photosynthetic pigments in barley plants which may be because of the increments of N

and Mg contents in the leaves which are the essential elements in the chemical composition of chlorophylls (Behboudi et al., 2018).

Endogenous phytohormones

Data in (Fig. 2, A, B, C, D) showed that, chitosan with different concentrations caused increases in IAA, ABA, GA, and Cyt as compared with untreated plants. The highest values of hormones were achieved with 100 mg L^{-1} chitosan treatment by 78%, 30%, 44%, and 73% in IAA, ABA, GA, and cyt respectively.

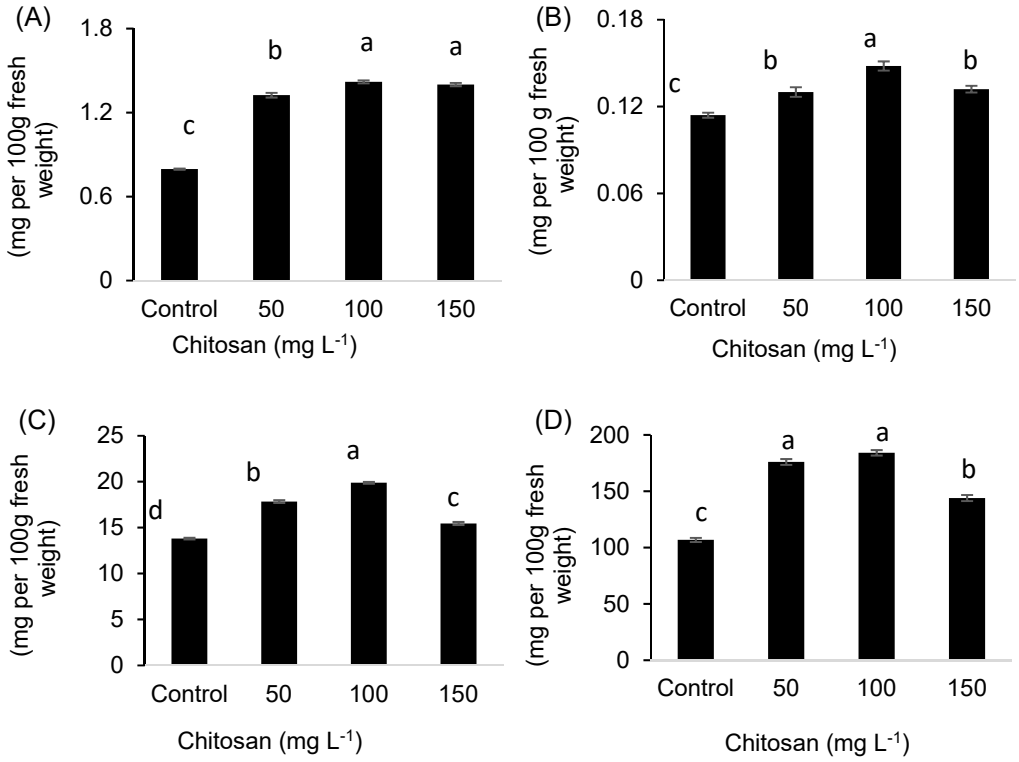

Figure 2. Effect of different concentrations of chitosan (50, 100 and 150 mg L^{-1}) on endogenous phytohormones as (mg/100 g fresh wt.) (A) IAA, (B) ABA, (C) GA, and (D) Cytokinin on wheat plant (Gimmeza 9) variety at 75 days from sowing. The different letters (a–d) show statistical significance at $p < 0.05$; vertical bars indicate ± SE.

Iglesias et al. (2019) and Ma et al. (2019) reported that chitosan plays an important role in plant hormone production and systemic gained resistance. Chitosan might improve growth and development through signaling pathway associated to auxin biosynthesis through the tryptophan-independent pathway (Uthairatanakij et al., 2007). Similarly, Oligochitosan was able to increase IAA concentration, which improved the growth of tobacco plants (Guan et al., 2009). Moreover, chitosan induce the accumulation of IAA in the root apex of the Arabidopsis (Lopez-Moya et al., 2017). Muthukrishnan et al. (2019) recognized the enhancing role of chitosan on the IAA contents of the chickpea plants. These increments may be because of the promoted

influence of chitosan on auxin-related gene expression, improved IAA biosynthesis and transport and decreased IAA oxidase activity (Li et al., 2019).

Moreover, chitosan elevated ABA activity, which plays an important role in the regulation of the stomatal aperture and decreased the rate of transpiration when the plant is subjected to stress condition (Lim et al., 2015). These results are in harmony with Iriti et al. (2009) who reported that the application of chitosan increased the ABA content in bean leaves. Srivastava et al. (2009) observed that chitosan treatment improved the plant under water deficit. The participation of ABA in this operation is also proposed through the notice that chitosan and ABA have approximate signaling components, similar to calcium and ROS. Uthairatanakij et al. (2007) stated that the treatment with chitosan increased the length of the stalks of the orchid *Dendrobium*. Chitosan might stimulate a signal to synthesize plant hormones like gibberellins.

Organic solutes

The impact of chitosan (50, 100 & 150 mg L^{-1}) increased significantly the organic solutes [proline (Pro) free amino acids (FAA) total soluble sugars (TSS) and total soluble protein (TSP)] in wheat plants when compared with untreated plants (Fig. 3, A, B, C, D). The maximum increases in organic solutes were noted by using 100 mg L^{-1} chitosan except proline was increased at 50 mg L^{-1} chitosan.

Rising the osmolytes progress plant cells tolerance to the growth in sandy soil through enhancing osmotic pressure in the cytoplasm in addition to relative water contents is vital for plant growth. The present results are in harmony with their obtained by Geng et al. (2020) after chitosan application in Creeping bent-grass plants. Rabêlo, et al. (2019) reported that, chitosan induced an increment in the contents of soluble sugars, and soluble proteins during its function in enhancing the expression of enzymes that participated in glycolysis. In addition, It seems that chitosan improved the concentration of osmoprotectant compounds, whichplay a role in regulating plant osmosis and subsequently leading to better growth, yield and improved plant tolerance to environmental stress conditions (Li et al., 2017). Treatment with chitosan increased the accumulation of proline levels in the thyme plant (Bistgani et al., 2017). Chibu & Shiayama (2001) stated that these favorable influences the greater availability of amino compounds emitted from chitosan. Li et al. (2017 and Geng et al. (2020) reported that chitosan treatment promoted the production of metabolites and amino acids such as proline,γ-aminobutyric acid, aspartic acid, threonine, serine, isoleucine, valine, lysine, and phenylalanine on white clover under drought stress. Also, Hidangmayum et al. (2019) demonstrated that chitosan treatment promotes antioxidant enzymes via nitric oxide and hydrogen peroxide signaling pathways. It promotes the production of organic acids, sugars, amino acids and other metabolites that are essential for osmotic adjustment, stress signaling, and energy metabolism under stress. Abdallah et al. (2020a) found that the application of chitosan increased the protein content in the stressed wheat leaves.

Total carbohydrates

Data in (Fig. 3, E) showed that, chitosan with different concentrations increased significantly *(*$P < 0.05$) total carbohydrates. The highest values of carbohydrates were obtained at treatment with 100 mg L^{-1}chitosan. These results are in agreement with those obtained by Abdallah et al. (2020b) andstated that foliar treatment of chitosan, significantly enhanced growth parameters, photosynthetic pigments and carbohydrate constituents in

sunflower plants. Leaf total carbohydrates were increased significantly in response to foliar chitosan application at 250 ppm on cowpea plants or 100 and 150 ppm on sour orange seedlings according to Farouk & Ramadan (2012) and Mohamed et al. (2018).

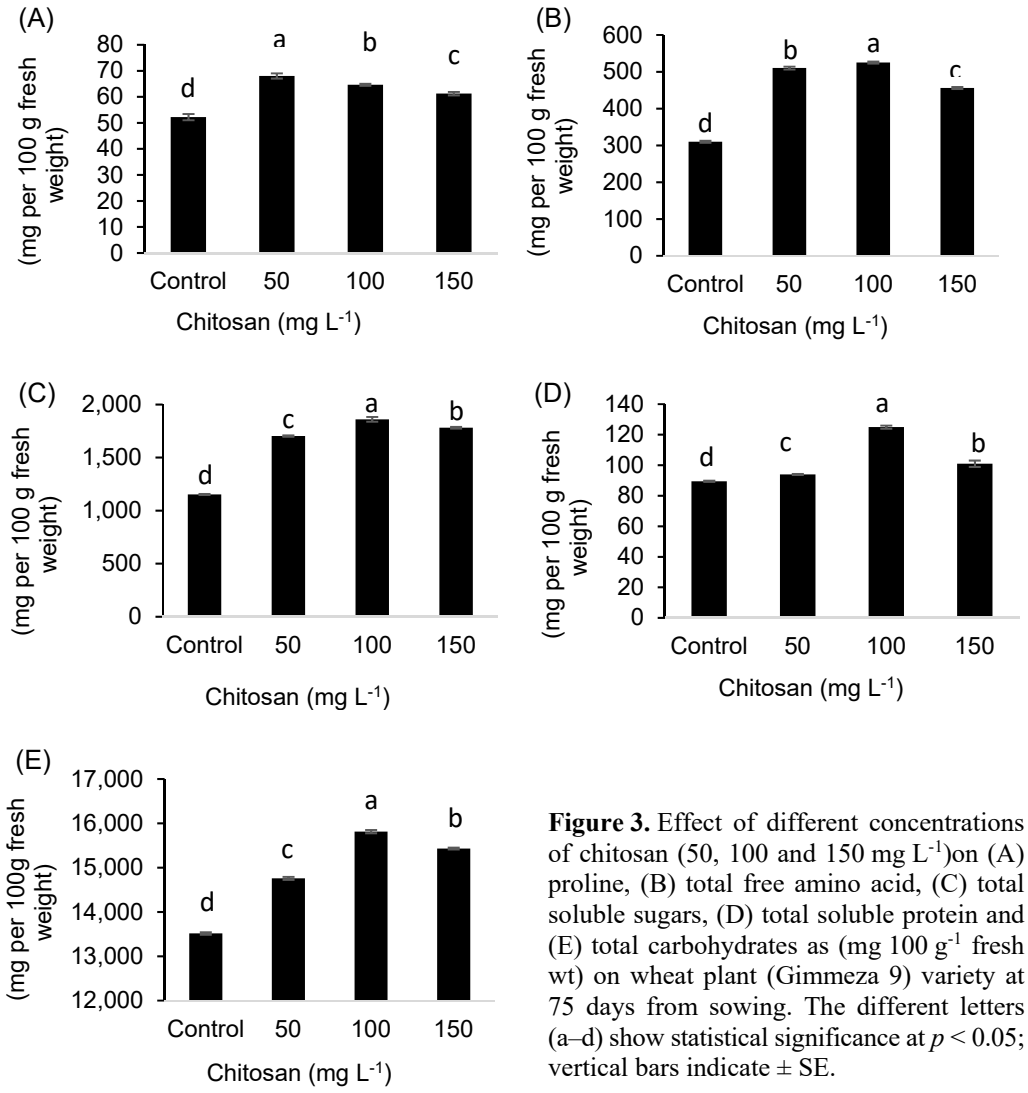

Figure 3. Effect of different concentrations of chitosan (50, 100 and 150 mg L^{-1})on (A) proline, (B) total free amino acid, (C) total soluble sugars, (D) total soluble protein and (E) total carbohydrates as (mg 100 g^{-1} fresh wt) on wheat plant (Gimmeza 9) variety at 75 days from sowing. The different letters (a–d) show statistical significance at $p < 0.05$; vertical bars indicate ± SE.

Flavonoids and total phenol contents:

The influence of various concentrations of chitosan (50, 100 & 150 mg L^{-1}) on flavonoids and total phenol contents of wheat plant are shown in (Fig. 4, A & B). Chitosan significantly increased flavonoids and phenolic contents. The highest increment of flavonoids contents was achieved via foliar treatment with chitosan 150 mg L^{-1} and phenol contents in 100 mg L^{-1}.

Phenolics are important constitutive with scavenging capability because of their hydroxyl groups, which might participate straight to their antioxidant properties that trigger a chain of secondary metabolites molded by shikimic acid or malonic acid cycles

like it has a cellular signaling function (Michalak, 2006). In this concern, Abdallah et al. (2020a) reported that chitosan application significantly raised phenolic compounds concomitantly with lipid peroxidation decrease (Fig. 4, D). Also, Hawrylak-Nowak et al. (2021) stated that, chitosan stimulated secondary metabolites as phenolic compounds by promoting specific genes that participate in the biosynthesis of secondary metabolites. Chitosan promotes metabolic pathways (e.g., phenylpropanoid) and may be involved in the signaling pathway for the biosynthesis of phenolics (Singh et al., 2020). Moreover, Chen et al. (2009) reported that chitosan treatment raised gene expression of phenylpropanoid and flavonoid biosynthesis in soybean sprouts.

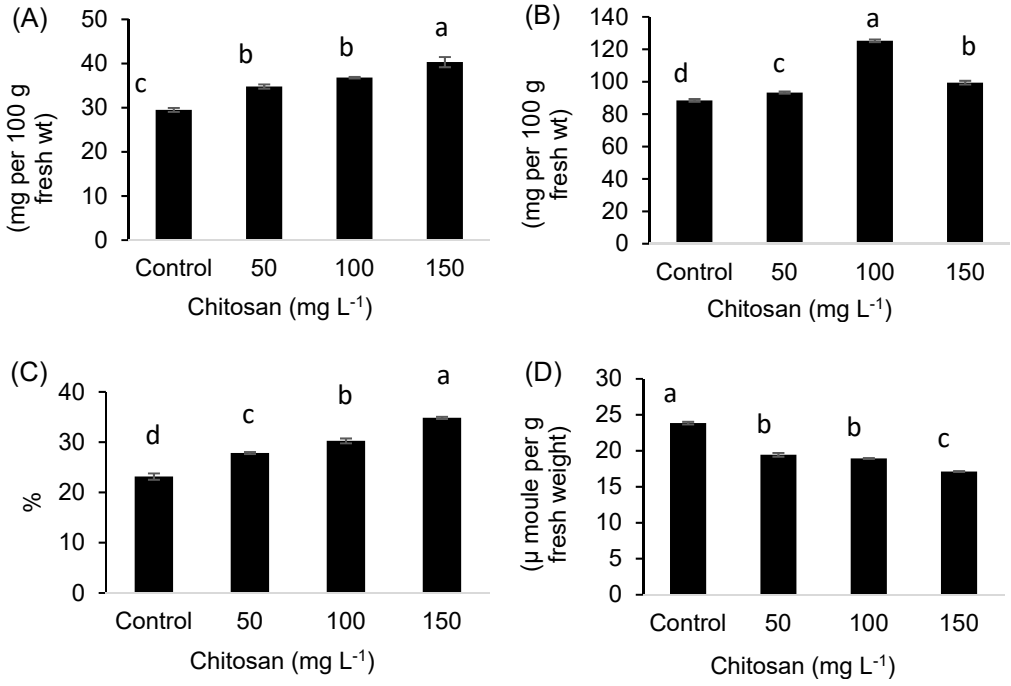

Figure 4. Effect of different concentrations of chitosan (50, 100 and 150 mg L^{-1}) on flavonoids (A), total phenols (B) as (mg per 100 g fresh wt), total antioxidant activities (DPPH%) (C), and lipid peroxidation (µ mole per g fresh weight) (D) on wheat plant (Gimmeza 9) variety at 75 days from sowing. The different letters (a–d) show statistical significance at $p < 0.05$; vertical bars indicate ± SE.

Total antioxidant activityand Lipid peroxidation

Chitosan significantly increased total antioxidant activities with the increase of chitosan concentration (Fig. 4, C). The highest value of antioxidant activities was achieved by foliar treatment with chitosan (150 mg L^{-1}). Fig. 4, D illustrated that foliar spraying with chitosan on wheat plant grown in sandy soil reduced significantly lipid peroxidation in treated plants than the untreated. This noticed gradual decrease in MDA contents was reported with rising concentrations of chitosan-sprayed plants.

The current study indicated that chitosan treatment in wheat plants significantly reduced the malondialdehyde (MDA) content and increased antioxidant activities (Fig. 4, C, D). Xie et al. (2001) proposed that the antioxidant characteristic of chitosan are attributed mostly to its plentiful active hydroxyl and amino groups, that could interact

with ROS to compose constant and comparatively nontoxic macromolecular radicals. Chitosan treatment showed an important role in the inhibition of malondialdehyde content which is one of the decomposition products of polyunsaturated fatty acids (PUFA) of bio-membranes (Seckin et al., 2010) where PUFA which are the major membrane lipid constituents liable to peroxidation and decay. Moreover, Al-Tawaha et al. (2018) and Geng et al. (2020) reported that the treatment of chitosan significantly reduced lipid peroxidation by enhancing the antioxidant activities, directing to inhibited membrane permeability.

The role of chitosan in inhibiting malondialdehyde contents may be due to that chitosan receptors are existing on the plasma membrane; however, through a signaling cascade, the chloroplast is the initial chitosan action organelle (Hadwiger, 2013). Charge-charge interactions among positively charged chitosan amine groups and negatively charged phospholipids stimulate a signal that will lead to the octadecanoid pathway activation; this metabolic pathway is straight correlated to reduced H_2O_2 forming (Almeida et al., 2020).

Minerals contents

Data in Fig. 5 (A, B, C, D) showed that, chitosan with different concentrations caused increases in percentage of nitrogen, phosphorus, potassium and calciumas compared with untreated ones. The highest levels of minerals were recorded with 100 mg L^{-1} chitosan application which caused an increase 47%, 11%, and 11% in N, K, and Ca respectively. Its worthy to mention that, 150 mg L^{-1} chitosan induced maximum increase of P by 54%.

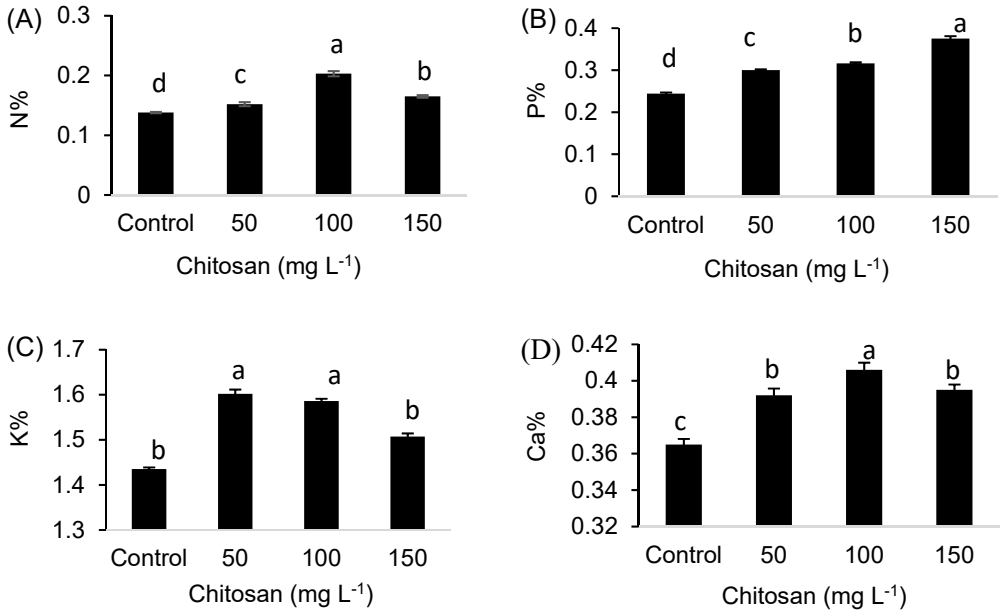

Figure 5. Effect of different concentrations of chitosan (50, 100 and 150 mg L^{-1}) on minerals contents (Nitrogen % (A), phosphorus % (B), potassium % (C) and calcium % (D) as on wheat plant (Gimmeza 9) variety at 75 days from sowing. The different letters (a–d) show statistical significance at $p < 0.05$; vertical bars indicate ± SE.

Abdallah et al. (2020a) observed that chitosan application raisedN, P, K and Ca contents in the wheat plants grown in saline soil. Likewise, Farouk & Ramadan (2012) observed that chitosan significantly improved N and K contents in cowpea plants. The positive role ofchitosan in plant growth may be related to its influence in increasing nutrient uptake like N, P and K which play a substantial function in the biosynthesis and translocation of carbohydrates and promotes cell division, cell turgor, DNA and RNA (Farouk & Ramadan, 2012).

Changes in yield and yield components

The influences ofvarious concentration of chitosan (50, 100 & 150 mg L^{-1}) on yield parameters of wheat plants are shown in (Table 4) Chitosan significantly improved ($P < 0.05$) all yield parameters e.g. [shoot length, spike length, spike weight, number of spikelets /spike, grain yield/ spike (g), 1,000 grains weight (g), straw yield, biological yield and grain yield (ton ha^{-1})] as compared with the control. The results indicated that the treatment with chitosan at the rate of (100 mg L^{-1}) on wheat was more efficient in enhancing yield component properties except for shoot length (cm) which showed their increase with 150 mg L^{-1} treatment. Grains yield (ton ha^{-1}) increased significantly ($P < 0.05$) by 31% with 100 mg L^{-1} chitosan foliar spray.

Table 4. Effect of different concentrations of chitosan (50, 100 and 150 mg L^{-1}) on yield component of wheatplant (Gimmeza 9) variety (combined analysis of two seasons)

Chitosan mg L^{-1}	Shoot length (cm)	Spike length (cm)	Spike wt. (g)	No. of spikelets /spike	Grain yield /spike(g)	1,000 grains weight (g)	Straw yield, ton ha^{-1}	Straw yield, ton ha^{-1}	Grain yield, ton ha^{-1}	WP, %
Control	85.4d	9.2d	2.74d	16.0d	2.247d	44.82d	5.195d	9.751d	4.505d	0.747d
50	86.5c	9.8c	3.36c	17.3c	2.96c	51.08c	6.501c	11.658c	5.153c	0.864c
100	89.3b	11.8a	4.09a	19.3a	3.283a	52.66a	7.058a	12.866a	5.832a	0.975a
150	92.4a	10.2b	3.58b	19.0b	2.993b	51.41b	6.68b	12.234b	5.550b	0.937b

Chitosan application led to the increment in wheat yield might be because of its influences in activating physiological processes, progressing vegetative growth (Table 3), photosynthetic pigments (Fig. 1), endogenous phytohormones content (Fig. 2) and mineral contents (Fig. 5) of treated wheat plants followed by active translocation of photo-assimilates from source to sink tissues. Moreover, foliar application of chitosan improved yield and grain quality as shown from the nutrient elements, protein and carbohydrate contents in the grains (Fig. 6). In this respect Abdallah et al. (2020b) showed that chitosan exerts a significant role in ameliorating growth, photosynthetic effectiveness and chlorophyll contents,improved yield & yield component and water productivity in sunflower plants.Chitosan is supposed to be a growth regulator and a signal molecule, as well as its function as a highly efficient biomolecule (Gornik et al., 2008). Moreover, The role of chitosan in improving the yield of the wheat plants may be due to increasing activities of key enzymes of nitrogen metabolism (nitrate reductase, glutamine synthetase, and protease) as well as meliorative translocation of nitrogen in the efficient leaves so raised plant growth and development (Sultana et al., 2017). Ghoname et al. (2010) found that chitosan foliar application in sweet pepper enhanced significantly the fruits quantity and quality. Also, seed soaking and foliar spraying with

chitosan at various growth stages in wheat (*Triticum aestivum* L.) improved the yield components (Ma et al., 2014).

Water productivity (WP)

The present work showed that wheat plants treated with various concentrations of chitosan raised significantly water productivity in contrast with the untreated plants (Table 4).

Abdallah et al. (2020a) observed that foliar treatment with chitosan raised the water productivity of wheat. This increase may be due to the chitosan inhibited transpiration by stimulating the closure of stomata. The obtained results suggested that chitosan mightbe effective as anti-transparent which means keeping water to utilize in agriculture (Abdallah et al., 2020b). Geng et al. (2020) found that chitosan application promoted WP and carbohydrates in creeping bent-grass.

Nutritious value of the grains yield

Foliar spraying for chitosan increased ($P < 0.05$) significantly carbohydrate %, protein %, lycopene %, flavonoids %, of the grains wheat (Fig. 6, A, B, C, D). It seems from data that 100 mg L^{-1} foliar treatment was the most efficient to increase carbohydrates, lycopene, flavonoids, by 12.3%, 51.1%, and 77.3%respectively. Meantime, protein contentincreased by 19.9%resulted from grains of plants treated with 150 mg L^{-1} chitosan (Fig. 6, B).

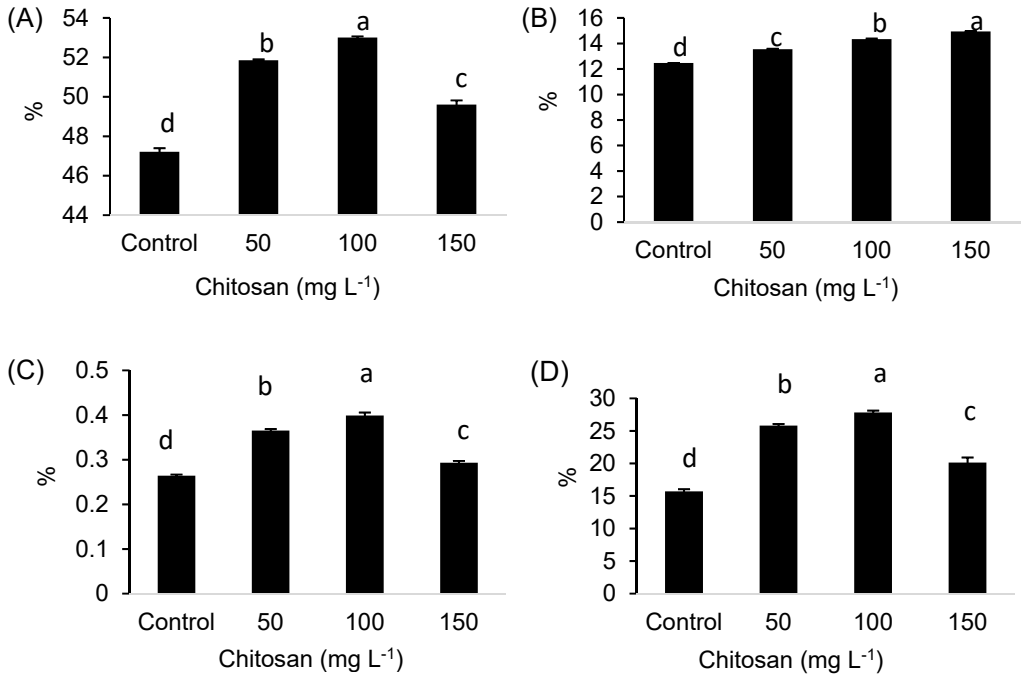

Figure 6. Effect of different concentrations of chitosan (50, 100 and 150 mg L^{-1}) on Carbohydrate % (A), Protein %(B), Lycopene % (C),Flavonoids % (D)in grain yield on wheat plant (Gimmeza 9) variety (combined analysis of two seasons). The different letters (a–d) show statistical significance at $p < 0.05$; vertical bars indicate ± SE.

Foliar spraying of chitosan increased ($P < 0.05$) significantly carbohydrate % and protein % of the wheat grains (Fig. 6, A, B). Abdallah et al. (2020b), found that application of chitosan increased carbohydrates % and protein %in the yielded wheat grains.

Antioxidant compounds (lycopene and flavonoids) increased in wheat grains in response to various concentrations of chitosan (Fig. 6, C, D). Lycopene and flavonoids are considered plant secondary metabolites of phenolic nature which havingantioxidant properties, include cell signaling and communication. These results agree with that of Abdallah et al. (2020a) on wheat cultivars. Padayatty et al. (2003) recorded that, flavonoids in the human diet inhibit the danger of different cancers and avoid menopausal symptoms so the high contents of flavonoids have a much significant influence on human health.

Mineral contents of the grains yield:

Foliar spraying of chitosan increased ($P < 0.05$) significantly N%, P%, K% and Ca% of the wheat grains (Fig. 7, A, B, C, D). It seems from the data in (Fig. 7, A, C) chitosan that 150 mg L^{-1} gave the most efficient increment inN and K% by 20.6 and 32.2% respectively. Meantime, the increase in P% and Ca% was 28.6% and 27.0%, respectively resulted from grains of plants treated with 100 mg L^{-1} chitosan (Fig. 7, B, D).

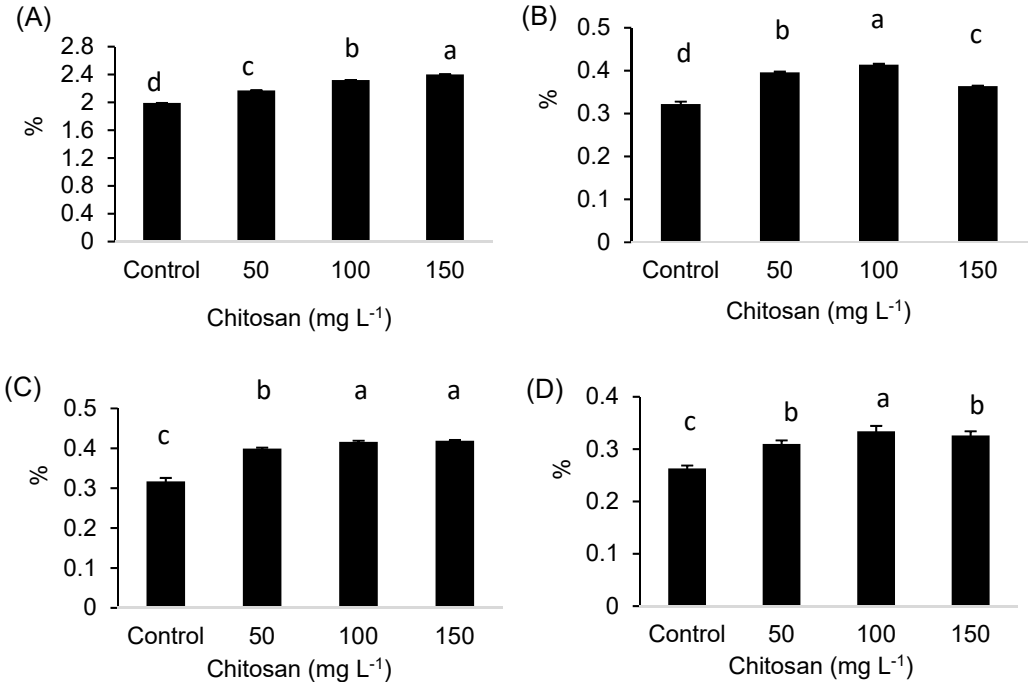

Figure 7. Effect of different concentrations of Chitosan **(**50, 100 and 150 mg L^{-1}) on Nitrogen % (A), Phosphorus % (B), Potassium % (C) and Calcium % (D) in grain yield on wheat plant (Gimmeza 9) variety (combined analysis of two seasons).The different letters (a–d) show statistical significance at $p < 0.05$; vertical bars indicate ± SE.

Foliar application of wheat plants grown in sandy soil with chitosan might work as fertilizer sources that enables the plant to overcoming the unfavorable conditions of thesoil such as deficiency in nutrients required for plant growth and development and consequently its yield and quality. Abdallah et al. (2020a) found that the application of chitosan increased the contents of N, P and K in wheat grains under water stress. Also, Farouk & Ramadan (2012) demonstrated that chitosan increased significantly N and K contents in cowpea plants. This may be due to the role of chitosan to stimulate plant growth, uptakeand transport of nutrients and photosynthesis efficiency (Guan et al., 2009).

Correlation Matrix

Pearson's correlation coefficients of grain yield among all deliberate characters of wheat plants growing in sandy soil conditions and three chitosan levels are presented in (Fig. 8). There was observed a potent correlation among grain yield and all of the related studied traits, i.e., shoot length, leaf No/plant, tiller fresh and dry wt, Chl a, Chl b, Car, total chl, IAA, ABA, GA, Cyt, FAA, TSS, total carbohydrates, protein, N%, P%, Ca%, flavonoids, total phenol, DPPH%, nutritional values in wheat grains (N%, P%, K%, Ca%, Carb%, Protein%, and flavonoids) which are highly positively associated with grain yield. However, RWC, proline andK% in wheat leaves and lycopene in nutritional value in grain yield are positive between grain yield.Moreover, there was a negative association among grain yield and lipid peroxidation.

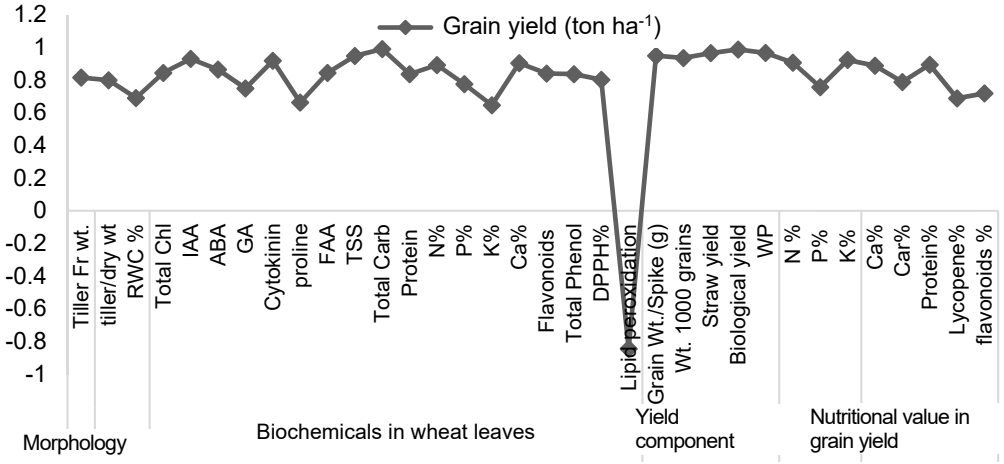

Figure 8. Pearson's correlation coefficients between all studied characters of wheat plants growing in three chitosan levels (50, 100 and 150 mg L^{-1}).

CONCLUSION

Chitosan is a low toxic and cheap substance that is biodegradable and environmentally friendly. Adding exogenous chitosan is an inexpensive and efficient measure which improved growth and yield of wheat plants. Chitosan significantly affects some metabolic processes; it enhances plant growth and development through enhancing photosynthesis, endogenous phytohormones, protein synthesis and ions uptake.

Chitosan-induced increase in osmoprotectants and secondary metabolites such as antioxidant compounds (lycopene, flavonoids and total phenol) and antioxidant activities played an essential role in contributing to enhanced growth, yield and water productivity in the wheat plants. Additionally, the antioxidants decrease the malondialdehyde levels in wheat plants. The application of chitosan increased significantly the carbohydrate, protein, flavonoids, N, P, K and Ca of the wheat grains. Chitosan at a concentration of 100 mg L^{-1}, was the most effective to be appliedas a foliar to wheat plants growing under sandy soil conditions. The perspective of sustainable crop production and rising agricultural yield with inexpensive contributions are farmers' requests for cultivating wheat plants under sandy soil conditions. This study illustrates the possible roles of chitosan in increasing the horizontal expansion of plant cultivation in the sandy soils. The results of the article can be implemented on a large scale in the new lands and the application of sustainable farming methods are safer to the environment. Thus using chitosan help to solve the problem of the bad properties of sandy soils and food gap. It can finally help in filling the food gap of grain crops in the third world.

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