Original Research Article

Effect of Foliar Application of Chitosan on Growth, Yield and Nutritional Qualities of Red Amaranth (*Amaranthusgangeticus*L.)

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ABSTRACT

Chitosan is a promising natural biopolymer that stimulates growth, increases yield, and boosts-up plants' immune systems.A field experiment was conducted to study the effect of foliar application of chitosan on growth, yield and quality attributes (viz. chlorophyll-a, chlorophyll-b, total chlorophyll, carotenoids, total ash and major nutrient contents) of red amaranth. The field experiment was laid out in a Randomized Completely Block Design (RCBD) with three replications and six treatments [viz. T0 = Control (no chitosan application); T1 = 100 mg L^{-1}; T2 = 150 mg L^{-1}; T3 = 200 mg L^{-1}; T4 = 250 mg L^{-1} and T5 = 300 mg L^{-1} chitosan solution]. The study results revealed that the application of chitosan at different doses had no significant effect on plant height, the number of leaves plant-1, and the moisture content of red amaranth. But there were significant variations in stem diameter, root length, and yield of red amaranth. Similarly, the application of chitosan at different doses also showed a significant effect on chlorophyll contents, carotenoids and total ash contents of red amaranth. The average chlorophyll-a, chlorophyll-b, total chlorophylls, carotenoids and ash content of red amaranth ranged from 6.02-9.51, 3.67-5.92 and 9.68-15.15 mg g^{-1} tissue, 0.20-0.28 µg g^{-1} sample (fresh wt.), and 16.36-17.69%, respectively. Among the major nutrient elements, the amount of Ca, Mg, Na, K and P in red amaranth was statistically insignificant among the treatments. On the contrary, the effect of foliar application of chitosan at different doses disclosed a statistically significant difference in S, Zn and Fe contents of red amaranth. The highest amounts of chlorophyll-a, carotenoids, total ash, S, and Fe in red amaranth were obtained from treatment T5. Finally, the study results concluded that foliar application of chitosan at 300 mg L^{-1} has a significant positive effect on the growth, yield, pigments, S, and Fe contents of red amaranth.

Keywords: Chitosan dose, field application, chlorophylls, carotenoids and nutrient elements

1. INTRODUCTION

"Chitosan is modified from chitin, the main structural component of marine crustaceans like shrimp, prawns, crab, and exoskeletons of most insects. Due to its high affinity and non-toxicity nature, it does not harm human beings and livestock. Chitosan is a natural biopolymer that stimulates growth, increases plants' yield, and induces plants' immune systems" [1]. "Moreover, chitosan not only activates plants' cells but also improves their disease and insect resistance ability" [2-3]. "The application of chitosan in agriculture, even without chemical fertilizer, can increase the microbial population by large numbers and transform organic nutrient into inorganic nutrient, which is easily absorbed by the plant roots"

[4]. “Furthermore, plants treated with chitosan may be less prone to environmental stress such as drought, salinity, and temperature” [5-6].

At the moment, consumers are looking for more natural, safe foods that are good in quality, have a long shelf-life, and do not include any artificial preservatives [7]. “Therefore, chitosan is widely regarded not only as a promising and cost-effective crop protection material but also as an environmentally friendly, biocompatible, and biodegradable polymer” [8-9] “with a wide range of applications in different fields of agriculture, viz. crop production and protection” [10-13], and storage [14-16] of different fruits and vegetables, etc.

“Amaranth has two morphological types (morphs), and red amaranth (*Amaranthusgangeticus*L.) is one of them. It can be grown throughout the year and harvested quickly, although the quality is best as vegetables when cultivated in the winter. Red amaranth is a popular vegetable in Bangladesh since it is both nutritious and delicious. Red amaranth is an excellent source of nutrients, antioxidant pigments, minerals, and phytochemicals, viz. phenolics, betalains, flavonoids, carotenoids, chlorophyll, vitamin C, and carotene” [17-18]. In addition, the leaves of red morph amaranth are an excellent source of dietary fiber, carbohydrates, moisture, and protein. These phytochemicals contributed significantly to the antioxidant potentials of red amaranth. As these substances serve as potential antioxidants in our daily diet to achieve nutritional and antioxidant sufficiency, red amaranth could be a potential source of nutrients, antioxidant pigments, minerals, and phytochemicals. However, to our knowledge, there is no systemic research report on the effect of chitosan foliar treatment on red amaranth production. As a result, it will be among the pioneer studies in Bangladesh on using chitosan in producing leafy vegetables. The findings of this study will be used to prescribe a chitosan application dose for red amaranth production in Bangladesh, which will open up new avenues for delivering healthy food while also protecting soil health and the environment as a whole. Furthermore, the research also studies the effect of foliar application of chitosan on growth, yield, and quality attributes (carotenoid, chlorophyll, ash, and major nutrients content) of red amaranth.

2. MATERIAL AND METHODS

2.1 Experimental Site

“The field experiment was carried out at the north-western side of Karim Bhabon, Bangladesh Agricultural University, Mymensingh, during the period from November 2019 to December 2019. Geographically the experimental site is located at 24^{0}75′ N latitude and 90^{0}50′ E longitudes at an elevation of 18 m above sea level” [19].The study region has a humid subtropical environment impacted by the monsoon season. It rains heavily during the monsoon, which starts in May or June and continues till August. During the monsoon, the temperature fluctuates between 25 and 31 °C, while it drops below 15 °C in winter (December and January). The months of April and May have the highest temperatures, which may get as high as 40 °C [20].

2.2Treatments for the Experiment

Chitosan used in the experiment was collected from Research-Lab Fine Chem Industries, Maharashtra, India (CAS No. 9012-76-4; Deacetylation>80%). There were 6 treatments for the experiment, viz. T0 meanscontrol (no chitosan application), while T1, T2, T3, T4 and T5 comprised 100, 150, 200, 250 and 300 mg chitosan L^{-1}, respectively. At first, the measured quantity for the respective concentration of chitosanwas dissolved in a beaker containing about 25 mL of glacial acetic acid and then made a volume of 1.0 L. Then, the pH of the solution was adjusted to 5.0 with 0.1 M NaOH solution. On the other hand, 25 mL glacial acetic acid solution (diluted to 1.0 L with distilled water and pH adjusted to 5.0 with 0.1 M NaOH solution) without chitosan was used as control. Foliar application of chitosan solution was started when the seedlings were at the age of 15 days (i.e., 15 days after sowing, DAS) and continued spraying solution at 7 days intervals up to harvesting (i.e., 43 DAS).

2.3Plant Material and Experimental Design

The experiment was conducted with the seeds of Amaranth cv. BARILalshak-1. The seeds were collected from Bangladesh Agricultural Development Corporation (BADC), Gabtali, Mirpur, Dhaka. The field experiment was laid out in Randomized Completely Block Design (RCBD) with 3 replications. Thus, the total numbers of plots were 18 (6×3). Each plot size was 2.0 m × 2.0 m, i.e., 4.0 m^2.The plot-to-plot distance was 50 cm. There were six rows in each plot, and row to row distance in each plot was 30 cm. Seeds were sown as the broadcast method in each row. After germination, ten (10) healthy seedlings were kept for the study, and others were uprooted.The treatments were randomly distributed to the aforementioned experimental field of the department. The plots were prepared 2-3 days prior to seed sowing by spading soils several times, and all kinds of weeds, stubbles and residues of crops and weeds were removed from the field during the preparation.

2.4Cultivation Practice

Fertilizers were applied in the plot as recommended for the high yield goal and medium soil fertility status as described in Fertilizer Recommendation Guide [21]. The recommended nitrogen, phosphorus, and potassium doses were 65, 15, and 25 kg ha^{-1}, and fertilizer sources were urea, TSP, and MoP, respectively. Among the fertilizers, TSP, MoP, and half of urea were applied to the individual plots during final preparation according to the recommendation. The remaining urea was applied as topdressing at 12 DAS. No manure was used in the field experiment.Intercultural operations viz. weeding, irrigation, disease and pest management were done using traditional methods as and when necessary.

2.5 Chemical Analyses of Red Amaranth

2.5.1 Moisture content

Moisture content in red amaranth was calculated using the following equation, and the obtained results are expressed in percent.

$$Moisture\ (\%) = \frac{[Fresh\ weight\ (g) - Dry\ weight\ (g)] \times 100}{Fresh\ weight\ (g)}$$

2.5.2 Ash content

To measure ash content in red amaranth, a specific amount (weighed quantity) of the oven-dried sample was taken in a porcelain crucible, and pre-ashing of the sample was done by placing the crucible in a muffle furnace maintained at 200^0C temperature for 2-3 hours. The temperature for ashing increased up to 600^0C, and the furnace operated for 4 hours. Then the crucible was cooled and kept in a desiccator for some time and weighed. From the weights recorded above, the percent ash content was calculated as follows-

$$\%\ Ash = \frac{A \times 100}{I}$$

Where, A = weight of ash afterbeing heated in a muffle furnace, and I = Initial weight of the oven-dried pulp.

2.5.3 Carotenoid content

Total carotenoids present in red amaranth were measured by spectrophotometry, following the technique outlined by Yang *et al*. [22] and Branisa*et al*. [23]. A specific amount of sample was ground using a ceramic mortar and pestle using acetone-water mixture (4:1) as a solvent. The extract was filtered through Whatman No. 1 filter paper into a volumetric flask and made up to a volume of 50 mL. Then the extract was stored in the dark until required. Absorbance reading for the clear extract was taken using a spectrophotometer (T60 Visible Spectrophotometer, PG instrument, UK) at 470 nm wavelength. Then the amounts of carotenoids present in red amaranth were calculated using the following equation-

$$Carotenoids\ (\mu g\ mL^{-1}) = \frac{1000 \times A_{470} - 2.27 \times (Chl-a) - 81.4 \times (Chl-b)}{227}$$

Finally, the obtained results were expressed as µg g^{-1} fresh weight of the sample.

2.5.4 Chlorophyll content

The photosynthetic pigment chlorophyll present in red amaranth was measured by spectrophotometry, following the techniques as Sadasivam and Manickam[24] mentioned. A specific amount of sample was ground using a ceramic mortar and pestle containing 90% acetone. The extract was filtered through Whatman No. 1 filter paper into a volumetric flask and made up to a volume of 50 mL. Then the extract was stored in the dark until required. Absorbance reading for the clear extract was taken using a spectrophotometer (T60 Visible Spectrophotometer, PG instrument, UK) at 663 and 645 nm wavelengths. Then the amounts of chlorophyll-a, chlorophyll-b and total chlorophyll present in red amaranth were calculated using the following equations-

$$Chlorophyll-a\ (mg\ g^{-1}\ tissue) = \frac{(12.7 \times A_{663} - 2.69 \times A_{645}) \times V}{1000\ \times W}$$

$$Chlorophyll-b\ (mg\ g^{-1}\ tissue) = \frac{(22.9 \times A_{645} - 4.68 \times A_{663}) \times V}{1000\ \times W}$$

$$Total\ Chlorophyll\ (mg\ g^{-1}\ tissue) = \frac{(20.2 \times A_{645} + 8.02 \times A_{663}) \times V}{1000\ \times W}$$

Where, A = absorbance at a specific wavelength, V = total volume of extract in mL, and W = fresh weight of tissue in g.

2.6 Preparation of Extract of Red Amaranth

"The red amaranth plant extract was prepared by wet oxidation method using the di-acid mixture described" by Singh et al. [25] and used to determine major mineral elements. In this method, 1.0 g of finely ground dried sample was taken into a 250 mL conical flask and 10 mL of the di-acid mixture (HNO_3:$HClO_4$ = 2:1) was added to it. The flask was then put on an electric hot plate and heated at a temperature between 180 and 200ºC until the solid particles disappeared and white fumes evolved from the flask. It was then allowed to cool to ambient temperature, rinsed with distilled water, and filtered through filter paper (Whatman No. 1) into a 100 mL volumetric flask. A blank extract was also prepared for quality control purposes by taking the same reagent without a sample. Finally, the volume was made up to the mark with distilled water and preserved for the determination of different mineral nutrients in the samples.

2.7 Determination of Macro Nutrients

"Among the major mineral nutrient elements, Ca and Mg were determined titrimetrically, P and S were measured spectrophotometrically (660 and 425 nm absorbance wavelength, respectively; T60 UV-Visible Spectrophotometer, PG Instrument, UK), and Na and K were estimated by flame photometrically (589 and 766 nm emission wavelength, respectively; 0.2 µg g^{-1} limit of detection; Jenway PFP7, Flame Photometer, UK)"[25]. The instrumental parameters were adjusted according to the manufacturer's recommendations.However, determinations of Fe and Zn in aqueous extractsof red amaranth were done by an atomic absorption spectrophotometer (AAS) (SHIMADZU, AA-7000; Japan).At first, the AAS was calibrated, followed by the manufacturer's recommendation, and the extract was run directly to determine the metal. A hollow cathode lamp of Zn and Fe was employed as a light source at wavelengths of 213.9 and 248.3 nm, respectively, for the determination of each metal.

2.8 Data Collection and Statistical Analysis

Data on plant height and number of leaves $plant^{-1}$ were recorded at 15, 22, 29, 36 and 43 days after seed sowing (DAS). In addition, stem diameter, root length and yield data were measured from each plot at harvesting, and then the average data were used in this study. Obtained data were analysed statistically and the mean differences of the treatments were adjusted by the least significant difference (LSD) test with the help of the computer package M-STAT.

3. RESULTS AND DISCUSSION

3.1 Effect of Chitosan on Agronomic Characteristics of Red Amaranth

3.1.1 Plant height

"Present study results revealed that plant height was greater in chitosan applied red amaranth plants than in control plants. However, there were no significant variations among the treatments at different DAS. The highest plant height was obtained from the T5 treatment (54.87 cm), while the control plant produced the shortest plant height (46.12 cm) at harvesting (Table 1). Many researchers reported a similar result at home and abroad"[26-29], and "they described that foliar application of chitosan at the early growth stages of tomatoes increased plant height. Chitosan has been reported as a high-potential biomolecule that had molecular signals that served as plant growth promoters"[30]."Recently, it has been reported that the stimulating effect of chitosan on plant growth might be attributed to an increase in key enzymes activities of nitrogen metabolism (nitrate reductase, glutamine synthetase, and protease) and improved the transportation of nitrogen in the functional leaves, which enhanced plant growth and development"[30].

3.1.2Number of leaves $plant^{-1}$

"Effects of foliar application and different concentrations of chitosan on the number of leaves $plant^{-1}$ of red amaranth at different DAS are presented in Table 1. There were insignificant variations among the treatments ondifferent days after sowing except for 43 DAS. The maximum number of leaves $plant^{-1}$ of red amaranth was obtained from the T4 and T5 treatments (15.65) at harvesting, while the treatment T0 produced the minimum number of leaves $plant^{-1}$ of red amaranth (14.90 cm). Thus, it can be inferred from the present study results that the number of leaves of red amaranth is positively affected by the foliar application of chitosan. The result obtained from the present study is consistent with the result” of Islam et al. [31], who stated that “leaf number in tomato plants increased with the application of chitosan than in control plants. The chitosan-treated plants had 35.1% more leaves than the control plants”[32]. Similar results were also reported by Boonlertnirun et al.[33] and Mondal et al. [34] in rice and mungbean, respectively.

Table 1: Effect of foliar application of chitosan on plant height, number of leaves plant^{-1}, stem diameter and root length of red amaranth at different days after sowing (DAS).

Treatments	Plant height (cm)					Number of leaves plant^{-1}					Stem diameter at 43 DAS (cm)	Root length at 43 DAS (cm)
	15 DAS	22 DAS	29 DAS	36 DAS	43 DAS	15 DAS	22 DAS	29 DAS	36 DAS	43 DAS		
T0	4.51	9.53	21.21	38.10	47.04	5.00	7.65	9.45	13.15	14.90c	0.68c	14.38c
T1	4.83	9.86	21.42	38.18	46.12	5.05	7.70	9.85	13.35	15.00bc	0.69bc	15.60bc
T2	5.17	9.72	23.57	39.80	48.53	5.05	7.25	9.50	13.25	15.15b	0.80ab	17.63ab
T3	5.03	10.06	22.73	39.35	47.47	5.10	7.40	9.65	13.40	15.45a	0.74bc	17.04ab
T4	5.41	11.81	25.20	42.68	51.20	5.30	7.55	9.75	13.35	15.65a	0.89a	17.30ab
T5	5.26	12.14	26.72	46.22	54.87	5.45	7.90	10.00	13.65	15.65a	0.89a	18.16a
SE±	0.13	0.47	0.88	1.29	1.34	0.07	0.09	0.09	0.07	0.13	0.039	0.578
CV (%)	0.06	0.11	0.09	0.08	0.07	0.03	0.03	0.02	0.01	0.02	0.12	0.08
Level of Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	**	**

** indicating significant at P ≤ 0.01, * means significant at P ≤ 0.05, and NS means non-significant.

3.1.3Stem diameter

The effect of foliar application and different concentrations of chitosan had a highly significant positive impact on the stem diameter of red amaranth. The average stem diameter of red amaranth ranged from 0.68-0.89 cm (Table 1). The highest stem diameter of red amaranth was obtained from the treatment T4 and T5 (i.e., foliar application of chitosan at 250 mg L^{-1} and 300 mg L^{-1}, respectively). In contrast, the lowest stem diameter of red amaranth (0.68 cm) was obtained from the T0 treatment (control). So, it can be concluded from the present study results that the stem diameter of red amaranth is positively affected by the foliar application of chitosan. A similar positive influence of chitosan on plant vegetative features was observed in multiple genera from the family*Orchidaceae*, such as *Cymbidium* by Nahar et al. [35] or *Dendrobium* by Tantasawat et al. [36].

3.1.4Root length

“The effect of foliar application and different doses of chitosan on the root length of red amaranth was statistically significant at a 1% probability level. The highest root length of red amaranth (18.16 cm) was obtained from the treatment T5 (i.e., foliar application of chitosan at 300 mg L^{-1}), followed by the T2 (17.63 cm), T4 (17.30 cm), and T3 (17.04 cm) treatments.

Maize (*Zea mays* L.) plants treated with Cu-chitosan nanoparticles showed enhanced root length and number in both pot and field conditions"[37]. Similarly, González Gómez et al. [38] reported that "chitosan-polyvinyl alcohol hydrogels with absorbed copper nanoparticles increased the root length of grafted watermelon". Tsugita et al. [39] suggested that "the application of chitosan in daikon radish triggered the growth of roots and shoots. Thus, it can be inferred that the foliar application of chitosan influence positively increases the root length of different crops".

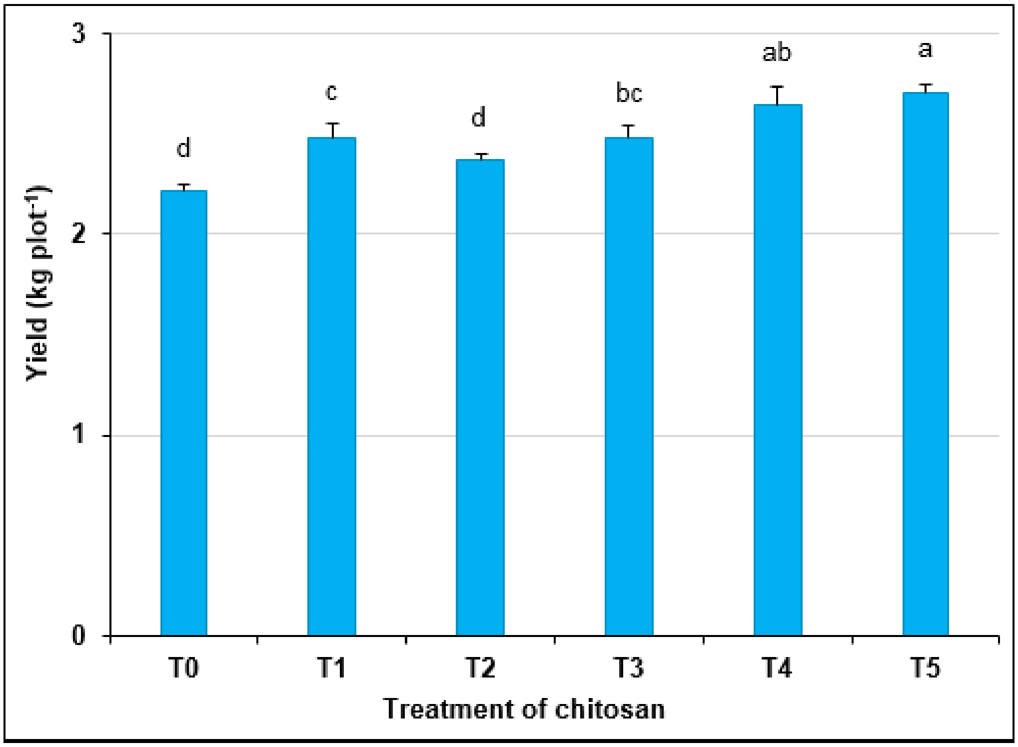

Figure 1: Effect of foliar application of chitosan on yield of red amaranth. Each value is the mean for three replicates, and the vertical bar indicates the standard error. Different letters are indicating the least significant difference (LSD) at P-value ≤ 0.05.

3.1.5 Totalyield

The effect of different doses of chitosan foliar application on the red amaranth yield was statistically significant at a 5% probability level. The average yield of red amaranth ranged from 2.22-2.71 kg $plot^{-1}$ (Fig. 1). The highest yield of red amaranth was obtained from the treatment T5 (i.e., foliar application of chitosan at 300 mg L^{-1}), followed by T4 (2.65 kg $plot^{-1}$), T3 (2.49 kg $plot^{-1}$) and T1 (2.48 kg $plot^{-1}$). On the other hand, the lowest yield of red amaranth was obtained from the control treatment. Similarly, several studies reported that foliar spraying of chitosan has a significant positive effect on yield and yield contributing characteristics of tomato [26-27], maize [37], and watermelon [38]. Furthermore, combining chitosan and plant probiotics enhanced the growth and yield of strawberries and bell peppers [40-41].Moreover, an increase in the weight of fresh fruit and yield of the kiwi was observed after spraying with chitosan in field conditions [42]. A positive effect on grain yield was also observed after using chitosan-silicon nano-fertilizer on maize [43]. Studies on wheat also showed that the foliar application of nano chitosan NPK fertilizer enhances yields

[44].Therefore, it can be concluded that foliar application of chitosan increased the yield of different crops.

3.2 Effect of Chitosan on Biochemical Components of Red Amaranth

3.2.1 Moisture content

The effect of foliar application of chitosan had no significant effect on the moisture content of red amaranth. The average moisture content in red amaranth varied from 89.74-90.81% (Table 2). The highest moisture content of red amaranth was obtained from treatment T5,followed by T4, T2, and T0 (control), while the lowest amount was obtained from the T3 treatment. Such types of variation in moisture content of red amaranth might be due to stage of maturity, size and environmental factors. However, it is evident from Table 2 that moisture content in red amaranth did not affect by the foliar application and different concentrations of chitosan.

Table 2: Effect of foliar application of chitosan on different biochemical qualities of red amaranth

Treatments	Moisture Content (%)	Chlorophyll-a (mg g^{-1} tissue)	Chlorophyll-b (mg g^{-1} tissue)	Total chlorophyll (mg g^{-1} tissue)	Carotenoid (µg g^{-1}fresh wt.)	Ash content (%)
T0	90.25	6.02b	3.67b	9.68b	0.20b	16.36b
T1	89.94	9.20a	5.53a	14.73a	0.27a	17.20ab
T2	90.33	9.23a	5.92a	15.15a	0.26a	16.54ab
T3	89.74	7.96a	4.72ab	12.69a	0.26a	17.11ab
T4	90.64	8.15a	4.54ab	12.19ab	0.25ab	16.95ab
T5	90.81	9.51a	4.75ab	12.76a	0.28a	17.69a
SE±	0.166	0.532	0.323	0.802	0.011	0.196
CV (%)	0.01	0.16	0.16	0.15	0.10	0.03
Level of Significance	NS	**	*	*	**	*

** indicating significant at P ≤ 0.01, * means significant at P ≤ 0.05, and NS means non-significant.

3.2.2 Chlorophyll content

Red amaranth is a good source of chlorophyll. Present study results found the average chlorophyll-a, chlorophyll-b, and total chlorophyll content of red amaranth ranged from 6.02-9.51, 3.67-5.92 and 9.68-15.15 mg g^{-1} tissue, respectively (Table 2). The effect of foliar application and different doses of chitosan on different types of chlorophylls of red amaranth were statistically significant.However, it can be seen from Table 2 that there were some inconsistencies in the obtained results, which might be due to stage of maturity, size and environmental factors. In experiments conducted in indoor climate-controlled chambers, rice plants soaked and sprayed with 0.05% chitosan showed a significant increase in photosynthesis and biomass of rice under elevated ozone conditions[45].Similarly, foliar application of chitosan could alleviate the toxic effects of cadmium (Cd) on the growth and leaf chlorophyll content of edible rape (*Brassica rapa* L.)[46]. In addition, chitosan nanoparticles combined with gibberellic acid significantly increased leaf area and the levels of chlorophylls in *Phaseolus vulgaris*[47]. These results also support the findings of the present study. Similarly, a research study conducted under moderate and severe drought conditions showed that wheat treated with chitosan achieved higher values of total chlorophyll as well as total carotenoid concentration [48]. In studies on rice under drought conditions, an improvement in chlorophyll a and b were obtained for plants treated with chitosan [49]. Moreover, after the application of chitosan at concentrations of 0.01 to 0.12%, an increase in chlorophyll a and b content was recorded in maize [37]. Similarly, according to Abdallah et al. [50], chitosan-treated wheat plants recorded higher chlorophyll a and b contents than the controlunder salinity stress.

3.2.3 Carotenoids content

Carotenoids are yellow, orange, and red organic pigments that are found in different fruits and vegetables. The foliar application of different concentrations of chitosan had a significantpositive effect on carotenoid contents of red amaranth.The highest amount of carotenoids in red amaranth (0.28 µg g^{-1} fresh wt.) was obtained from treatment T5, while the lowest amount of carotenoids in red amaranth (0.20 µg g^{-1} fresh wt.) was obtained from the control plant (Table 2). Thus, similar to chlorophyll content, it can be inferred from the present study results that the contents of carotenoids in red amaranth increased by the foliar application of chitosan at different doses. Pereira et al. [47] also stated that the application of chitosan nanoparticles significantly increased the amount of carotenoids in *Phaseolus vulgaris*. Furthermore, it has been reported that total polyphenols in several fruits had increased due to chitosan-coating that activated the key enzyme such as phenylalanine ammonia-lyase (PAL) in the phenol synthesis pathway[51]. Similarly, chitosan application consistently produced remarkably higher levels of total flavonoids and phenolics in strawberry fruit [52].

3.2.4 Ash content

Ash content represents the total mineral content in any food item. Present study results revealed that the effect of foliar application and different doses of chitosan on theash content of red amaranth was statistically significant at a 5% level of probability (Table 2). The highest amount of ash (17.69%) was found at T5 (i.e., foliar application of chitosan at 300 mg L^{-1}) treatment, followed by T1 (17.20%), T3 (17.11%) and T4 (16.95%) treatments. On the other hand, the lowest amount of ash (16.36%) was obtained from the control treatment. Thus, it can be inferred from this study's results that the application of chitosan also increased ash content in red amaranth.

3.3 Effect of Chitosan on Major Nutrient Contents of Red Amaranth

Mineral nutrients are essential for human nutrition. However, there is a minimal number of research found in the literature which shows the effect of foliar application of chitosan on major nutrient elements. To the best of our knowledge, this study is one of few reports that demonstrated the effect of foliar application of chitosan biopolymer on major nutrient elements of any crop/vegetables grown in the field in a dose-dependent manner. However, the present study measured 8 (eight) major nutrient elements in red amaranth: Ca, Mg, Na, K, P, S, Zn, and Fe. However, the effects of foliar application of chitosan at different concentrations on these nutrient elements of red amaranth are presented in Table 3. Among these nutrient elements, the amount of Ca, Mg, P, Na, and K in red amaranth varied from 2.17-2.49, 0.60-0.85, 0.56-0.61, 0.98-1.05, and 2.97-3.26%, respectively (Table 3).

However, the study results revealed that the effect of different doses of foliar application of chitosan had no significant impact on the nutrient contents of red amaranth.

Table 3: Effect of foliar application of chitosan on major nutrient contents of red amaranth

Treatments	Ca (%)	Mg (%)	P (%)	Na (%)	K (%)	S (%)	Zn (µg g^{-1})	Fe (µg g^{-1})
T0	2.17	0.85	0.60	1.02	2.97	0.84b	12.36a	771.27e
T1	2.49	0.60	0.59	1.05	3.15	0.75b	6.29bc	791.82d
T2	2.29	0.61	0.60	1.05	3.04	0.77b	7.41bc	799.23cd
T3	2.47	0.64	0.56	0.99	3.02	0.89ab	5.96c	802.63c
T4	2.36	0.68	0.56	0.99	3.14	0.87ab	8.79b	823.17b
T5	2.45	0.65	0.61	0.98	3.26	0.99a	8.45bc	846.43a
SE±	0.05	0.04	0.01	0.01	0.04	0.03	0.66	11.02
CV (%)	0.05	0.13	0.04	0.03	0.03	0.10	0.28	0.08
Level of significance	NS	NS	NS	NS	NS	*	**	**

** indicating significant at $P \leq 0.01$, * means significant at $P \leq 0.05$, and NS means non-significant.

On the contrary, the effect of foliar application and different levels of chitosan on S, Zn, and Fe contents of red amaranth were statistically significant among the treatments. The highest amount of S was found at T5 treatment (0.99%). On the other hand, the lowest amount of S was obtained from the T1 treatment, which was statistically similar to T2 and control treatments. Similar to S, there were significant positive variations in Fe content of red amaranth among the treatment combinations due to the effect of foliar application of chitosan (Table 3). The Fe content in red amaranth ranged from 771.27-846.43 µg g^{-1}. The highest amount of Fe was obtained from the T5 treatment, while the lowest amount was found in T0 (control). It has also been reported that the application of chitosan positively affected major nutrient elements of tomato fruits [26]. However, the Zn content in red amaranth ranged from 5.96-12.36 µg g^{-1} (Table 3). The highest amount of Zn was obtained from treatment T0 (control). On the other hand, the lowest amount of Zn was found in T3. It is evident from Table 3 that all chitosan treatments had a significant negative effect on the total Zn content of red amaranth. Thus, the current study suggests pinpointing an investigation to explore the reasons behind such effects of chitosan treatment.

4. CONCLUSION

This research work was done to study the effect of foliar application of chitosan on growth, yield, and quality attributes of red amaranth, as well as to find a suitable dose of chitosan for foliar application. The study results summarized that foliar application of chitosan at 300 mg L^{-1} has a significant positive effect on the growth, yield, and biochemical characteristics of red amaranth. However, before the final recommendation of the application dose of chitosan, further study is needed in different years and agro-ecological zones of Bangladesh. Furthermore, various types of chitosan from different manufacturers have varying physicochemical properties and perhaps from multiple sources. Thus, the proper initiative is required to ensure the supply of quality products with consistent properties and nominal prices.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

'Author HN' conducted field experiment, collected samples, performed analysis,collect data and wrote the first draft of the manuscript. 'Author HMZ' designed the study,supervise the work, and corrected the final draft of the manuscript.'Author NAN and NRP' helped in field experiment, data collection and analysis.'Author QFQ' helped to design the study and corrected the final draft of the manuscript. All authors read and approved the final manuscript.

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