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Research Article
Amino Acids Sources and Chitosan Enhance Cauliflower Yield and Quality under Heat Stress

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Abstract

Background and Objective: Heat stress is one of the abiotic stresses that limit vegetable cultivations in the summer season especially with the extreme temperature due to climate changes. Therefore, this study established for evaluating the effect of glycine betaine and arginine as amino acids and concentrations of chitosan as a foliar application on cauliflower plants grown under high temperature. **Materials and Methods:** This work was carried out during the two summer seasons of 2019 and 2020 to investigate the effect of different amino acids sources (Control, glycine betaine at 250 ppm and arginine at 250 ppm) and different concentrations of chitosan(0, 250, 500 and 750 ppm) as a foliar application for alleviating the heat stress on cauliflower plants grown in sandy soil. **Results:** In both seasons, the results pointed out that glycine betaine significantly increased vegetative growth parameters, yield, head physical quality, leaves mineral contents (N, P and K), Leaf Relative Water Content (LRWC) (%), Membrane Stability Index (MSI) (%), total chlorophyll and nutritional values (vitamin C and crude protein) as compared to the other treatments. Adding 750 ppm chitosan was the most effective on growth parameters and pre-mentioned parameters. **Conclusion:** For alleviating the high-temperature effects on cauliflower plants grown in sandy soil, it should be used glycine betaine and/or 750 ppm of chitosan as a foliar spray.

Key words: Cauliflower, yield, nutritional value, heat stress, amino acids, glycine betaine, arginine, chitosan

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cauliflower (*Brassica oleracea* L. var. botrytis) is a widespread international winter vegetable crop that belongs to the family Brassicaceae. Cauliflower plays an important role in the human diet due to its high nutritional value. It is a rich source of mineral elements such as calcium and magnesium, high-quality proteins, carbohydrates, vitamin-B and C which are necessary for human health[1]. Cauliflower is very sensitive to environmental stresses. Weather parameters affect every stage of plant growth and thereby affect the yield. Heat stress (high and low temperatures) is considered one of the most important obstacles facing cauliflower crop during plant growth which causes buttoning and blind bud or no head. For reducing the effect of heat stress on cauliflower plants, many investigators used some amino acids as foliar spraying. Where the amino acid application is a prevalent practice in vegetable cultivation throughout the world. It is biostimulants that improve plant growth, enhance nutrient availability and promote fruit yield and quality[2,3]. Amino acids work as hormone precursors[2]; cofactors of several physiological progressions, like glutamate receptors[4,5]; controller of nitrogen uptake[6]; root development and antioxidant metabolism[7,8]. Furthermore, Teixeira *et al*.[9] found that different effects on soybean crops when treated by foliar application of amino acids. Several investigations have observed a positive effect of foliar spray of amino acid mixtures on plants. In this concern, Saeed *et al*.[10] suggested that foliar spray of amino acids significantly enhanced vegetative growth parameters and pods yield of soybean. Glycine betaine is an organic molecule that plays a vital role in osmoregulation which allow a variety of plant species to tolerate environmental stresses (drought, salinity and extreme temperatures), the structure of complex proteins, enzymes, photosynthetic machinery and maintaining the membrane structures[11,12]. Furthermore, Ragab *et al*.[13] found that foliar application of GB at 10 mM L^{-1} ameliorated the negative effects of water stress and produced the highest significant values of vegetative growth parameters of tomato plants as well as significantly increased LRWC and photosynthetic pigments. Arginine is a great important amino acid inside plants. It has many benefits one of them, it acts as a store of nitrogen, as well as it acts as a precursor to the biosynthesis of amines and nitric oxide, which participate in all physiological and biochemical processes within the plant, growth and development and plant resistance to stress conditions[14].

Also, chitosan is an environmentally friendly product that has been widely used in the agricultural sector to stimulate plant defense[15], due to its many-sided biological, chemical and physical properties against invading pathogens[16]. Chitosan has many advantages such as it is naturally available, non-degradable and also a non-toxic compound[17]. The exoskeletons of crustaceans are the main source of chitosan[18]. Chitosan is generally used in agriculture as coatings for controlling decay of minimally processed fruits and vegetables[19] to protect plants against microorganisms[20] and enhance plant growth as well as increase the production of okra[21]. Several studies have been focused on the positive effects of chitosan on the growth of roots, shoots and leaves of various plants[22]. Plants that treated with chitosan appeared an increase in the yield of tomato trials. They found also the chitosan foliar treatment tended higher yield than the yield from other treatments. Fresh and dry shoot weights of lettuce plants that treated with chitosan were significantly higher than control.

Therefore, the objective of this study was to evaluate the effect of the separate application of glycine betaine and arginine with foliar concentrations of chitosan on vegetative growth, yield, heads physical quality, leaf mineral contents, LRWC (%), MSI (%) and nutritional value of cauliflower plants which cultivating under high-temperature condition.

MATERIALS AND METHODS

Study area: This work was carried out at the Experimental and Production Station of National Research Centre, El-Noubaria region, Beheira Governorate, north of Egypt, during the two summer seasons of 2019 and 2020 to investigate the effect of different amino acids sources and different concentrations of chitosan as a foliar application for alleviating the heat stress on cauliflower plants grown in sandy soil.

Plant materials: Seeds of cauliflower cv. Varsnowball was sown at the first of July in foam trays (208 eyes) filled with a mixture of peat moss: vermiculite (1:1 volume). This mixture is the recommended transplant production media for protected cultivation. Seedlings at 45 days of age were transplanted in the field in sandy soil. Physical and chemical analysis of soil samples took place according to Wilke[23]and illustrated in Table 1.

Forty day-old seedlings were field transplanted, then healthy transplants 40 days age the area of the experimental plot was 16.8 m consisted of three rows; each row was 8 m length and 0.7 m width. Cauliflower transplants were sown 0.5 m apart on one side of the irrigation line, one seedling was adjacent to every irrigation eye. Ditches of 20 cm width and 20 cm depth were prepared beside every irrigation line. Calcium superphosphate and agricultural sulphur at a rate of

Table 1: Physical and chemical properties of the experimental soil

Sand (%)			Clay (%)			Silt (%)			Texture
				Physical properties					
73.15			6.1			20.75			Sandy
Cations (Meq L^{-1})					Anions (Meq L^{-1})				
EC (dS m^{-1})	pH	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{-}	HCO^{-}	Cl^{-}	SO_4^{-}
				Chemical properties					
2.18	7.81	4.60	2.20	3.79	0.91	Nil	1.40	1.6	8.5
N				P					K
				Available nutrient (ppm)					
19.40				70.89					165.75

100 kg per feddan were spread through the ditches and covered by sand. Drip irrigation lines were established over the ditches and soil was irrigated continuously three days before transplanting. The recommended horticultural practices of growing cauliflower were followed according to the Ministry of Agriculture.

Experimental design and statistical analysis: The experiment was arranged in a split-plot design with three replicates. Amino acids sources were assigned in the main plots, whereas concentrations of chitosan were allotted in the sub-plots. Every experiment contained three replicates. Each replicate consisted of 12 treatments which were the combinations of three amino acids treatments multiplied by four concentrations of chitosan. Data of the experiment was statistically analyzed using M-static (M.S.) software. The comparison among means of the different treatments was determined[24]. Means followed by the same alphabetical letters are not statistically different at a 5% level of significance[25].

Experimental treatments: Cauliflower plants were exposed to different treatments as foliar spraying for alleviating the heat stress (Daily maximum and minimum air temperatures in 2019 and 2020 seasons were shown in Fig. 1a-b.

Amino acids sources:

- Control (using distilled water)
- Glycine betaine at a concentration of 250 ppm
- Arginine at a concentration of 250 ppm

Concentrations of chitosan:

- 0 ppm chitosan (using distilled water, control)
- 250 ppm chitosan
- 500 ppm chitosan
- 750 ppm chitosan

Plants were treated as a foliar application 3 times; 21 days after transplanting, 10 and 20 days after the first time.

Data sampling and analysis: Samples of three plants were randomly chosen from every plot after 45 days from transplanting and directly transferred to the Laboratory. The following data were recorded:

Vegetative growth characteristics

Plant length: Plant length was measured from the soil surface to the longest leaf.

Number of leaves per plant: Total number of leaves of the three selected plants was counted and their mean was recorded as the number of leaves per plant.

Leaves fresh weight (g/plant): The leaves were collected and weighted then recorded as the leaves fresh weight in grams.

Leaves dry matter (%): A sample of 100 g of leaves was oven-dried at 70°C till constant weight.

Determination of plant total chlorophyll: Plant pigments are important indicators of plant health and nutrient status as it has a pivotal role in plant photosynthesis. In this study plant leaf, total chlorophyll was measured by Minolta Chlorophyll Meter SPAD handheld device.

Leaf relative water content (LRWC) percentage: For the estimation of LRWC, 20 leaf discs samples (10 mm in diameter) were taken to determine the fresh weight (F.Wt.), discs were floated for 24 hrs in distilled water to determine the turgid weight (T.Wt.). Finally, the leaf discs were placed in a pre-heated oven at 70°C (for 48 hrs) to obtain discs dry weight (D.Wt.). Therefore, LRWC percentage was calculated according to the equation of Kaya *et al.*[26] as:

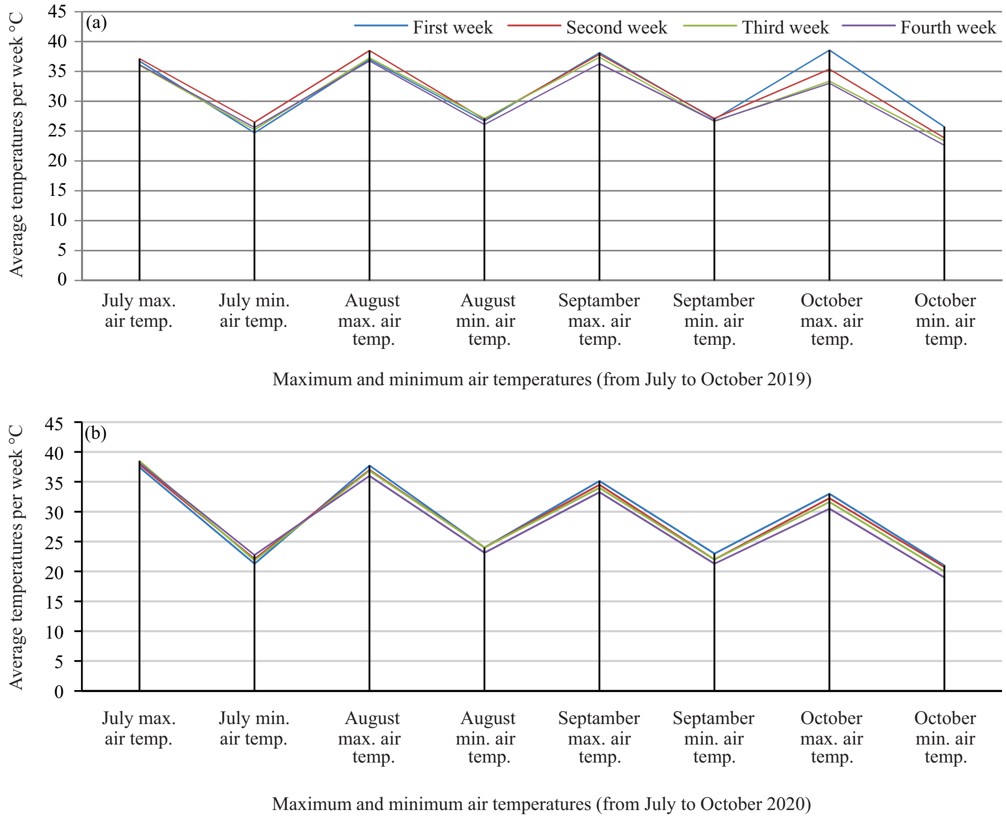

Fig. 1(a-b): Maximum and minimum weekly air temperatures (a) From July to October in 2019 and (b) From July to October in 2020

$$LRWC\ (\%) = \frac{FW\text{-}DW}{TW\text{-}DW} \times 100$$

Membrane stability index (MSI): Ten leaf discs (10 mm in diameter) were obtained and placed in the tube containing 10 mL of distilled water in two sets. One set was subjected to 40°C for 30 min and its electrical conductivity (EC1) was determined using an electrical conductivity meter (HANNA H199301). The second set of tubes were boiled in a temperature-controlled water bath at 100°C for 15 min and then the electrical conductivity (EC2) was measured[27]. Membrane stability index was calculated as percentage:

$$MSI\ (\%) = 1\text{-}\frac{EC1}{EC2} \times 100$$

Determination of nitrogen, phosphorus and potassium (N, P and K) percentage of leaves: A dried sample of 0.1 g was taken in 500 mL Kjeldahl flask and then 10 mL of Conc. H_2SO_4 was added and digested till a colourless solution appeared. The content was cooled down and diluted to about 25 mL with distilled water (solution 1).

Percentage of nitrogen: Total nitrogen was determined in solution (1) according to the Kjeldahl method as Motsara and Roy[28].

Percentage of phosphorus: Total phosphorus was determined calorimetrically by the NH4-Metavanadate method Motsara and Roy[28].

Percentage of potassium: Total potassium was determined in solution (1) by using a flame photometer according to Junsomboon and Jakmunee[29].

Physical properties of head: After harvesting the cauliflower plants, total yield was collected for different measurements and assessments. These measurements were as follow:

- **Total yield (kg):** Three plants were randomly selected per replicate and each plant head were collected and weighted individually. The average of the three was calculated then multiplied by the number of plants per replicate to calculate the yield for each replicate. Then calculating cauliflower production (ton/Fadden)

- **Head diameter:** All cauliflowers collected from the individual selected plants were measured using a calliper which gives diameter in cm. The average of them all was calculated and reported as the replicate head diameter
- Head fresh weight (g)
- Head height (cm)
- Head dry matter (%)

Determination of the nutritional value of heads

Percentage of crude protein: Crude Protein is the total protein equivalent including nitrogen from both protein and non-protein sources. Crude protein is calculated as mineral nitrogen multiplied by the protein factor, which is 6.25:

$$\text{Crude protein} = N \times 6.25$$

Vitamin C contents: Vitamin C contents (mg/100 g F.w) were determined in heads (fresh weight basis) according to Tantray *et al.*[30].

RESULTS AND DISCUSSION

Effect of amino acids sources on vegetative growth: The response of vegetative growth of cauliflower plants for different amino acids sources was assessed. Vegetative growth characteristics (plant length, leaves number/plant, leaves fresh weight and dry matter) positively responded to all foliar spray with amino acids as clear from Table 2. Plant length, leaves number/plant, fresh and dry weights significantly increased in response to foliar spray with glycine betaine, compared to control treatment (sprayed with water). Cauliflower plant length increased from 52.2 and 53.7 cm in the case of control treatment to 63.8 and 67 in the case of glycine betaine treatment in the first and second seasons, respectively. Similarly, leaves number/plant also increased under glycine betaine to 12.7 and 11.4. Leaves fresh weight ranged between 782.1, 760.8, 970.4 and 953.9 g in case of control and glycine betaine treatments in the first and second seasons, respectively.

Effect of amino acids sources on yield and physical quality: Likewise, physical quality (heads diameter, height, fresh and dry weights) as well as head yield of cauliflower plants were positively affected by amino acids treatment and negatively with control treatment as cleared in Table 3. The results showed that a significant increase in physical quality and head yield in response to glycine betaine and arginine treatments. The highest values of heads height (23.3 cm) have been attained in the case of glycine betaine treatment. Also, plants treated with arginine came at the second order in enhancing the heads height within (20.7 cm). The highest significant values of head diameter (cm) have been obtained due to spray with glycine betaine treatment (25.2 cm). In the case of spraying with glycine betaine, head fresh weight accounted for 1189.4 and 1139.6 g compared with control 869.1 and 853.3 g in the first and second season, respectively. Head dry matter percentage of plants that spray with glycine betaine reached 18, 18.8% and head yield of cauliflower plants which treated with glycine betaine accounted by 6.6 and 6 ton/fed compared with control 4.4 and 4.5 ton/fed in the first and second season, respectively.

Effect of amino acids sources on leaf mineral content and nutritional values: There were significant differences among different amino acids treatments in terms of leaf mineral contents i.e., N, P and K and nutritional values (protein and vitamin C). Leaf mineral contents and nutritional values also had an encouraging response to amino acids application in

Table 2: Effect of different amino acids sources on vegetative growth of cauliflower plants during 2019 and 2020 seasons

	Plant length (cm)		Leaves number/plant		Leaves fresh weight g/plant		Leaves dry matter (%)	
Treatments	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	52.2^{C}	53.7^{C}	9.7^{C}	8.6^{C}	782.1^{C}	760.8^{C}	12.2^{C}	11.6^{C}
Arginine	56.7^{B}	59.7^{B}	11.3^{B}	9.6^{B}	910.5^{B}	860.9^{B}	14.5^{B}	14.2^{B}
Glycine	63.8^{A}	67.0^{A}	12.7^{A}	11.4^{A}	970.4^{A}	953.9^{A}	16.3^{A}	16.6^{A}

Values followed by the same letter(s) are not significantly different at 5%

Table 3: Effect of different amino acids sources on heads physical quality and total yield of cauliflower plant during 2019 and 2020 seasons

	Head height (cm)		Head diameter (cm)		Head fresh weight (g)		Head dry matter (%)		Head yield (t fed^{-1})	
Treatments	1st season	1st season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	18.6^{C}	18.6^{C}	18.6^{C}	20.4^{C}	869.1^{C}	853.3^{C}	14.0^{C}	14.4^{C}	4.4^{C}	4.5^{C}
Arginine	20.7^{B}	20.7^{B}	21.2^{B}	22.0^{B}	979.1^{B}	973.7^{B}	16.2^{B}	17.1^{B}	5.3^{B}	5.3^{B}
Glycine betaine	23.3^{A}	23.3^{A}	25.2^{A}	25.2^{A}	1189.4^{A}	1139.6^{A}	18.0^{A}	18.8^{A}	6.6^{A}	6.0^{A}

Values followed by the same letter(s) are not significantly different at 5%

Table 4: Effect of different amino acids sources on leave mineral content and nutritional values of cauliflower plants during 2019 and 2020 seasons

	N (%)		P (%)		K (%)		Vitamin C (mg/100 g FW)		Protein (%)	
Treatments	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	2.19^{C}	2.20^{C}	0.35^{B}	0.32^{C}	2.92^{C}	2.85^{C}	69.8^{C}	68.8^{C}	15.5^{C}	15.5^{C}
Arginine	2.53^{B}	2.43^{B}	0.41^{AB}	0.39^{B}	3.21^{B}	3.07^{B}	76.3^{B}	75.5^{B}	16.8^{B}	17.2^{B}
Glycine	2.75^{A}	2.58^{A}	0.49^{A}	0.45^{A}	3.53^{A}	3.41^{A}	81.4^{A}	79.9^{A}	17.7^{A}	18.4^{A}

Values followed by the same letter(s) are not significantly different at 5%

Table 5: Effect of different amino acids sources on LRWC, MSI percentages and total chlorophyll content of cauliflower plants during 2019 and 2020 seasons

	LRWC (%)		MSI (%)		Total chlorophyll (SPAD)	
Treatments	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	69.45^{C}	67.84^{C}	56.32^{C}	56.62^{C}	13.7^{C}	14.6^{C}
Arginine	75.17^{B}	73.85^{B}	63.79^{B}	63.51^{B}	14.8^{B}	15.3^{B}
Glycine	80.30^{A}	79.20^{A}	69.51^{A}	69.37^{A}	16.1^{A}	16.6^{A}

Values followed by the same letter(s) are not significantly different at 5%

Table 6: Effect of different concentrations of chitosan on vegetative growth of cauliflower plants during 2019 and 2020 seasons

Rates of	Plant length (cm)		Leaves number/plant		Leaves fresh weight (g/plant)		Leaves dry matter (%)	
chitosan	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	54.4^{D}	54.3^{D}	8.8^{D}	8.2^{D}	844.7^{D}	821.9^{D}	13.6^{D}	13.1^{D}
250 ppm	57.5^{C}	59.8^{C}	10.2^{C}	9.4^{C}	871.8^{C}	836.2^{C}	14.1^{C}	13.8^{C}
500 ppm	58.7^{B}	62.6^{B}	11.6^{B}	10.7^{B}	901.6^{B}	877.3^{B}	14.5^{B}	14.4^{B}
750 ppm	59.7^{A}	63.8^{A}	14.2^{A}	12.5^{A}	932.9^{A}	898.6^{A}	14.9^{A}	15.1^{A}

Values followed by the same letter(s) are not significantly different at 5%

Table 4. The lowest nitrogen, phosphorus and potassium concentrations values in cauliflower leaves were achieved in the case of control (2.19, 2.20, 0.35, 0.32, 2.92 and 2.85%), while the highest significant values (2.75, 2.58, 0.49, 0.45, 3.53 and 3.41%) were obtained with spraying glycine betaine in the first and second season, respectively. The highest protein percentages were observed with the previous treatment (17.7-18.4%). Also, glycine betaine treatment produced the highest values of vitamin C (81.4-79.9 mg/100 g F.W.) which is far from control treatment values (69.8-68.8 mg/100 g F.W.).

Effect of amino acids sources on LRWC (%), MSI (%) and total chlorophyll: There were significant differences between different amino acids in terms of leaf relative water content, membrane stability index and total chlorophyll. Where, LRWC, MSI and total chlorophyll also had an encouraging response to amino acids application in Table 5. Foliar spray for glycine betaine resulted in the highest significant values of LRWC % (80.30, 79.20%) and MSI (%) (69.51, 69.37%) in the first and second seasons, respectively. Total chlorophyll (SPAD) varied within the slight range 13.7-14.6 and 16.1-16.6 in the case of control and glycine betaine in the two investigated seasons, respectively.

Enhancement of vegetative growth of cauliflower plants treated by amino acids may be due to the role of amino acids for providing the nitrogen source consequently better roots growth. The better growth of roots increases the absorption of nutrients from the soil which encouraged the photosynthetic activity of the treated plants. The mentioned photosynthesis activity encouragement caused more different metabolic substances production consequently, more dry matter accumulation in plant tissues, more nutrients in plant tissues and more heads yield. The higher content of K in plant tissue explains the higher chemical and physical quality of the cauliflower heads due to the presence of K which acts on photosynthate translocation from the leaves to the storage organs. These results come along with many previous studies which have proven that amino acids can directly or indirectly affect the vital activities of plants, as well as amino acids, play a big role in increasing the chlorophyll content in plants[31,32]. The foliar application of amino acids caused an increment of N, P, K and other micro-elements[31,32]. Shekari and Javanmardi[33] found that foliar application of amino acid at suitable concentration had positive effects on the physical and chemical properties of broccoli plants. These effects strengthened the role of the applied amino acids in the metabolism of broccoli plants. Also, Abou El Magd[34] found that the highest values of vegetative growth, total yield and quality of kohlrabi and broccoli plants were recorded by the foliar application of amino acids and seaweed extract. The exogenous application of amino acids has been shown to induce growth promotion effects on many crops[35,36].

Effect of different chitosan concentrations on vegetative growth: Data in Table 6 illustrated that increasing chitosan concentrations from 0-750 ppm significantly increased plant

Table 7: Effect of different concentrations of chitosan on yield and head physical quality of cauliflower plants during 2019 and 2020 seasons

Rates of chitosan	Head height (cm)		Head diameter (cm)		Head fresh weight (g)		Head dry matter (%)		Total yield (t fed^{-1})	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	19.2^{C}	19.8^{D}	20.2^{C}	19.6^{C}	963.5^{D}	925.5^{C}	15.4^{D}	16.1^{D}	4.4^{D}	4.7^{D}
250 ppm	19.8^{C}	20.4^{C}	22.6^{B}	21.8^{B}	1004.8^{C}	970.6^{C}	15.8^{C}	16.5^{C}	5.4^{C}	5.5^{B}
500 ppm	21.4^{B}	23.2^{B}	23.7^{A}	22.7^{A}	1031.4^{B}	1014.8^{B}	16.2^{B}	17.0^{B}	5.7^{B}	5.2^{C}
750 ppm	23.2^{A}	24.0^{A}	23.7^{A}	22.9^{A}	1050.5^{A}	1044.4^{A}	16.7^{A}	17.4^{A}	6.2^{A}	5.6^{A}

Values followed by the same letter(s) are not significantly different at 5%

Table 8: Effect of different concentrations of chitosan on leaves mineral content and nutritional values of cauliflower plants during 2019 and 2020 seasons

Rates of chitosan	N (%)		P (%)		K (%)		Vitamin C (mg/100 g FW)		Protein (%)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	2.15^{B}	1.95^{D}	0.32^{D}	0.31^{D}	2.96^{D}	2.78^{D}	71.7^{D}	71.1^{D}	14.3^{D}	14.8^{D}
250 ppm	2.38^{B}	2.22^{C}	0.38^{C}	0.36^{C}	3.04^{C}	2.84^{C}	75.1^{C}	73.6^{C}	16.4^{C}	15.5^{C}
500 ppm	2.71^{A}	2.88^{B}	0.45^{B}	0.42^{B}	3.25^{B}	3.11^{B}	77.4^{B}	75.7^{B}	17.7^{B}	18.6^{B}
750 ppm	2.73^{A}	2.58^{A}	0.50^{A}	0.46^{A}	3.63^{A}	3.70^{A}	79.2^{A}	78.5^{A}	18.3^{A}	19.3^{A}

Values followed by the same letter(s) are not significantly different at 5%

Table 9: Effect of different concentrations of chitosan on LRWC, MSI percentages and total chlorophyll content of cauliflower plants during 2019 and 2020 seasons

Rates of chitosan	LRWC (%)		MSI (%)		Total chlorophyll (SPAD)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	66.68^{D}	64.96^{C}	58.05^{C}	56.60^{D}	12.4^{D}	13.3^{D}
250 ppm	77.86^{C}	76.71^{B}	57.95^{C}	57.80^{C}	14.7^{C}	15.4^{C}
500 ppm	76.79^{B}	76.38^{B}	62.98^{B}	63.33^{B}	15.5^{B}	16.3^{B}
750 ppm	78.60^{A}	77.46^{A}	73.86^{A}	75.03^{A}	16.9^{A}	17.0^{A}

Values followed by the same letter(s) are not significantly different at 5%

length, leaves number, leaves fresh weight and leaves dry matter, this was true in both seasons. The highest values of plant length leave number leave fresh weight and leave dry matter were achieved in the case of application of 750 ppm chitosan where it reached 59.7, 63.8 cm (plant length), 14.2, 12.5 (leaves number/plant), 932.9, 898.6 g/plant (leaves fresh weight) and 14.9, 15.1% (leaves dry matter) in the first and second seasons respectively.

Effect of different chitosan concentrations on yield and physical quality: Regarding the effect of different chitosan concentrations, it was found that spray cauliflower plants with 750 ppm of chitosan significantly improved yield and physical quality (heads height, diameter, fresh and dry weights) as clear in Table 7. The highest values of total yield, head height, head diameter, head fresh weight and head dry matter were achieved with plants that treated with a higher concentration of chitosan (750 ppm). However, the lowest values were recorded with control. The application of 750 ppm chitosan significantly increased total yield to 6.2, 5.6 ton/fed., head height to 23.2, 24 cm, head diameter to 23.7, 22.9 cm, head fresh weight to1050.5, 1044.4 g and head dry matter to 16.7, 17.4% in both seasons respectively.

Effect of different chitosan concentrations on leaf mineral contents and nutritional values: Adding chitosan at the concentrations of (250, 500 and 750 ppm) increased leaf mineral contents and nutritional values of cauliflower heads compared to control as cleared in Table 8. In both seasons, the most increases in nitrogen, phosphorus and potassium percentages in cauliflower leaves, have been attained by the spraying of 750 ppm chitosan compared with control and other treatments. Vitamin C reached 79.2, 78.5 mg/100 g F.W. and protein percentage (18.3 and 19.3%) in case of spraying cauliflower plants with 750 ppm chitosan.

Effect of different chitosan concentrations on LRWC (%), MSI (%) and total chlorophyll: Adding chitosan at the concentrations of (250, 500 and 750 ppm) increased LRWC (%), MSI (%) and total chlorophyll of cauliflower leaves compared to control as clear in Table 9. In both seasons, the highest significant values of leaf LRWC (%) (78.6, 77.46%) and MSI% (73.86, 75.03%) were obtained, due to spraying 750 ppm chitosan. The lowest leaf LRWC and MSI (%) values were achieved in the case of control (66.68, 64.96% and 58.05, 56.60%) in the first and second seasons, respectively. Moreover, the Application of 750 ppm chitosan by spraying, resulted in the highest value of total chlorophyll (SPAD), (16.9, 17). However, the lowest value recorded with control (12.4, 13.3).

Increased vegetative growth, yield and physical quality of cauliflower heads with the high concentration of chitosan-treated plants could be attributed to the higher chlorophyll content and denser number of leaves which encouraged the photosynthetic activity of the treated plants caused more different metabolic substances production consequently, more dry matter accumulation in plant tissues and more heads yield. Other researchers have been attributed this enhancement of growth by chitosan treatments increased key enzymes activities of nitrogen metabolism (nitrate reductase, glutamine synthetase and protease), which enhanced plant growth and development in okra[21]. These results come along with those obtained by Shaikha[37] and Rahman *et al.*[38] who mentioned that higher chlorophyll content in the plants treated by chitosan. The increase in dry matter reflected in the yield components such as heads weight, height and diameter. These were also clear in the higher content of protein and vitamin C in cauliflower heads. The degree of responses differed according to plants and the applied concentration of chitosan[37-39]. Also, El Bassiony *et al.*[40] found that the highest values of tuber height, diameter and fresh weight of tubers, as well as total yield of tubers of Kohlrabi plants, were recorded by foliar spray of chitosan. The mineral content of cauliflower leaves was influenced by the concentration of chitosan. Application of 750 ppm chitosan resulted in a significant increase in percentages of N, P and K. The increased nutrients in leaves of cauliflower plants may be due to imparted by the amino components in chitosan and also higher efficiency of the plant to absorb nutrients from the soil when chitosan was degraded. These results are compatible with El Bassiony *et al.*[40] who found that the highest values of N, P and K% in leaves and N (%) in tubers of Kohlrabi plants were recorded with foliar spray of chitosan.

Effect of the interaction between amino acids sources and concentrations of chitosan: Taking into dual effect of studied factors, results showed that interaction treatments between amino acids sources and concentrations of chitosan had augmented vegetative growth, yield parameters, leaf mineral contents and nutritional values of cauliflower plants.

Where the interaction between glycine betaine as amino acid and chitosan increased vegetative growth parameters; plant length leaves number per plant, leaves fresh weight and leaves the dry matter of cauliflower plants compared to the other treatments. Data in Fig. 2a and b showed that cauliflower plants that received glycine betaine as amino acid and chitosan at 750 ppm produced the highest significant values of plant length (67.4 and 71.5 cm) and No. of leaves/plant (16.2 and 14.1), while the lowest values for plant length (49.8 and 45.4 cm) and No. of leaves/plant (7.1 and 6.3) were obtained with control treatment in the both studied seasons, respectively.

In addition, the maximal values of leaves fresh weight and leaves dry matter (1010.6, 974.6 g and 16.9, 17.0%) have been recorded by addition glycine betaine+750 ppm of chitosan compared to minimum values for the control treatment (714.3, 710.7 g and 11.6, 10.3%) in the first and second seasons, respectively in Fig. 3a and b.

For the effect of the interaction of studied treatments on fruit yield and quality of cauliflower heads. Cauliflower plants that received glycine betaine as amino acid and chitosan at 750 ppm produced the highest significant values of head fresh weight, head height, head diameter, head yield and head dry matter in both tested seasons. In the case of spraying cauliflower plants with glycine betaine as a source of amino acid+750 ppm of chitosan, head fresh weight accounted by 1214.6 and 1210.3 g compared with control 812.5, 810.6 g in the first and second season respectively in Fig. 4a. As well as, head height (26.5, 28.9 cm) and head diameter (27.6, 26.5 cm) increased with the same interaction treatment during the two successive seasons respectively in Fig. 4b and 5a.

While The lowest total head yield values were achieved in the case of control (3.2-4.1 t fed^{-1}), while the highest significant values (7.1-6.3 t fed^{-1}) were obtained, due to spraying glycine betaine+750 ppm chitosan treatment, followed by spraying with glycine betaine+500 ppm of chitosan (6.9-6.3 t fed^{-1}) and did not reach the level of significance in the second season in Fig. 5b.

On the other hand, the highest significant head dry matter values were observed with spraying cauliflower plants by glycine betaine+750 ppm chitosan, followed by plants treated by glycine betaine+500 ppm chitosan and did not reach the level of significance in both seasons (Fig. 5c).

Cauliflower plants which treated by the interaction treatments of glycine betaine as an amino acid with chitosan at 750 ppm showed the highest concentrations of leaf mineral contents (N, P and K %) and nutritional values of cauliflower heads (head protein percentage and head vitamin C content) compared to the other treatments. Dealing with the amino acids effect and chitosan treatments on the abovementioned parameters, data reveal distinctive differences between them with control. One can reveal the following aspects:

- Nitrogen is ranged between 1.99-1.81% and 3.09-2.90 in the case of control treatment and glycine betaine+ 750 ppm chitosan treatment, respectively in Fig. 6a

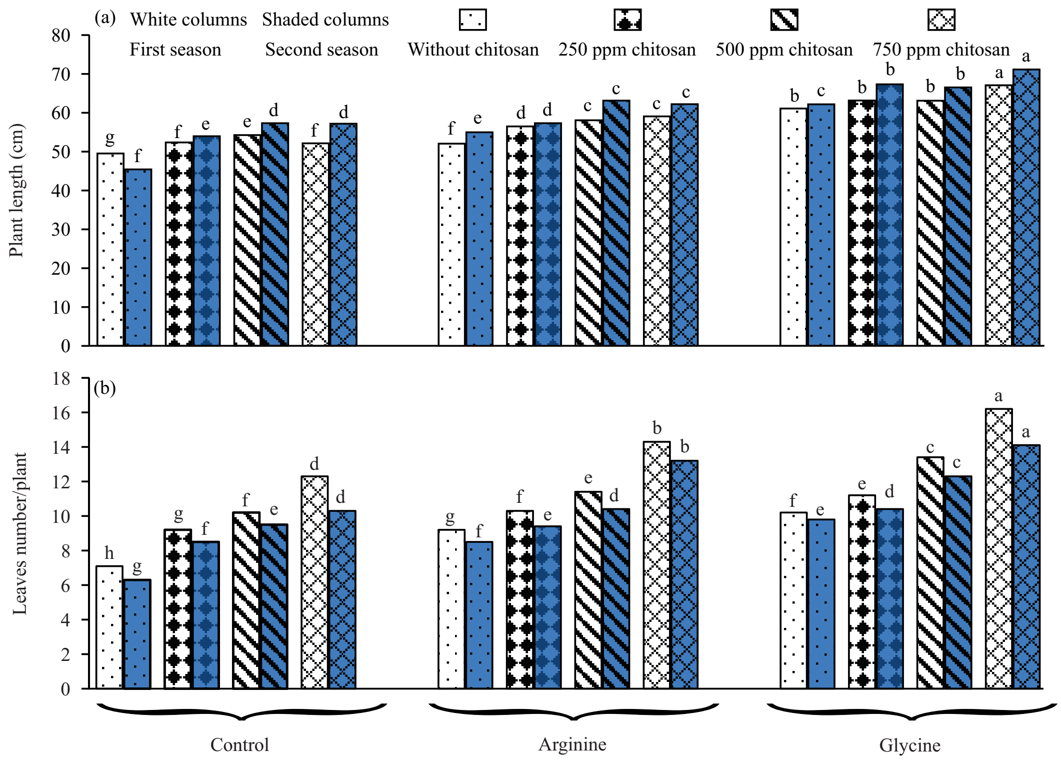

Fig. 2(a-b): Interaction effect of studied treatments on (a) Plant length and (b) Leaves number/plant of cauliflower plants during 2019 and 2020 seasons

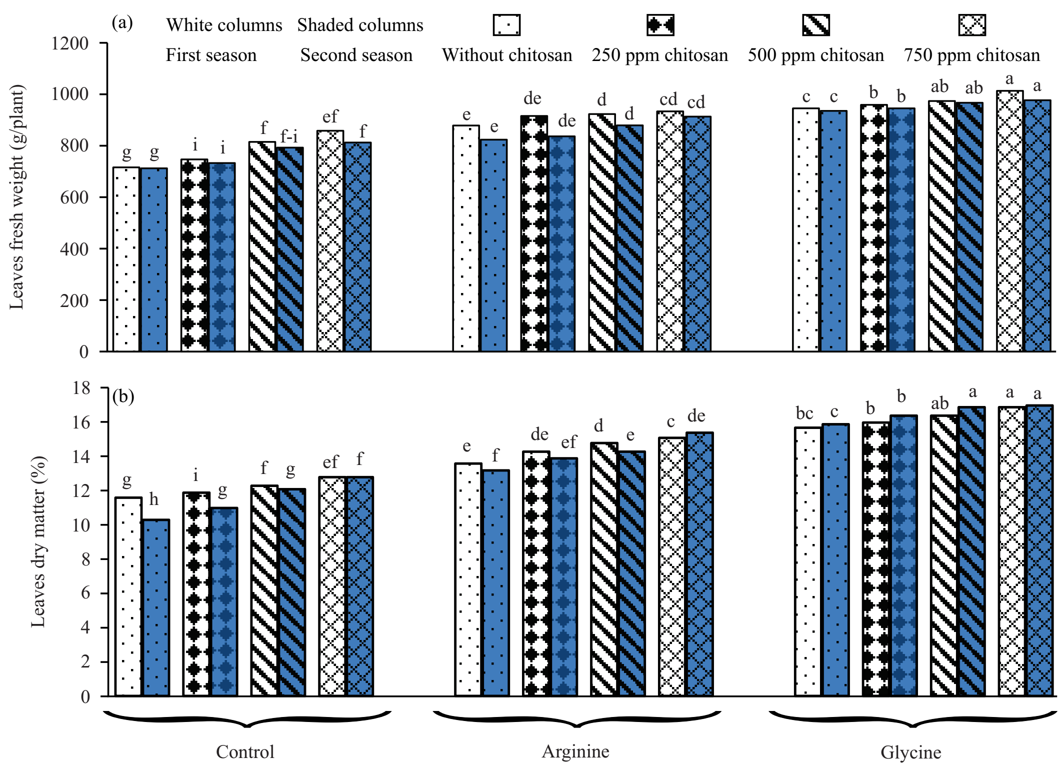

Fig. 3(a-b): Interaction effect of studied treatments on leaves (a) Fresh weight and (b) Dry matter of cauliflower plants during 2019 and 2020 seasons

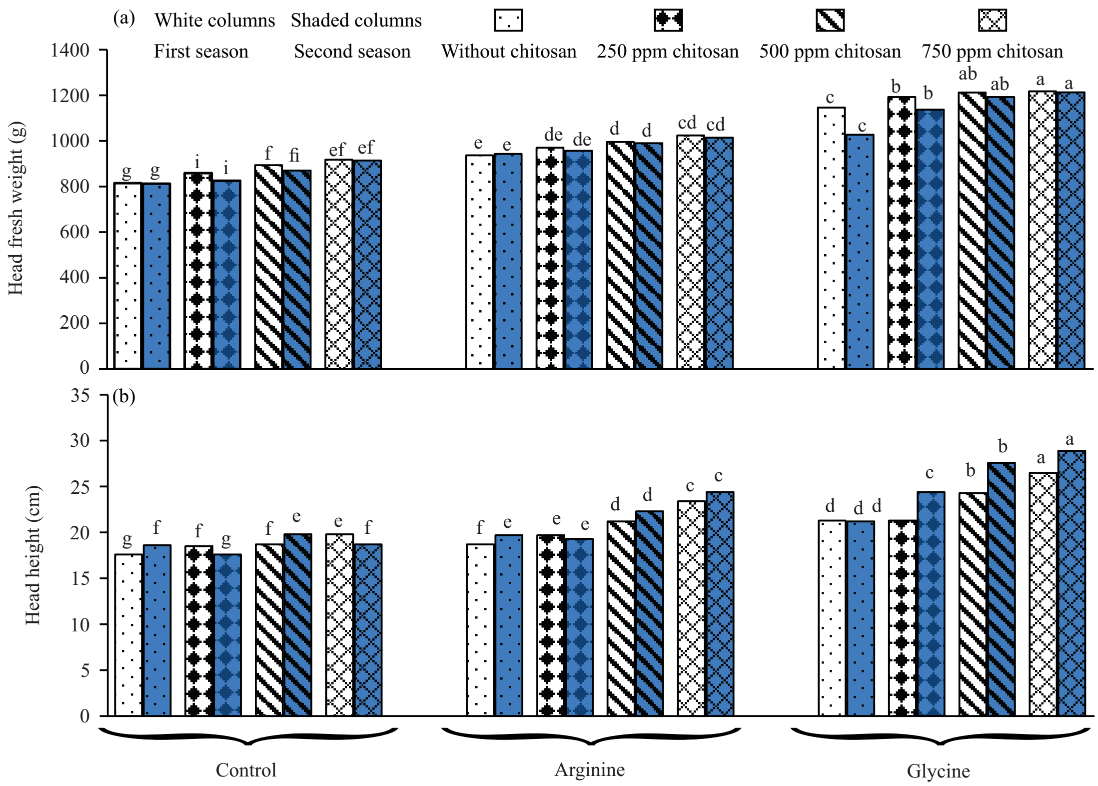

Fig. 4(a-b): Interaction effect of studied treatments on the head of (a) Fresh weight and (b) Height of cauliflower plants during 2019 and 2020 seasons

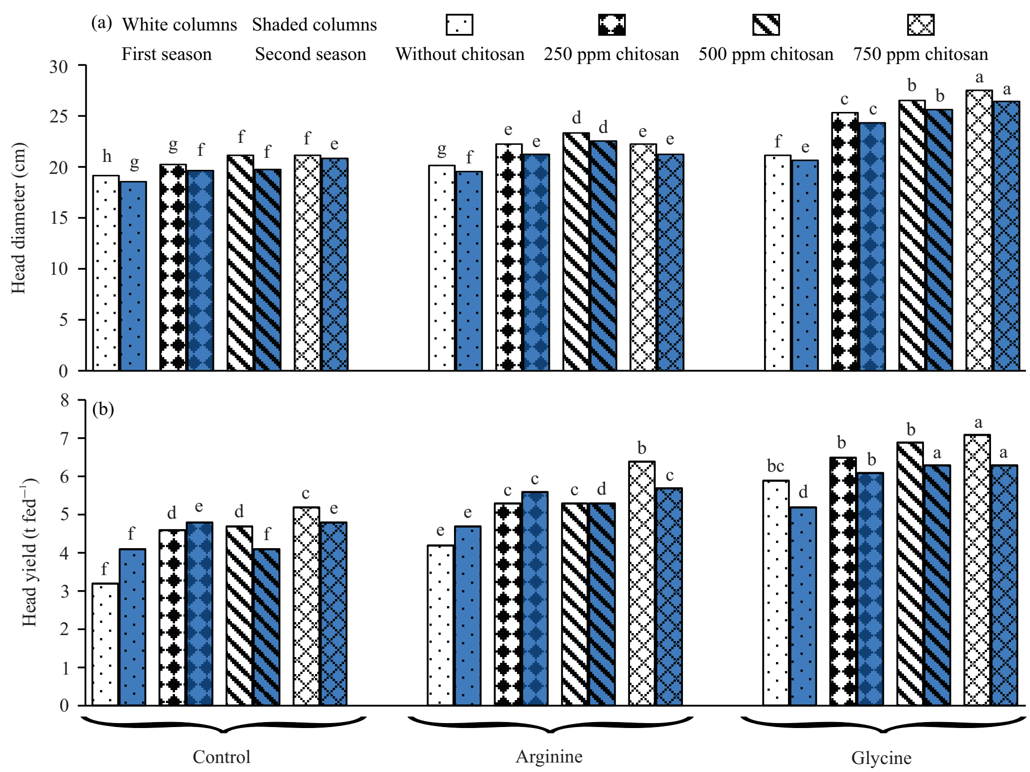

Fig. 5(a-c): Continued

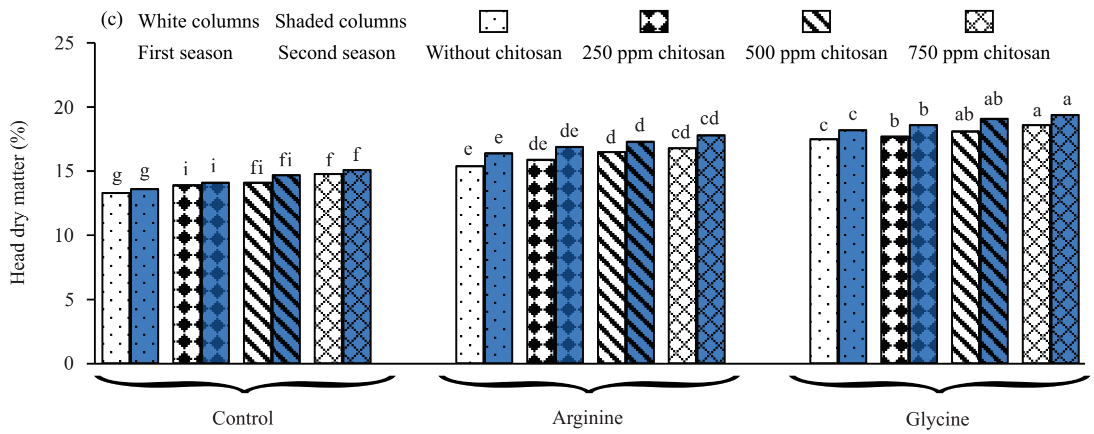

Fig. 5(a-c): Interaction effect of studied treatments on the head (a) Diameter, (b) Yield and (c) Dry matter of cauliflower plants during 2019 and 2020 seasons

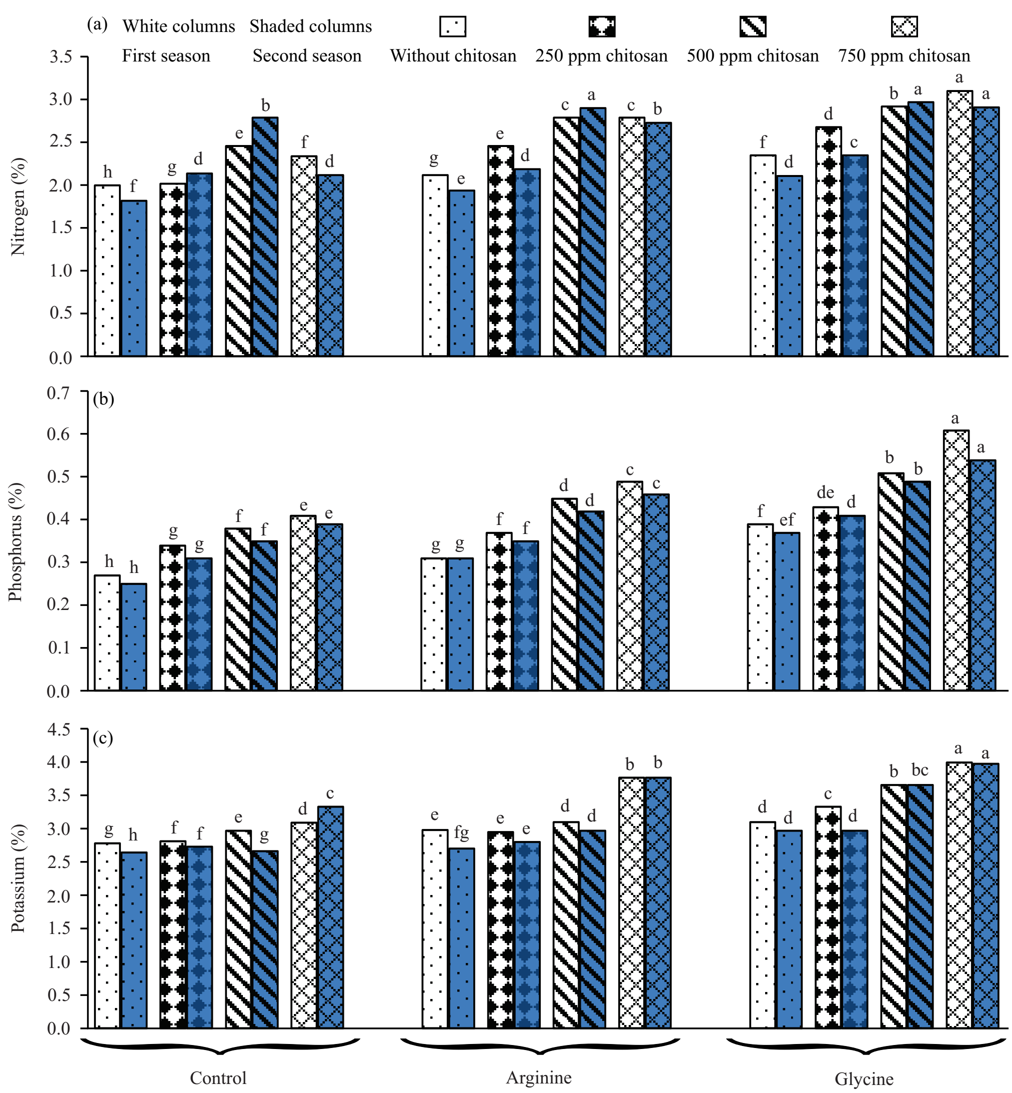

Fig. 6(a-c): Interaction effect of studied treatments on leaves (a) N, (b) P and (c) K percentage of cauliflower plants during 2019 and 2020 seasons

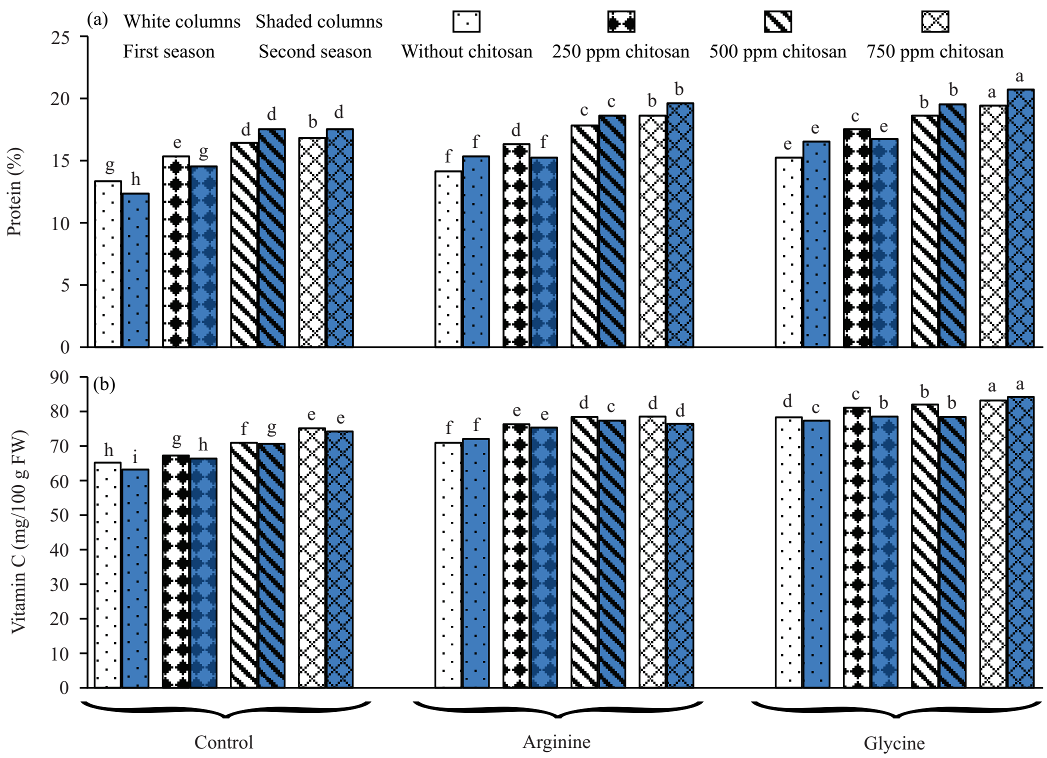

Fig. 7(a-b): Interaction effect of studied treatments on head (a) Protein percentage and (b) Vitamin C content of cauliflower plants during 2019 and 2020 seasons

- Phosphorus is ranged between 0.27-0.25% and 0.61-0.54% in the case of control treatment and glycine betaine+750 ppm chitosan treatment, respectively in Fig. 6b
- In Fig. 6c, the relatively highest values have been detected in potassium (4.01-3.99%) with the same interaction treatment

Furthermore, Fig. 7a and b data revealed that the highest significant values for protein (19.5-20.8%) and vitamin C (82.3-78.7 mg/100 g F.W) were obtained with cauliflower plants that received glycine betaine+750 ppm chitosan treatment.

In Fig. 8a, it's clear to notice that the maximum mean values of LRWC had been recorded with adding glycine betaine+750 ppm chitosan as a foliar application in both seasons (85.48, 85.09%), followed by plants treated by glycine betaine+500 ppm chitosan (83.12-84.12%) and did not reach the level of significance in both seasons.

In the same trend, the highest significant values of MSI percentage and total chlorophyll (SPAD) were observed with the treatment of glycine betaine+750 ppm chitosan, while the lowest values were obtained with control treatment in both seasons in Fig. 8b and c.

Vegetative growth enhancement may be due to the dual effect of amino acids and chitosan concentrations which are important for plant growth and have a well-known effect on physiological activities. One of the most important physiological effects of amino acids inside plants, the effect on resistance to unfavourable environmental conditions like high temperature. When adding amino acids to the plant in the free form before, during or after these unfavourable conditions, it provides the plant with amino acids directly, which leads to the provision of energy for the plant. Amino acids also play an important role in the photosynthesis process inside the plant, where the use of amino acid glycine and arginine increases the concentration of chlorophyll, which leads to an increase in the synthesis of chlorophyll units inside the plant and thus increase the efficiency of the photosynthesis process. Chitosan also plays a role in the synthesis of chlorophyll inside the plant and encouraged the photosynthetic activity of the treated plants. Since head yield is the function of the vegetative growth, photosynthetic activity, nutrient uptake and dry matter accumulation. The resulting increases in head yield are the function of the increases in the vegetative growth, photosynthetic activity, nutrient uptake and dry matter accumulation. These results are in harmony with these

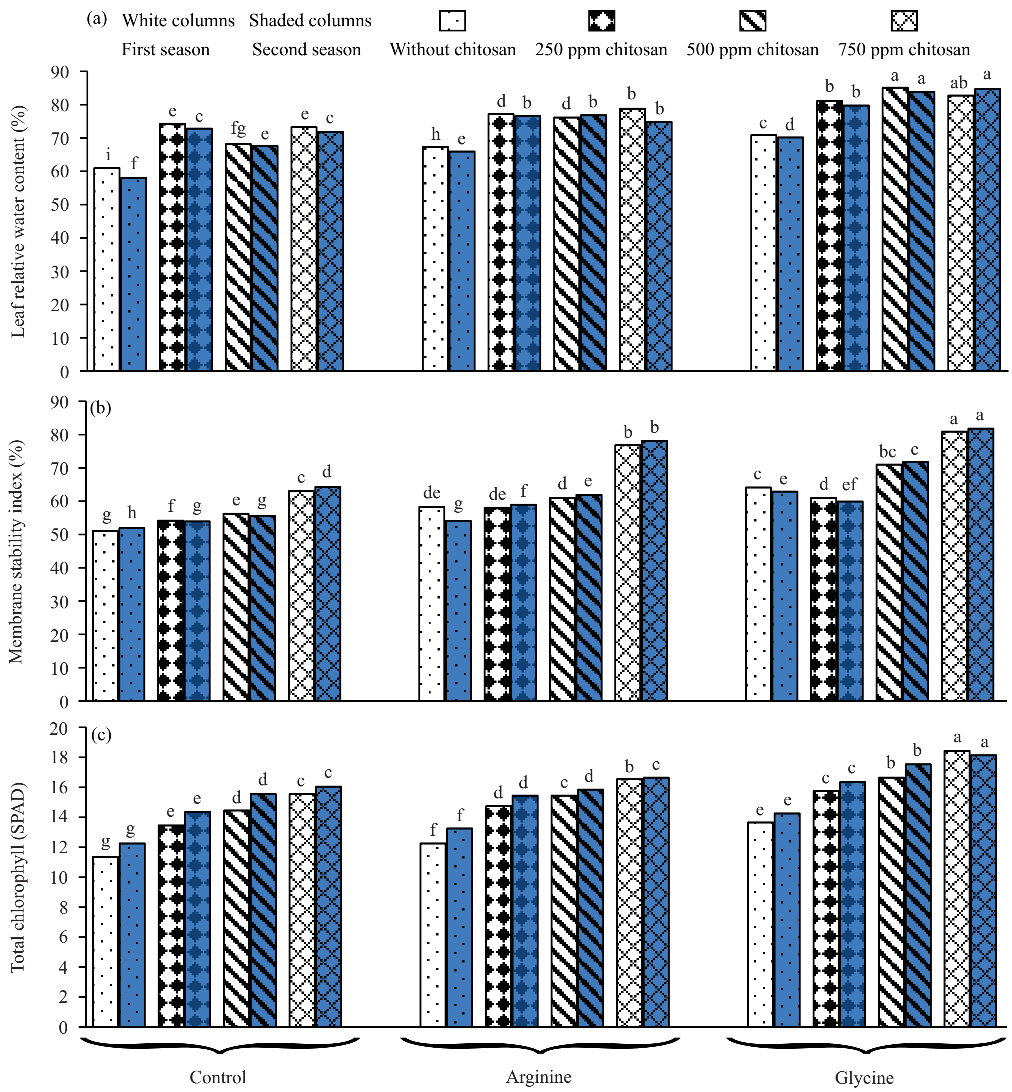

Fig. 8(a-c): Interaction effect of studied treatments on (a) LRWC percentage, (b) MSI percentage and (c) Total chlorophyll (SPAD) of cauliflower plants during 2019 and 2020 seasons

obtained by El Bassiony *et al.*[40] who found that foliar application of yeast, amino acid and chitosan enhanced vegetative growth and yield of kohlrabi.

CONCLUSION

Amino acids and chitosan used as a foliar application in vegetable cultivations for alleviating heat stress. So under Egyptian conditions in the sandy farm, for producing cauliflower in the summer season under high temperature, it recommended using glycine betaine (500 ppm) as amino acid and/or chitosan (750 ppm) as a foliar application for improving the vegetative growth, increasing the yield, head physical quality and nutritional values.

SIGNIFICANCE STATEMENT

This study confirmed that foliar spraying of amino acids such as glycine betaine or arginine separated or mixed with chitosan are effective agriculture methods for alleviating the high temperature during the summer season and improving the vegetative growth, increasing the head yield and enhancing the head physical quality and nutritional values for cauliflower.

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