Original Research Article

Growth, yield and biochemical qualities of spinach *(Spinacia oleracea)*as influenced by the foliar application of chitosan

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ABSTRACT

TheA(remove the A) study was carried out to evaluate the effects of various doses of foliar application of chitosan on spinach growth, yield, and quality characteristics (including chlorophyll-a, chlorophyll-b, total chlorophyll, carotenoids, total ash, and main nutrient contents).The Randomized Completely Block Design (RCBD) was used to set up the field experiment, with six treatment combinations [T0 = Control (no chitosan application), T1 = 100 ppm chitosan solution, T2 = 150 ppm chitosan solution, T3 = 200 ppm chitosan solution, T4 = 250 ppm chitosan solution, and T5 = 300 ppm chitosan solution] and three replications. The results revealed that applying chitosan at different doses significantlyaffected growth (viz., plant height, the number of leaves plant^{-1}) and yield of spinach. Moreover, due to the application of chitosan at various doses, there were notable differences observed in chlorophyll, total phenol, and ash contents of spinach.The spinach sample contained an average of 13.61–15.82 mg g^{-1} of chlorophyll–a, 9.16–10.18 mg g^{-1}of chlorophyll–b, and 22.77–25.85 mg g^{-1}of total chlorophyll, respectively.On the contrary, the effect of foliar application of chitosan at different doses showed a statistically significant difference in the P, K, Zn, and Fe contents of spinach. The highest amounts of chlorophyll-a, total ash, P, and K in spinach were obtained from treatment T3. Finally, according to the study's findings, foliar application of chitosan at the rate of 200 ppm significantly affects the growth, yield, pigments, total ash, P, and K contents of spinach.

Keywords: Chitosan dose, chlorophylls, total phenols, ash content and nutrient elements

1. INTRODUCTION

Chitosan is derived from chitin, known as a natural biopolymer, that can induce plants to produce defense enzymes (chitosanases) with antimicrobial activity [1]. It is a sustainable and environmentally beneficial molecule due to its low cost of manufacturing and other biological qualities, including non-toxicity, biocompatibility, and biodegradability [2-4]. Chitosan used in agriculture can dramatically increase the microbial population, also converting organic nutrients into inorganic minerals that are quickly absorbed by the plant roots [5]. Furthermore, Chitosan has a broad range of applications in agriculture, including crop protection, storage, and nutritional value. As a bioactive fungicide, chitosan has also been widely used in the past [6-8].However, chitosan treatments may make plants more resistant to environmental stresses like heat, salinity, and drought [9-11] and also able to prevent the growth of several pathogenic bacteria, viz. *Xanthomonas* [12], *Pseudomonas syringae*[13], *Agrobacterium tumefaciens* and *Erwinia carotovora*[14].

The leafy green vegetable spinach *(Spinacia oleracea)*, native to central and western Asia, is a member of the *Amaranthaceae* family and subfamily *Chenopodioideae*. Spinach leaves are a popular edible vegetable that can be eaten raw or cooked after being preserved through canning, freezing, or dehydration. Green leafy vegetables like spinach are abundant in nutrients and belong to a large group of vegetables known as "nature's anti-aging wonders" and have therapeutic benefits [15]. According to Bose *et al*.,[16], the plant is reported to contain1.9% protein, 3.0% carbohydrates, 1.4 mg of iron per 100 grams, 3250 IU of vitamin A per 100 grams, and 0.15% calcium. In addition, traditional uses for spinach leaves include cooling, emollient, wholesome, antipyretic, diuretic, laxative, digestible, lungs and bowel inflammation, sore throat, joint pain, thirst, sore eye, ringworm, scabies, arrest vomiting, biliousness, flatulence, and febrile [17-18]. In the food and pharmaceutical industry, spinach extract can be utilized as a natural antibacterial and preservative [19]. However, to our knowledge, there has yet to be a systemic research report on the effect of chitosan foliar treatment on spinach production. There are published reports on the effect of chitosan application on the production of red amaranth [20] and tomato [21] in Bangladesh condition. The results of this study will be utilized to recommend a chitosan application dosage for spinach production in Bangladesh, creating new possibilities for supplying nutrient-dense food while simultaneously preserving soil health and the environment as a whole. Furthermore, the research also studies the effect of foliar application of chitosan on the growth, yield, and some biochemical quality attributes,viz., total phenolics, chlorophylls, ash, and major nutrients content of spinach.

2. MATERIAL AND METHODS

2.1 Experimental Site

The research was conducted in the northwest side of Karim Bhabon, Bangladesh Agricultural University, in Mymensingh, between November to December 2020. The experimental site is situated 18 meters above the sea level at 24043′20′ N latitude and 90026′32′ E longitudes [22].

2.2 Treatments for the Experiment

Chitosan was applied to the foliar parts of spinach plants. The treatment combinations for the experiment were, T_0 = Control (no chitosan application), T_1 = Foliar spraying of chitosan (@ 100 ppm & at 7 days interval), T_2 = Foliar spraying of chitosan (@ 150 ppm & at 7 days interval), T_3 = Foliar spraying of chitosan (@ 200 ppm & at 7 days interval), T_4 = Foliar spraying of chitosan (@ 250 ppm & at 7 days interval) and T_5 = Foliar spraying of chitosan (@ 300 ppm & at 7 days interval).

2.3 Plant Material and Experimental Design

The experiment was carried out using spinach seed (Kupipalong- *Spinacea oleracea*). The seeds were obtained from the Seed Testing Laboratory of Bangladesh Agricultural Development Corporation (BADC), Beej Bhaban, Gabtoli, Mirpur-1216, Dhaka. A Randomized Completely Block Design (RCBD) was used to set up the field experiment with three replications. Thus the total numbers of plots were 18 (6×3). Each plot has dimensions of 2.0 m by 2.0 m, i.e., 4.0 m^2. In the aforementioned experimental field of the department, the treatments were dispersed at random. Then the research field was prepared by spading the soil to eliminate all types of weeds, stubbles, and crop residues.

2.4 Cultivation Practice

Fertilizers are supplied to the plot as recommended by the Fertilizer Recommendation Guide for high-yield goals and low soil fertility status [23]. The recommended dose of nitrogen, phosphorus, and potassium are 60.0 kg ha^{-1}, 18.0 kg ha^{-1}, and 18.0 kg ha^{-1}, respectively. Fertilizers such as urea, TSP, and MoP were used as sources for N, P, and K, respectively. Among the fertilizers, TSP, MoP, and gypsum were applied to the individual plots during final land preparation according to the recommendation. Urea as the source of nitrogen was applied as topdressing in two installments at 21 and 42 days after sowing (DAS) under moist soil conditions. No manure was used in the experiment.

2.5 Chemical Analyses of Spinach

2.5.1 Moisture content

The following equation was used to determine the moisture content in spinach, and the results are presented as percentages.

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

2.5.2 Ash content

A precise amount (weighed quantity) of the oven-dried sample of spinach was placed in a porcelain crucible, and the sample was pre-ashed by placing the crucible in a muffle furnace kept at 200ºC temperature for 1-2 hours. Up to 600ºC was reached for ashing, and the furnace ran for four hours. The crucible was then cooled, dried out for a while, and weighed. The percentage of ash content was computed using the weights listed above.

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

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

2.5.3 Chlorophyll content

By using the methods described by Sadasivam and Manickam [24], the photosynthetic pigment (chlorophyll) contained in spinach was quantified. About 90 percent acetone was added to a ceramic mortar and pestle, which was used to grind a precise quantity of sample. The extract was put into a volumetric flask and filled to a capacity of 50 mL after being filtered through Whatman No. 1 filter paper. The extract was then kept in the dark until it was needed. A spectrophotometer (T60 Visible Spectrophotometer, PG Instrument, UK) was used to measure the absorbance of the clear extract at 663 and 645 nm wavelengths. The following equations were used to determine the amounts of chlorophyll-a, chlorophyll-b, and total chlorophyll present in spinach-

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

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

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

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

2.5.4 Phenol content

According to Sadasivam and Manickam [24], the Folin-Ciocalteau reagent was used to estimate the total phenol in spinach. The concentration of phenols in the spinach was calculated against the catechol standard curve and expressed as mg phenols/100 g material.

2.6 Preparation of Extract of Spinach

The spinach plant extract was prepared using a di-acid mixture of the wet oxidation process described by Singh et al. [25]. In this method, exactly 1.0 g of dried and finely ground spinach plant samples were taken into a 250 mL conical flask and 10 mL of the di-acid mixture (conc. HNO_3: $HClO_4$ = 2:1) was added to it. Then the flask was placed on an electric hot plate for heating at 180-200 ºC temperature until the solid particles disappeared and white fumes evolved from the flask. Then, it was cooled at room temperature, washed with distilled water, and filtered into 100 mL volumetric flasks through filter paper (Whatman No. 1). Finally, the volume was made up to the mark with distilled water. This solution was used for the determination of different nutrient elements present in samples of the spinach seedlings.

2.7 Determination of Major Mineral Elements

Among the major mineral nutrient elements, Calcium and magnesium concentrations of plant samples were determined by the complexometric method of titration. The P and S contents in plant samples were determined colorimetrically using a spectrophotometer (Model: TG-60 U) 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]. However, determinations of Zn and Fe in aqueous extracts of spinach were done by an atomic absorption spectrophotometer (AAS) (SHIMADZU, AA-7000; Japan). After the manufacturer's recommendation, and the AAS calibration, the extract was run straight to identify the metal. A hollow cathode lamp built for each of Zn and Fe was utilized to determine the composition of each metal, with light sources at wavelengths of 213.9 and 248.3 nm, respectively.

2.8 Data Collection and Statistical Analysis

Data on plant height and number of leaves $plant^{-1}$ were recorded at 15, 30, 40, 50, and 60 days after seed sowing (DAS). Additionally, each plot's root length and yield data were measured at harvest (60 DAS), and the average data were then used in this study. The data were statistically analyzed using the computer program M-STAT, and the least significant difference (LSD) test was employed to adjust the mean treatment differences.

3. RESULTS AND DISCUSSION

3.1 Effect of Chitosan on Agronomic Characteristics of Spinach

3.1.1 Plant height

The experiment results inferred that spinach plant height was greatly influenced by the application of chitosan than in control plants. However, there were significant variations among the treatments at different DAS. The highest plant height was obtained from the T5 treatment (47.45, 175.70, 325.35, 452.60 and 470.10 cm at 15, 30, 40, 50, and 60 DAS, respectively), while the control plant produced the shortest plant height at most cases (Table 1). Numerous studies both domestically and abroad reported a similar outcome [20–21, 26], and they stated that adding chitosan to tomato plants' and red amaranths' leaves during their early growth stages increased plant height. Chitosan is a high-potential biomolecule with molecular signals that promote plant development [27]. According to recent research, nitrate reductase, glutamine synthetase, and protease activity increases and improved nitrogen transportation in functional leaves may be responsible for the stimulating effect of chitosan on plant growth [27].

3.1.2 Number of leaves plant^{-1}

The effects of foliar application and different chitosan concentrations on the quantity of spinach leaves at various DAS are shown in (Table 1).The maximum number of leaves plant^{-1} of spinach at 60 DAS was obtained from the T4 treatment (14.70), while the treatment T0 (control) produced the minimum number of leaves plant^{-1} (13.57). As a result, it can be concluded from the findings of the current study that the foliar application of chitosan has a positive impact on the quantity of spinach leaves.The outcome of the current study is consistent with those of Nasrin *et al.*[20] and Islam et al. [28], who found that the application of chitosan enhanced the number of leaves onred amaranth and tomato plants relative to control plants.Boonlertnirun *et al.*[4] and Mondal et al. [29] showed similar findings for rice and mungbean, respectively. According to Salachna and Zawadzinska [30], the chitosan-treated plants had 35.1% more leaves than the control plants.

4.1.3 Root length

Foliar application and different concentrations of chitosan had no significant effect on the root length of spinach. The highest root length (21.20 cm) was recorded from the treatment T0 (control) followed by the treatments T4 (21.05 cm), T1 (20.81 cm), and T2 (20.75 cm). On the other hand, the lowest root length was obtained from the treatment T3 (19.54 cm) (Table 1). A similar result was also reported by Razavizadeh and Adabavazeh [31]. On the contrary, several studies reported a positive effect, in both pot and field conditions, viz., Nasrin *et al.*[20] reported that the highest root length (18.16 cm) of red amaranth was obtained from the foliar application of chitosan @ 300 ppm, while the lowest root length (14.38 cm) was recorded from the control plant. Similar to this, González Gómez et al. [32]found that the root length of grafted watermelon was increased by chitosan-polyvinyl alcohol hydrogels with absorbed copper nanoparticles. Therefore, it can be concluded that foliar chitosan application influences positively increase the root length of various crops.

4.1.4 Total yield

The effect of foliar application and different doses of chitosan on the yield of spinach was statistically significant at a 0.1% level of probability. The average yield of spinach ranged from 7.53-10.28 kg plot^{-1}. The highest yield of spinach was obtained from the treatment T3 (i.e., foliar application of chitosan @ 200 ppm), followed by T4 (9.90 kg plot^{-1}), T5 (9.03 kg plot^{-1}) and T2 (8.85 kg plot^{-1}). On the other hand, the lowest yield of spinach (7.53 kg plot^{-1}) was obtained from the control treatment (Table 1). Several studies reported that foliar spraying of chitosan has a significant positive effect on yield and yield-contributing characteristics of red amaranth and tomatoes [20, 21, 28]. Similarly, numerous studies have found that spraying chitosan onto plant leaves significantly improves yield and yield-contributing traits in maize [33], and watermelon [32]. Furthermore, after spraying chitosan in field conditions, an increase in the weight of fresh fruit and the yield of the kiwi was reported by Scortichini[34]. Following the application of chitosan-silicon nano-fertilizer to maize, a favorable impact on grain yield was also noted by Kumaraswamy*et al.*[35]. So, it can be inferred that the different doses of foliar applications of chitosan increase the yield of different crops including spinach.

Table 1: Effect of different doses of foliar application of chitosan on plant height, number of leaves plant^{-1}, root length and yield plot^{-1} of spinach

Treatment	Plant height (cm)					Number of leaves plant^{-1}					Root length (cm)	Yield plot^{-1} (kg)
	15 DAS	30 DAS	40 DAS	50 DAS	60 DAS	15 DAS	30 DAS	40 DAS	50 DAS	60 DAS		
T0	35.10c	144.75bc	255.15b	396.05b	442.30ab	3.67c	5.70c	9.80c	11.93	13.57b	21.20	7.53e
T1	37.55bc	138.65c	264.90b	403.60b	428.95b	3.80bc	6.10c	10.10bc	12.50	14.27ab	20.81	8.73d
T2	43.35ab	162.10ab	294.75ab	408.10b	441.85ab	3.80bc	6.90b	10.43a	12.17	14.43ab	20.75	8.85d
T3	43.65ab	146.95bc	263.95b	401.55b	458.65ab	3.93ab	7.33a	10.47a	12.30	14.10b	19.54	10.28a
T4	46.08a	157.10abc	290.45ab	419.35ab	466.80ab	3.87ab	6.90b	10.50a	12.37	14.70a	21.05	9.90b
T5	47.45a	175.70a	325.35a	452.60a	470.10a	4.00a	6.80b	10.30ab	12.20	14.10b	19.99	9.03c
CV (%)	0.115	0.088	0.093	0.050	0.036	0.030	0.091	0.026	0.016	0.014	5.20	1.17
LSD (0.5)	6.53	17.85	39.7	22.00	38.55	1.38	1.13	1.15	5.68	3.31	1.74	0.17
Level of significance	**	**	**	*	*	*	**	*	NS	*	NS	***

***, ** and * indicating significant at P ≤ 0.001, P ≤ 0.01, and P ≤ 0.05, respectively. NS means non-significant.

3.2 Effect of Chitosan on Biochemical Components of Spinach

3.2.1 Moisture content

The moisture content of spinach was significantly impacted by the foliar application of chitosan. In spinach, the average moisture content ranged from 92.69 to 93.42% (Table 2). The T3 treatment (foliar application of chitosan @ 200 ppm) produced spinach contained the maximum moisture, whereas the T0 treatment (control) produced spinach with the lowest moisture content. However,Table 2 makes it clear that foliar applications and various concentrations of chitosan have an impact on the moisture content of spinach.

3.2.2 Chlorophyll content

A good source of chlorophyll is spinach. According to the current findings, chlorophyll-a, chlorophyll-b and total chlorophyll contents in spinach ranged from 13.61–15.82 mg g^{-1} tissue, 9.16–10.18 mg g^{-1} tissue, and 22.77–25.85 mg g^{-1} tissue, respectively. However, Table 2 showed some discrepancies in the results, which may be related to the maturity stage, size, and environmental factors. However, the study found that adding chitosan to spinach leaves significantly increased the amounts of various types of chlorophylls. Similar outcomes were achieved in the case of red amaranth, reported by Nasrin et al. [20]. In experiments conducted in climate-controlled indoor chambers, rice plants treated with 0.05% chitosan through soaking and spraying showed a considerably increased rate of photosynthesis and biomass under high ozone conditions [36]. Additionally, *Phaseolus vulgaris* had significantly more leaf area and chlorophyll levels after being treated with chitosan nanoparticles and gibberellic acid [37]. The outcomes of the present study findings are also supported by these results. Similar to this, wheat treated with chitosan produced increased total chlorophyll and total carotenoid concentrations, according to a study carried out under moderate and severe drought circumstances [38]. Plants treated with chitosan produced more chlorophyll a and b in experiments on rice grown in drought conditions [39]. Furthermore, an increase in the content of chlorophyll a and b was observed in maize following the application of chitosan at concentrations of 0.01 to 0.12% [33]. Similar to this, Abdallah et al. [40] reported that under salinity stress, wheat plants treated with chitosan had higher chlorophyll a and b contents than the control.

Table2: Effect of different doses of foliar application of chitosan on some biochemical qualities of spinach

Treatments	Chlorophyll-a (mg g^{-1})	Chlorophyll-b (mg g^{-1})	Total chlorophyll (mg g^{-1})	Phenol (mg/100g material)	Moisture Content (%)	Ash content (%)
T0	13.61d	9.16e	22.77c	5.28a	92.69c	9.84c
T1	15.66ab	10.18a	25.85a	4.57b	92.94b	11.89ab
T2	15.13c	9.68c	24.81b	3.56c	93.34a	12.54a
T3	15.82a	9.80bc	25.62a	3.53c	93.42a	12.46a
T4	15.14c	9.81b	24.95b	3.40c	92.86b	10.61c
T5	15.27bc	9.42d	24.69b	2.66d	92.89b	10.70bc
CV (%)	1.76	0.72	1.02	5.32	0.06	6.59
$LSD_{0.05}$	0.483	0.126	0.462	0.370	0.087	1.22
Level of Significance	***	***	***	***	***	**

*** and ** indicating significant at P ≤ 0.001 andP ≤ 0.01, respectively.

3.2.3 Total phenol content

The data obtained for the total phenol content in spinach after harvesting showed statistically significant variations at a 0.10% level of probability because of the foliar application of chitosan (Table 2). The range of total phenol in spinach was 2.66-5.28 mg $100g^{-1}$ fresh sample. It is apparent from Table 3 that the highest amount of total phenol (5.28 mg $100g^{-1}$) in spinach was obtained from treatment T0 (control). On the other hand, the lowest amount of phenol in spinach (2.66 mg $100g^{-1}$) was obtained from the T5 treatment. The study results revealed that total phenolic contents in the spinach decreased significantly by applying chitosan. Phenolic compounds play an important role at a physiological level, increasing the stress tolerance of plants due to their antioxidant properties and ability to scavenge free radicals, protecting plant cells from the detrimental effects of oxidative stress [41]. However, the response of phenolic compounds to chitosan application depended both on the family of compounds studied and on the concentration of the bio-stimulant [42]. Similarly, Deng *et al.*[43] stated that with higher enzyme (polyphenol oxidase) activity oxidation of phenolics may be accelerated. Thus, it can be inferred that several factors, viz.,crop genotypes, processing methods, environmental conditions, and others play a vital role in the total phenolic contents in different crops including spinach.

3.2.4 Ash content

The inorganic residue that is left behind either after complete oxidation or after the ignition of organic molecules in a food product is referred to as ash. According to Bakkali *et al*. [44], the ash content measures the total quantity of minerals in a diet, whereas the mineral content measures the amount of specific inorganic components, such as Ca, Na, and K, present in a cuisine. The foliar treatment of chitosan had a statistically significant effect on spinach's ash content at a 1.0% level of probability. The maximum amount of ash (12.54%) was produced by the foliar application of chitosan at 150 ppm, which was statistically equal to the applications of chitosan at 200 ppm (12.46%) and 100 ppm (11.89%). On the other hand, the lowest amount of ash (9.84%) was obtained from the control treatment (Table 2). Thus, it can be inferred from this study's results that the foliar application of chitosan increased the ash content in spinach. This result is at par with the finding of Nasrin et al. [20], who stated that foliar application of chitosan at different doses in red amaranth significantly increased the ash content.

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

Treatments	Major nutrient contents in spinach							
	Ca (%)	**Mg (%)**	**P (%)**	**Na (%)**	**K (%)**	**S (%)**	**Zn (µg g^{-1})**	**Fe (µg g^{-1})**
T0	1.52a	0.39a	0.37b	2.05a	2.59b	0.79a	12.73a	597.18f
T1	1.39a	0.31a	0.42ab	2.31a	2.63b	0.84a	10.42bc	657.79d
T2	1.37a	0.46a	0.40ab	2.44a	3.11a	0.81a	9.73c	712.12a
T3	1.26a	0.49a	0.45a	2.38a	2.86ab	0.82a	9.69c	689.37b
T4	1.28a	0.55a	0.43a	2.35a	3.05a	0.97a	10.91b	632.63e
T5	1.33a	0.47a	0.37b	2.52a	2.61b	0.91a	10.47bc	667.69c
CV (%)	14.82	21.13	8.63	8.24	6.43	21.26	6.16	0.37
LSD0.05	0.32	0.17	0.05	0.31	0.29	0.29	1.17	4.46
Level of significance	NS	NS	*	NS	*	NS	***	***

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

3.3 Effect of Chitosan on Major Nutrient Contents of Spinach

For human nutrition, minerals are crucial. The literature only has a very small number of studies that examined the impact of chitosan treatment on the main nutrients. We are aware of very few studies that have shown the influence of the foliar application of chitosan biopolymer on the main nutritional components of spinach grown in the field in a dose-dependent manner. However, the 8 (eight) major nutrients measured in the current study were Ca, Mg, Na, K, P, S, Zn, and Fe. The amounts of Ca, Mg, Na, and S in spinach were statistically insignificant among these nutrient components (Table 3). On the other hand, among the treatments, the impact of various doses of foliar application of chitosan on the Zn and Fe contents of spinach was highly significant (P≤ 0.001). Table 3 also showed that spinach is a very rich source of iron, and adding chitosan to the leaves makes spinach's iron content even higher. For red amaranth, Nasrin *et al.*[20] made a related observation. However, chitosan applied topically reduces the zinc content of spinach, which may be related to the adsorption of zinc metal ions in field soils. According to Seyedmohammadi et al. [45], the use of both macro and nanoparticles of chitosan resulted in the highest removal of zinc ions through adsorption from an aqueous solution at normal temperature. However, Nasrin et al. [20] also reported that foliar application of different doses of chitosan decreases Zn content in red amaranth under field conditions.

4. CONCLUSION

The goal of this study was to determine the best chitosan dose for foliar treatment while also examining how chitosan application affected spinach growth, yield, and quality characteristics. According to the study's findings, spinach's growth, yield, and the majority of its biochemical properties are significantly impacted by the foliar application of chitosan at the rate of 200 ppm. However, additional research in different years and agro-ecological zones of Bangladesh is required before the application dose of chitosan is finally recommended. Furthermore, different brands of chitosan from different suppliers may come from different sources and have different physicochemical properties. To secure the delivery of high-quality goods with reliable characteristics and affordable pricing, the appropriate initiative is necessary.

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