

Effect of Spraying Chitosan and *Spirulina platensis* Extract on the Growth and Flowering of *Dianthus barbatus* L.

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Abstract

This study was conducted in plastic house of Research Station, Department of Horticulture and Landscape Gardening, College of Agriculture, University of Diyala, during the 2025–2026 growing season to evaluate the effect of foliar application of chitosan and *Spirulina platensis* extract on the growth, flowering, and ornamental quality of carnation cv. 'Husan Yousif'. The experiment included three concentrations of chitosan (0, 200, and 400 mg L⁻¹) and three concentrations of *Spirulina* extract (0, 100, and 200 mg L⁻¹) arranged in a factorial experiment according to a Randomized Complete Block Design (RCBD) with three replications. The results showed that foliar application of chitosan at 200 mg L⁻¹ accelerated flowering, reducing the number of days to inflorescence emergence to 67.04 days, whereas chitosan at 400 mg L⁻¹ produced the highest inflorescence diameter (60.68 mm), flowering stem length (32.02 cm), and number of florets per inflorescence (41.91 florets). Likewise, foliar application of *Spirulina* extract at 200 mg L⁻¹ significantly improved flowering characteristics, recording the largest inflorescence diameter (61.44 mm), the longest flowering stem (33.46 cm), the highest number of florets per inflorescence (41.72 florets), and the earliest flowering (65.85 days). The interaction between chitosan at 400 mg L⁻¹ and *Spirulina* extract at 200 mg L⁻¹ produced the best flowering performance, achieving the greatest flowering stem length (38.33 cm) and the highest number of florets per inflorescence (43.25 florets). These findings indicate that the combined application of chitosan and *Spirulina* extract effectively enhances the flowering performance and ornamental quality of carnation plants.

Keywords: Biostimulants, Chitosan, *Spirulina*, Carnation (*Dianthus caryophyllus* L.).

Introduction

Dianthus barbatus L. c.v 'Husan Yousif' is one of the most widely cultivated ornamental flowering plants worldwide due to its remarkable diversity in flower colors and attractive floral displays. The genus *Dianthus* comprises approximately 300 species belonging to the family Caryophyllaceae, with a natural distribution mainly concentrated in the Mediterranean region. It is extensively grown in gardens and landscapes as an ornamental plant.

Plants may reach up to 90 cm in height and are characterized by erect stems and flat, lanceolate leaves with five prominent veins, ranging from 7.5 to 15 cm in length [1]. The flowers are arranged in dense corymbose inflorescences, each cluster bearing numerous flowers of diverse colors. The petals are typically serrated, and flowers occur in both single and double forms, exhibiting a wide spectrum of colors including pink, white, red, purple, and

various mixed color combinations [2]. Chitosan, the principal deacetylated derivative of chitin, is considered one of the most abundant naturally occurring biopolymers. It can be extracted from a wide range of biological sources, including insects, mollusks, fungi, fish, and algae [3]. Numerous studies have highlighted the importance of chitosan as a safe, environmentally friendly, and non-toxic biostimulant that plays a significant role in sustainable agriculture. Its strong metal-chelating capacity enhances its effectiveness in plant and biological applications, while its ability to activate nitric oxide- and hydrogen peroxide-dependent antioxidant systems contributes to improved plant performance under various environmental conditions [4]. Furthermore, chitosan can interact with diverse cellular components, including cell wall constituents, DNA molecules, and specific receptors involved in defense signaling pathways. These interactions stimulate the expression of defense-related genes and promote the synthesis of organic compounds associated with stress adaptation and tolerance, thereby supporting its widespread use as an agricultural biostimulants [5].

Blue-green microalgae, commonly referred to as cyanobacteria, particularly *Spirulina platensis*, have attracted considerable attention owing to their ability to enhance soil fertility, improve crop growth and productivity, and contribute to environmental sustainability. In recent years, their application as plant biostimulants and biofertilizers has increased substantially due to their positive effects on plant development and agricultural productivity [6]; [7]. *Spirulina* is a filamentous, multicellular cyanobacterium belonging to the group of blue-green microalgae. The organism typically reaches approximately 0.5 mm in length and grows optimally under alkaline conditions with a pH range of 8.3–11.0. The name *Spirulina* is derived from its characteristic spiral morphology. As a photosynthetic microorganism, it naturally inhabits alkaline and saline environments rich in bicarbonates and carbonates, particularly within tropical and subtropical regions [8]. *Spirulina* cells reproduce through binary fission and contain chlorophyll pigments similar to those found in higher plants. Although traditionally classified as a microalga because of its photosynthetic capacity and pigmentation,

its prokaryotic cellular organization places it within the bacterial domain. Its biochemical composition is exceptionally rich, containing approximately 55–70% proteins, 15–25% carbohydrates, about 18% essential fatty acids, in addition to substantial quantities of minerals, vitamins, and photosynthetic pigments such as chlorophyll, carotenoids, and phycocyanin [9].

Materials and Methods

The experiment was conducted in a plastic house at the Research Station of the Department of Horticulture and Landscape Gardening, the experimental period extended from December 21, 2025, to May 6, 2026. The study commenced with the sowing of Sweet William seeds, imported from Pan American Seed Company, on October 22, 2025. Seeds were sown in polystyrene seedling trays filled with peat moss, with one seed placed in each cell, and the trays were maintained under greenhouse conditions until transplanting. The growing medium used in experiment consisted of a mixture of soil and peat moss at a ratio of 3:1 (v/v)

Prior to planting, random samples of the growing medium were collected and analyzed for selected physical and chemical properties at the Central Laboratory for Soil, Water, and Plant Analysis, College of Agricultural Engineering Sciences, University of Baghdad. Seedlings were transplanted on December 21, 2025, when they had developed 2–3 true leaves. Transplanting was carried out into plastic pots measuring 24 cm in diameter and 19 cm in height, with one plant per pot. The experiment included three concentrations of chitosan (0, 200, and 400 mg L⁻¹) and three concentrations of *Spirulina* extract (0, 100, and 200 mg L⁻¹). Both chitosan and *Spirulina* extract were applied as foliar sprays on twice. First application was performed when plants reached 4–5 pairs of true leaves, whereas the second application was carried out two weeks after first spray. A two-day interval was maintained between the application of each treatment. The experiment was arranged as a factorial experiment within a Randomized Complete Block Design (RCBD) with three replications. A total of 54 experimental units were included, with each experimental unit consisting of eight pots containing one plant per pot, resulting in a total of 432 plants.

inflorescence using a measuring tape. Measurements were recorded for each plant within the experimental unit, and the mean was calculated.

- 4- **Number of florets per inflorescence:** The total number of fully opened florets per inflorescence was counted for three plants per experimental unit, and the average value was recorded.
- 5- **Inflorescence dry weight (g):** Three inflorescences from each experimental

Data obtained from experiment were subjected to analysis of variance (ANOVA), and treatment means were compared using Duncan's Multiple Range Test at 0.05 probability level.

Experimental Measurements

The following growth and flowering parameters were recorded during the experiment:

- 1- **Days to inflorescence emergence (day):** This parameter was determined by calculating the number of days from transplanting to the appearance of the first inflorescence, and the mean value was subsequently computed.
- 2- **Inflorescence diameter (mm):** The diameter of the inflorescence was measured using a digital Vernier caliper by recording the distance between the two widest points of the inflorescence. Measurements were taken from three plants within each experimental unit, and the average was calculated.
- 3- **Flowering stem length (cm):** The length of the flowering stem was measured from the soil surface of the pot to the base of the unit were harvested and air-dried at room temperature for two weeks. Subsequently, the samples were weighed using a sensitive balance, and the mean dry weight was calculated.
- 6- **Carbohydrate percentage (%):** Total carbohydrate content was estimated following the method described by Herbert et al. (1971). Briefly, 1 g of dried and ground plant material was mixed with 10 mL of distilled water and centrifuged at 3000 rpm for 15 minutes.

The supernatant was collected and the volume was adjusted to 50 mL in a volumetric flask. Then, 1 mL of the diluted extract was mixed with 1 mL of phenol and 5 mL of concentrated sulfuric acid (H_2SO_4). Absorbance was measured at 490 nm using a spectrophotometer, and carbohydrate percentage was calculated using the standard equation.

$$\text{Carbohydrate percentage (\%)} = (\text{concentration} \times \text{dilution} \times 100) / (10 \times 1 \text{ mL} \times \text{sample weight}).$$

7- Vase life (days): Fresh inflorescences were harvested early in the morning at the stage when approximately one-third The containers were kept in a controlled environment chamber at $25 \pm 5^\circ\text{C}$ and $35 \pm 5\%$ relative humidity, using an air-conditioning and hygrometer system. The water was replaced daily. Vase life was recorded as the number of days until the inflorescences lost their visual quality and commercial value.

1- Days to inflorescence emergence (day)

The results presented in table (1) revealed significant differences in response to chitosan application at 200 mg L^{-1} in carnation plants. This treatment significantly accelerated inflorescence emergence, recording 67.04 days compared with control treatment, which recorded 71.33 days. Foliar application of *Spirulina* extract significantly accelerated inflorescence emergence at 200 mg L^{-1} , recording 65.85 days compared with control treatment, which recorded 71.46 days. Results of the two-way interaction between chitosan and *Spirulina* extract revealed significant differences in promoting earlier inflorescence emergence. The combined application of chitosan and

of the florets (8–13 florets) were open. The stems were immediately placed in bunches and transferred to the storage room within one hour. Upon arrival at the laboratory, stems were pre-cooled by placing them in cold water for 30 minutes to reduce field heat effects [11]. Stems were then trimmed to 40 cm length after removing the lower one-third of leaves, ensuring that only healthy and disease-free stems were used. The cut stems were placed in containers filled with 250 mL of distilled water, and a diagonal cut was made at the base of each stem under water.

Spirulina extract at 200 mg L^{-1} resulted in the earliest flowering, recording 61.70 days compared with control treatment, which recorded 73.35 days.

2- Inflorescence diameter (mm)

In table 2 foliar application of chitosan at 400 mg L^{-1} resulted in a significant increase in inflorescence diameter, reaching 60.68 mm compared with 58.03 mm in control treatment. The foliar application of *Spirulina* extract at 200 mg L^{-1} significantly increased inflorescence diameter, reaching 61.44 mm compared with control treatment reaching 57.79 mg L^{-1} . Results of two-way interaction between chitosan and *Spirulina* at 200 mg L^{-1} extract showed significant differences in inflorescence diameter, reaching 62.21 mm compared with control treatment reaching 54.35 mg L^{-1} .

3- Flowering stem length (cm)

Foliar application of chitosan at 400 mg L^{-1} resulted in a significant increase in flowering stem length reaching 32.02 cm

compared with control treatment reaching 28.31cm. Foliar application of *Spirulina* extract at 200 mg L⁻¹ significantly

increased flowering stem length reaching 33.46 cm, compared with control treatment reaching 28.49 cm.

Results of two-way interaction between chitosan and *Spirulina* extract showed significant differences in inflorescence diameter reaching 38.33 cm, compared with control treatment reaching 25.68 cm.

4- Number of florets per inflorescence

Results presented in table (1) revealed significant differences in response to chitosan application at 400 mg L⁻¹ in carnation plants. This treatment significantly number of florets recording 41.91 florets , compared with control treatment which recorded 39.59 florets. Foliar application of *Spirulina* extract significantly number of florets per inflorescence at 200 mg L⁻¹ recording 41.72 florets compared with control treatment which recorded 39.55. Results of the two-way interaction between chitosan and *Spirulina* extract revealed significant differences in number of florets. The combined application of chitosan at 400 mg L⁻¹ and *Spirulina* extract at 200 mg L⁻¹ resulted florets per inflorescence, recording 43.25 florets, compared with control treatment, which recorded 39.37 florets.

Results presented in table (1) foliar application of chitosan did not show any significant effect on studied trait compared with control treatment. Foliar application of *Spirulina* extract significantly Inflorescence dry weight at 100 mg L⁻¹ recording 3.79g, compared with control treatment recorded 3.18 g. Results of two-way interaction between chitosan at 400 mg L⁻¹ and *Spirulina* extract 200 mg L⁻¹ revealed significant differences in inflorescence dry weight recording 4.07g, compared with control treatment which recorded 4.03g.

6- Carbohydrate percentage (%)

Results presented in table (1) foliar application of chitosan did not show any significant effect on studied trait compared with control treatment. Foliar application of *Spirulina* extract significantly carbohydrate percentage at 100 mg L⁻¹ recording 28.87%, compared with control treatment recorded 27.14%. Results of two-way interaction between chitosan at 200 mg L⁻¹ and *Spirulina* extract no sprayind revealed significant differences in inflorescence dry weight recording 30,91% compared with control treatment which recorded 23.16%.

5- Inflorescence dry weight (g)

7- Vase life (days)

Results presented in table (1) foliar application of chitosan did not show any significant effect on studied trait compared with control treatment. Foliar application of *Spirulina* extract significantly vase life at 100 mg L⁻¹ recording 12.94 day, compared

with control treatment recorded 12.22 day. Results of two-way interaction between no spraying chitosan and *Spirulina* extract at 200 mg L⁻¹ revealed significant differences vase life recording 13.67 day, compared with chitosan at 100 mg L⁻¹

treatment and *Spirulina* extract at 200 mg L⁻¹ which recorded 11.50 day.

Table (1): Effect of foliar application of chitosan, Spirulina extract, and their interaction on the growth and flowering of carnation (cv. Husan Yousif)

Characters Treatments		Days to inflorescence emergence (day)	Inflorescence diameter (mm)	Flowering stem length (cm)	Number of florets per inflorescence	Inflorescence dry weight (g)	Carbohydrate percentage (%)	Vase life (days)		
CH0		71.33 a	58.03 b	28.31 b	39.59 c	3.48 a	26.87 a	12.72 a		
CH1		67.04 c	60.47 a	31.21 a	40.65 b	3.38 a	27.62 a	12.50 a		
CH2		68.49 b	60.68 a	32.02 a	41.91 a	3.62 a	27.27 a	12.50 a		
Effect of S										
S0		71.46 a	57.79 c	28.49 b	39.55 b	3.18 c	27.14 b	12.22 b		
S1		69.56 b	59.95 b	29.60 b	39.81 b	3.79 a	25.75 b	12.56 ab		
S2		65.85 c	61.44 a	33.46 a	41.72 a	3.51 b	28.87 a	12.94 a		
Effect of CH and S										
CH0	S0	73.35 a	54.35 c	25.68 c	39.60 b	3.35bc	23.16 d	12.00 cd		
	S1	69.75 b	59.66 ab	28.73 bc	39.37 b	3.81 ab	28.23 ab	12.50 acd		
	S2	70.90 b	60.09 ab	30.53 b	39.80 b	3.27 bc	29.23 ab	13.67 a		
CH1	S0	70.60 b	59.88 ab	31.20 b	40.05 b	3.17 c	30.91 a	13.00 abc		
	S1	68.82 b	59.32 b	30.93 b	39.81 b	3.50 bc	23.97 d	13.00 abc		
	S2	61.70 d	62.21 a	31.50 b	42.09 a	3.48 bc	27.97 abc	11.50 d		
CH2	S0	70.42b	59.15 b	28.60 bc	42.21 a	3.03 c	27.34 bc	11.67 d		
	S1	70.10b	60.86 ab	29.13 bc	40.25 b	4.07 a	25.06 cd	12.17 cd		
	S2	64.95c	62.04 a	38.33 a	43.25 a	3.77 ab	29.42 ab	13.67 ad		

Means with the same letter are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test

The results presented in table 1 indicated that foliar application of chitosan led to earlier flowering and an increase in chlorophyll content. This effect may be attributed to the role of chitosan as a biostimulant that regulates plant

physiological activity by enhancing photosynthetic efficiency, improving mineral nutrient uptake, and modulating endogenous plant hormone levels. Consequently, this accelerates the transition from the vegetative to the reproductive stage [13], in agreement with the findings of [14].

Foliar application of chitosan also resulted in significant differences in flowering stem length, number of florets per inflorescence, inflorescence weight, and diameter. This may be due to its role in stimulating cell division and elongation, as well as activating enzymatic activities [15]. Additionally, chitosan acts as an antioxidant, thereby improving growth characteristics through enhanced photosynthetic activity and increased carbohydrate accumulation. This leads to improved solubility of sugars, which serve as an energy source within the plant, ultimately enhancing organic matter synthesis and increasing the dry weight of the inflorescence. These findings are consistent with those reported in *Strelitzia reginae* L. [16].

number of florets per inflorescence, which is consistent with studies on *Polianthes tuberosa* L. [17] [18]. Furthermore, Spirulina treatment improved chlorophyll content, in agreement with previous findings [19]. Results also indicated an increase in carbohydrate content and inflorescence dry weight, which is consistent with studies on *Zinnia* spp. [20]. Overall, the use of biostimulants such as chitosan and Spirulina extract significantly enhanced the floral traits of carnation cv. Husan Yousif. Chitosan regulates plant physiological growth and improves nutrient uptake, while Spirulina serves as a rich source of essential nutrients that enhance photosynthetic activity. The synergistic effect of these treatments resulted in earlier flowering, increased inflorescence diameter, higher carbohydrate content, and improved vase life.

References

Results also demonstrated that foliar application of *Spirulina* extract in carnation plants significantly improved growth traits due to its rich content of auxins, cytokinins, gibberellins, amino acids, as well as macro- and micronutrients. These components meet the plant's physiological requirements for photosynthesis, respiration, cell division, and elongation. Moreover, they are essential for chlorophyll biosynthesis and stimulate indole acetic acid (IAA) production, thereby enhancing the regulation of photosynthesis and nutrient and water uptake [17]. *Spirulina* extract application also promoted early flowering and increased inflorescence diameter, flowering stem length and

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