

Effect of Extract Concentrations of the Cyanobacterium *Anabaena variabilis* and Application Methods on Growth and Yield of Cucumber Infected with Cucumber green mottle mosaic virus (CGMMV)

¹Zeayd Tareq Alangood & ²Maadh A. Al-Fahad

¹ Department of Plant Protection, College of Agriculture, Tikrit University, Iraq

² Department of Plant Protection, College of Agriculture, Tikrit University, Iraq

¹ Zeadt63@gmail.com & ² maadh.alfahd@tu.edu.iq

Abstract

This study aimed to evaluate the effects of different concentrations (50%, 75%, and 100%) of *Anabaena variabilis* extract and application methods (seed soaking, foliar spraying, and soil drenching) on vegetative growth and yield traits of cucumber (*Cucumis sativus*) plants infected with Cucumber green mottle mosaic virus (CGMMV). The experiment was conducted using a randomized complete block design (RCBD) with three replicates. The selected concentrations were designed to represent a range from moderate to high levels in order to assess the dose–response relationship of the algal extract under viral stress conditions. Growth parameters, including plant height, leaf area, fresh and dry biomass of the vegetative parts, and fruit weight per ten plants at the first and second harvests, were recorded. Results revealed that the 75% concentration consistently produced superior performance across most traits. Plant height and leaf area reached maximum averages of 70.93 cm and 98.83 cm², respectively. Fresh and dry weights of the vegetative biomass peaked at 262.22 g and 79.44 g per plant with soil drenching, while fruit weight per ten plants reached 3436 g at the first harvest and 2429 g at the second harvest when applied via seed soaking. Foliar spraying and seed soaking also showed high efficiency, whereas the lowest values were observed at 50% concentration and in the infected control. Interestingly, the intermediate concentration (75%) outperformed both lower and higher concentrations, indicating that optimal plant responses were achieved at moderate levels, while higher concentrations did not necessarily enhance performance. This suggests that excessive concentrations may not improve efficiency and could potentially limit the positive effects of the extract. The intermediate concentration of *A. variabilis*

appeared optimal for enhancing vegetative growth and yield under viral stress, likely by improving photosynthetic efficiency, nutrient uptake, and delivery of bioactive compounds. Overall, *A. variabilis* extract proved an effective biostimulant for mitigating the negative impacts of CGMMV on cucumber plants. Among the treatments, 75% concentration applied through foliar spraying or seed soaking was the most efficient in promoting growth, biomass accumulation, and fruit production. These findings highlight the potential of cyanobacterial extracts as sustainable biostimulants for enhancing productivity in cucurbit crops under biotic stress conditions.

Keywords:

Cucumber, Viral stress, *Anabaena variabilis*, Biostimulant, Vegetative growth

Introduction

Cucumber (*Cucumis sativus* L.) is an economically and nutritionally important vegetable crop, belonging to the Cucurbitaceae family, which also includes zucchini, squash, and watermelon [1]. It is believed to have originated in South Asia, but its cultivation has expanded worldwide, including in Iraq, and it can be grown year-round using various production systems, both open-field and protected. Expansion of cultivated areas has increased crop exposure to diverse pathogens, including viruses, fungi, bacteria, nematodes, and phytoplasmas [2]. Iraq is considered an active producer of cucumber, especially in the central and southern governorates, where data from the Central Statistical Organization [3] indicated that in 2022 the total cultivated area reached 81,524 donums, with a total production of

195,924 tons and an average yield of 2403.3 kg/donum. The nutritional importance of cucumber lies in its high water content, reaching up to 95%, along with dietary fiber, vitamins C and K, and bioactive compounds with antioxidant properties [4]. Cucumber green mottle mosaic virus (CGMMV) is one of the most significant pathogens threatening cucurbit production, particularly cucumber, causing leaf mottling, fruit deformation, hardening, internal browning, reduced quality, and yield losses. CGMMV belongs to the genus Tobamovirus and is characterized by high mechanical transmissibility, seed-borne dissemination, and prolonged survival in soil and on contaminated tools, which complicates disease management [5]. Recent research has explored sustainable approaches to enhance plant resistance, including the use of microorganisms such as algae.

Anabaena variabilis, a nitrogen-fixing cyanobacterium, has shown potential to promote plant growth and immunity against pathogens through the production of bioactive compounds such as cytokinins and phytoalexins, in addition to improving nutrient uptake [6]. These microorganisms can stimulate natural plant defense mechanisms, as plants treated with biotic agents, including microbial extracts and active compounds, exhibit enhanced systemic acquired resistance and increased efficiency of innate defense responses. Recently, there has been growing interest in natural methods to induce systemic resistance in plants against viral infections. Studies have shown that algae such as *Spirulina platensis* can reduce viral infection severity and promote plant growth. In a recent study, [7] produced algal biomass and used it to stimulate potato plant resistance against Potato virus Y, achieving a notable reduction in infection rate. Previous studies also suggest that microbial inoculation can trigger the plant defense system through systemic acquired resistance (SAR), reducing viral severity and improving physiological performance under stress [8]. Given the lack of studies on CGMMV in Iraq and neighboring regions, and the limited information on the use of biostimulants for viral disease management, the present study aimed

to evaluate the effect of biopriming cucumber plants with *A.variabilis* on enhancing resistance to CGMMV infection. The study assessed changes in viral symptoms, physiological indicators, and growth and yield parameters, focusing on measured traits such as plant height, leaf area, fresh and dry weight, and weight of ten plants. Therefore, the present study aimed to evaluate the effect of different concentrations of *Anabaena variabilis* and their application methods on enhancing resistance, as well as improving growth and yield performance of cucumber plants infected with CGMMV. In addition, this study provides the first report of CGMMV occurrence in Iraq under field conditions.

Materials and Methods

Experimental Site and Period

The field study was conducted during the spring growing season of 2025 at an open-field agricultural site in Al-Shirqat District, Salah Al-Din Governorate, Iraq, using a local hybrid cucumber cultivar (F1). The experiment was arranged in a randomized complete block design (RCBD) with three replicates. The treatments consisted of nine factorial combinations derived from three concentrations of dried *A.variabilis* biomass (50%, 75%, and 100%) applied using three different methods:

seed soaking, soil drenching, and foliar spraying. Healthy and infected control plants without treatment were included. Within each replicate, all eleven treatments were randomly assigned to experimental plots to minimize spatial variability. Each experimental unit consisted of ten plants, and the plot mean (average of ten plants) was considered the experimental unit for statistical analysis. Plants were spaced 20 cm apart within rows, and buffer zones were maintained between plots and replicates to avoid treatment interference or cross-contamination. All other agronomic practices were conducted according to the technical recommendations of the Iraqi Ministry of Agriculture.

CGMMV Diagnosis

Infection with Cucumber Green Mottle Mosaic Virus (CGMMV) was diagnosed based on observation of characteristic symptoms on cucumber plants. Symptoms included leaf mottling with alternating light and dark green areas, leaf deformation, plant stunting, and overall growth reduction, reflecting the virus's impact on plant health. To confirm infection, virus-specific ImmunoStrip assays were used. These strips contain monoclonal antibodies directed against the CGMMV coat protein. Prior to experimental inoculation, five

representative leaf samples were collected from the inoculum source to verify virus identity, four of which tested positive while one was negative. Following mechanical inoculation of the plants, an additional five leaf samples were randomly collected from inoculated plants during the post-inoculation period to verify successful infection. Leaf tissues were homogenized in extraction bags, and the lower end of each ImmunoStrip was immersed in the extract for approximately 30 minutes at room temperature, following the manufacturer's instructions. Young, newly developed leaves were selected to maximize detection of viral accumulation. Samples were considered positive when both the control and test lines appeared, whereas samples showing only the control line were considered negative. It should be noted that no additional molecular diagnostic methods, such as ELISA or RT-PCR, were used in this study. The analysis relied solely on this qualitative serological approach combined with symptom observation to confirm CGMMV presence in the plants.

Cultivation and Biomass Preparation of *A. variabilis* for Experimental Crop Applications

The cyanobacterium *A. variabilis*, previously identified, was obtained

from the Virology Laboratory, College of Agriculture, Tikrit University, and identified based on standard morphological and taxonomic characteristics. It was then cultivated to produce sufficient biomass for experimental crop applications. Cultivation was carried out under aseptic conditions with controlled environmental factors essential for optimal growth, including light intensity and oxygen exchange, following a 16-hour light and 8-hour dark photoperiod in accordance with standard cyanobacterial cultivation protocols. The synthetic BG-11 growth medium was prepared and sterilized to obtain a clear, particle-free solution. The cyanobacterium was inoculated into the medium and maintained under stable conditions to ensure healthy and uniform growth, with gentle agitation to evenly distribute nutrients and dissolved oxygen. Biomass propagation continued for 7–14 days until reaching the exponential growth phase, with continuous monitoring to ensure the stability of biological characteristics. The resulting biomass was then directly used in experimental applications, including seed soaking, soil drenching, and foliar spraying, at the designated experimental concentrations.

Preparation and Application of *A. variabilis* Extract on Cucumber Plants

Cyanobacterium *A. variabilis* extract was prepared and applied to cucumber plants using three experimental concentrations calculated on a volume/volume (v/v) basis: 50%, 75%, and 100%. Three application methods were used:

$$V_s = C/100 \times V_t$$

[24]

Seed Soaking: Seeds were immersed in the extract at each specified concentration for 12 hours under dark conditions to ensure absorption of bioactive compounds. After soaking, seeds were air-dried for 10 minutes to remove excess moisture and then directly sown in experimental units.

Soil Drenching: Plants were irrigated at the root zone with the extract at the specified concentration, using 300 mL per plant, after the formation of the first true leaf. Each treatment was applied twice: the first application during early vegetative growth, and the second following the appearance of initial viral symptoms. Care was taken to evenly distribute the extract around the root zone.

Foliar Spraying: Extracts were sprayed directly onto the leaves using a fine mist sprayer to ensure complete

coverage of the above-ground plant parts, at 300 mL per plant to maintain consistency with soil drenching. Each treatment was applied twice: the first application at the fourth true leaf stage, and the second after observing initial viral symptoms. All concentrations were freshly prepared for each application, and the volume applied was kept consistent across all experimental units to ensure uniform coverage. Treatments were based on v/v percentages, i.e., the ratio of extract volume to total solution volume, without reference to dry weight.

Experimental Design

The study was arranged as a randomized complete block design (RCBD) with three replicates. Nine factorial treatment combinations were evaluated, representing three concentrations of *A. variabilis* biomass applied through three different methods, along with two control treatments (healthy and infected), resulting in a total of eleven treatments. Within each block, all treatments were randomly allocated to plots to maintain full plot-level randomization and reduce potential field variability. No split-plot or hierarchical design was applied; each treatment combination was treated as an independent experimental unit. Each plot contained 10 plants, and

data were collected from individual plants before calculating the mean for statistical analyses. The mean value of each plot was used as the experimental unit in all analyses.

Mechanical Inoculation with CGMMV

Mechanical inoculation of cucumber plants with Cucumber green mottle mosaic virus (CGMMV) was performed under open-field conditions. Infected leaf samples were collected from cucumber plants showing typical CGMMV symptoms, including leaf mottling and deformation. The presence of CGMMV in the source plants was confirmed using CGMMV-specific ImmunoStrip assays (Agdia, France), which showed clear positive reactions. The viral inoculum was prepared by grinding 1 g of infected leaf tissue in phosphate buffer (pH 7) containing KH_2PO_4 (1.36 g L^{-1}) and NaHPO_4 (1.42 g L^{-1}). The homogenate was filtered through two layers of cheesecloth followed by filter paper to obtain a clarified extract. Mechanical inoculation was performed at the four-true-leaf stage by gently rubbing the upper surface of one fully expanded leaf per plant with a sterile cotton swab soaked in the viral extract, in the presence of carborundum to facilitate mechanical injury and enhance viral entry. All inoculations were conducted

on the same day and at the same physiological growth stage to ensure comparability. To prevent cross-contamination, treatments were inoculated sequentially, and all tools were thoroughly washed between treatments. Negative control plants were not exposed to the viral extract and were spatially isolated from inoculated plants. To verify infection establishment and assess uniformity across inoculated treatments, representative leaf samples were randomly collected from different treatments, including the infected control, during the post-inoculation period and tested using CGMMV ImmunoStrip assays. Positive reactions were consistently observed in inoculated plants, and disease incidence in the infected control approached 100%, indicating successful and uniform infection pressure across experimental units. Plants were monitored daily for symptom development for 7–14 days following inoculation, according to the method described by [9].

Assessment of Plant Growth Traits

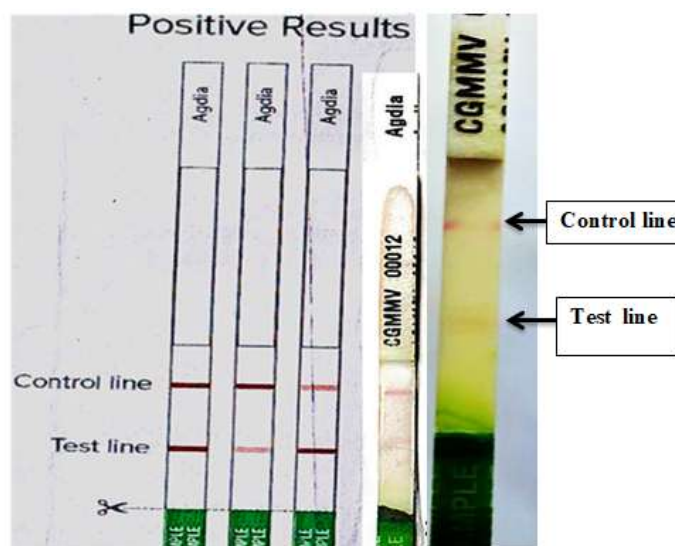
Plant growth parameters, including plant height (cm) and leaf area (cm²), were measured using standard procedures. Leaf area was determined using ImageJ software based on scanned leaf samples.

Assessment of Vegetative Biomass

Fresh and dry shoot biomass (g/plant) were recorded using standard measurement methods.

Assessment of Yield Traits

Fruit yield (g/10 plants) was measured over two consecutive harvests according to the experimental design.



Results and Discussion

Immunological Confirmation of CGMMV Infection

Immunological confirmation of Cucumber green mottle mosaic virus (CGMMV) was conducted using virus-specific ImmunoStrip assays (Agdia, France). Initial suspicion of infection was based on the appearance of characteristic symptoms, including

mosaic patterns, leaf mottling, deformation, and plant stunting. To verify the identity of the inoculum source, five representative symptomatic leaf samples were tested prior to mechanical inoculation. Four samples produced positive reactions, indicated by the presence of both control and test lines, while one sample showed only the control line and was considered negative. These results confirmed that the majority of source plants carried CGMMV and were suitable for inoculum preparation. Following mechanical inoculation, five randomly selected leaf samples from different inoculated treatments, including the infected control, were tested during the post-inoculation period using the same ImmunoStrip protocol. All tested samples produced positive reactions, confirming successful infection establishment under field conditions (Figure 1 and 2). Although the number of tested samples represents a small proportion of the total experimental population, the consistent positive reactions, together with uniform symptom development across inoculated plots, support confidence in infection establishment and treatment comparability. No additional molecular confirmation (e.g., ELISA or RT-PCR) was performed. Therefore, viral presence was verified based on combined symptom expression and serological detection.

This limitation is acknowledged and considered in the interpretation of resistance-related claims. [10,11].

Figure 1. ImmunoStrip detection of CGMMV in cucumber leaves; only positive samples are shown.



Figure 2. Symptoms of Cucumber green mottle mosaic virus (CGMMV) on leaves and fruits of infected cucumber plants in the experimental field, showing

distortion, chlorotic spots, and characteristic mosaic patterns.

Effect of *A. variabilis* concentrations and application methods on plant

The results presented in Table 1 revealed significant effects of *A. variabilis* extract concentrations, application methods, and their interaction on cucumber plant height under CGMMV infection. Among the tested concentrations, 75% *A. variabilis* extract produced the highest mean plant height (69.92 cm), significantly exceeding 50% (53.11 cm) and 100% (63.96 cm). This indicates that the intermediate concentration provided an optimal

height of CGMMV-infected cucumber plants

balance between bioactive compound availability and plant utilization efficiency. The lowest concentration was insufficient to elicit a strong growth response, while the highest concentration resulted in a slight decrease in height, likely due to a hormesis effect, where low to moderate doses stimulate growth, but excessive doses may induce physiological stress or saturation of uptake mechanisms [12].

Table 1 . Effect of *A. variabilis* concentrations and application methods on plant height (cm) of CGMMV-infected cucumber plants.

	Concentrations				
Treatment	50%	75%	100%	Mean of treatments	
Seed soaking	52.44 E	69.88 BC	65.44 BCD	62.59 A	
Foliar spraying	52.89 E	70.93 B	64.05 CD	62.62 A	
Soil drenching		53.99 E	68.95 BC	62.39 D	61.78 A
Healthy control	78.92 A				
Infected control	41.50 F				
Mean of concentrations	53.11 C	69.92 A	63.96 B		

Different letters indicate significant differences among means according to the LSD test ($P \leq 0.05$).

Regarding application methods, foliar spraying resulted in the tallest plants (62.62 cm), followed closely by seed soaking (62.59 cm) and soil drenching (61.78 cm). The superior performance

of foliar spraying can be attributed to the direct absorption of bioactive compounds, including amino acids, vitamins, and natural plant growth regulators, which promote cell

division, elongation, and overall physiological activity. The interaction between concentration and application method was also significant. The combination of foliar spraying with 75% extract yielded the highest plant height (70.93 cm), outperforming all other treatment combinations. This finding emphasizes that optimal growth is not solely concentration-dependent but also relies on efficient delivery of the extract to the plant. These results support the role of *A. variabilis* extract as a biostimulant

enhancing vegetative growth by improving photosynthetic efficiency and metabolic activity, consistent with previous reports indicating that algal extracts can enhance nutrient uptake, activate cell division and elongation, and improve vegetative traits under both normal and stress conditions [13,14,7]. Overall, 75% extract applied via foliar spraying was the most effective treatment for maximizing plant height under CGMMV infection in this study.

Effect of *A. variabilis* concentrations and application methods on leaf

Significant differences were observed among *A. variabilis* concentrations, application methods, and their interaction for leaf area, as shown in Table 2. The 75% concentration resulted in the highest leaf area across application methods. At this concentration, foliar spraying produced the maximum value (98.83 cm²), followed by seed soaking (88.75 cm²) and soil drenching (76.83 cm²).

area of CGMMV-infected cucumber plants

Lower (50%) and higher (100%) concentrations led to smaller leaf areas, indicating that the intermediate concentration provided the most effective balance of bioactive compounds, including polysaccharides, vitamins, and phytohormones, which stimulate cell expansion and leaf surface development.

Table 2. Effect of *A. variabilis* concentrations and application methods on leaf area (cm²) of CGMMV-infected cucumber plants.

Treatment	Concentrations			Mean of treatments
	50%	75%	100%	
Seed soaking	76.06 E	98.83 B	73.98 E	82.96 A
Foliar spraying	82.45 D	88.75 C	82.71 D	84.64 A
Soil drenching	74.24 E	76.83 E	76.66 E	75.91 B
Healthy	124.82 A			

control				
Infected control	51.73 F			
Mean of concentrations	77.58 B	88.14 A	77.78 B	

Different letters indicate significant differences among means according to the LSD test ($P \leq 0.05$).

Among application methods, foliar spraying was generally the most effective, likely because it bypasses root uptake limitations and delivers bioactive compounds directly to leaves, enhancing cellular expansion and division. Soil drenching was less effective in some cases due to partial loss or lower concentration of active compounds in the root zone. These findings are consistent with previous studies reporting that algal

Effect of *A. variabilis* concentrations and application methods on fresh

Significant differences were observed among *A. variabilis* concentrations, application methods, and their interaction for both fresh and dry vegetative weight (Tables 3 and 4). Cyanobacterial treatments significantly outperformed the infected control, confirming the positive effect of *A. variabilis* extract on vegetative growth under CGMMV infection. For fresh weight, the 75% concentration produced the highest mean ($262.22 \text{ g plant}^{-1}$), followed by 100% ($259.11 \text{ g plant}^{-1}$), while the

biostimulants enhance leaf expansion and vegetative growth by activating physiological pathways and improving photosynthetic efficiency [15,16,17] Overall, the combination of 75% *A. variabilis* concentration with foliar spraying was the most effective treatment for increasing leaf area, contributing to improved vegetative growth and mitigating the negative effects of CGMMV infection.

and dry vegetative weight of CGMMV-infected cucumber plants

50% concentration resulted in the lowest value ($172.44 \text{ g plant}^{-1}$). Among application methods, soil drenching achieved the highest average fresh weight ($258.67 \text{ g plant}^{-1}$), followed by seed soaking ($240.89 \text{ g plant}^{-1}$) and foliar spraying ($194.22 \text{ g plant}^{-1}$). The superior performance of soil drenching is likely due to direct delivery of the extract to the root zone, enhancing water status and nutrient absorption, thereby increasing vegetative biomass.

Table 3. Effect of *A. variabilis* concentrations and application methods on fresh vegetative weight (g plant⁻¹) of CGMMV-infected cucumber plants.

	Concentrations			
Treatment	50%	75%	100%	Mean of treatments
Seed soaking	192 D-G	253.33 B-E	277.33 B-D	240.89 AB
Foliar spraying	181.67 E-G	226.67 C-F	174.33 E-G	194.22 B
Soil drenching	143.67 FG	306.67 BC	325.67 B	258.67 A
Healthy control	460.67 A			
Infected control	112.67 G			
Mean of concentrations	172.44 B	262.22 A	259.11 A	

Different letters indicate significant differences among means according to the LSD test ($P \leq 0.05$).

For dry weight, a similar trend was observed. The 75% concentration produced the highest mean (79.44 g plant⁻¹), followed by 100% (77.44 g plant⁻¹), while 50% was lowest (52.11 g plant⁻¹). Soil drenching again outperformed other methods

(77.78 g plant⁻¹), followed by seed soaking (72.33 g plant⁻¹) and foliar spraying (58.88 g plant⁻¹), indicating that this method not only enhances temporary water-dependent growth but also promotes true dry matter accumulation.

Table 4. Effect of *A. variabilis* concentrations and application methods on dry vegetative weight (g plant⁻¹) of CGMMV-infected cucumber plants.

	Concentrations			
Treatment	50%	75%	100%	Mean of treatments
Seed soaking	57.67 DE	76.33 B-D	83 BC	72.33 AB
Foliar spraying	55 D-F	68.67 CD	53 D-F	58.88 B
Soil drenching	43.67 EF	93.33 B	96.33 B	77.78 A
Healthy control	130 A			
Infected control	32.67			
Mean of concentrations	52.11 B	79.44 A	77.44 A	

Different letters indicate significant differences among means according to the LSD test ($P \leq 0.05$).

Comparison with controls showed that the healthy control had the highest fresh and dry

weights due to normal growth, whereas the infected control had significantly reduced

values, reflecting the negative effect of CGMMV on photosynthetic efficiency and metabolite translocation, leading to reduced vegetative growth and dry matter accumulation. These findings align with previous studies showing that algal extracts improve fresh and dry vegetative weight by enhancing nutrient uptake, photosynthetic activity, and biomass accumulation

Effect of *A. variabilis* concentrations and application methods on total fruit weight

Significant differences were observed in total fruit weight per 10 plants among *A. variabilis* concentrations, application methods, and their interaction (Tables 5 and 6). Cyanobacterial treatments significantly outperformed the infected control, highlighting the positive effect of *A. variabilis* extract on total fruit yield under CGMMV infection. First harvest: The 75% concentration consistently resulted in the highest total fruit weight, with soaking producing 3436 g/10 plants, followed by

[18,19,20]. Overall, 75% *A. variabilis* concentration with soil drenching was the most effective treatment for increasing both fresh and dry vegetative weight, demonstrating the ability of cyanobacterial extract to enhance vegetative growth and mitigate the adverse effects of CGMMV infection.

per 10 cucumber plants (first and second harvests)

foliar spraying (2876.7 g/10 plants) and soil drenching (2313 g/10 plants). The 50% concentration yielded the lowest values (1600–1708.7 g/10 plants). These results indicate that moderate extract concentrations provide optimal physiological conditions for dry matter accumulation in fruits, consistent with [16] who reported that algal extracts enhance fruit yield and biomass through improved carbohydrate translocation and photosynthetic efficiency.

Table 5. Effect of *A. variabilis* concentrations and application methods on fruit weight of the first harvest (g per 10 plants).

Treatment	Concentrations			Mean of treatments
	50%	75%	100%	
Seed soaking	1600 E	3436 B	2411.3 CD	2482.4 A
Foliar spraying	1708.7 E	2876.7 C	2805 C	2463.4 AB
Soil drenching	1617.7 E	2313 D	2550 CD	2160.2 B
Healthy control	4253.3 A			
Infected control	880.3 F			
Mean of concentrations	1642.1 B	2875.2 A	2588.8 A	

Different letters indicate significant differences among means according to the LSD test ($P \leq 0.05$).

Second harvest: A similar trend was observed, although absolute values decreased due to plant maturation.

The 75% concentration remained superior, achieving the highest weight via soaking (2429 g/10 plants),

followed by foliar spraying (2123.3 g/10 plants), while 50% remained the least effective. This consistency across harvests suggests that *A. variabilis*

extract effects extend beyond a single developmental stage, supporting sustained production via bioactive compounds.

Table 6. Effect of *A. variabilis* concentrations and application methods on fruit weight of the second harvest (g per 10 plants).

Treatment	Concentrations			Mean of treatments
	50%	75%	100%	
Seed soaking	1110 D	2429 A	1273.3 CD	1604.11 A
Foliar spraying	1110.3 D	1439 C	2123.3 B	1557.56 A
Soil drenching	1030 D	1112 D	1271.7 CD	1137.89 B
Healthy control	2563.3 A			
Infected control	585 E			
Mean of concentrations	1083.44 B	1660 A	1556.11 A	

Different letters indicate significant differences among means according to the LSD test ($P \leq 0.05$).

Regarding application methods, soaking was most effective during the first harvest, whereas in the second harvest, its performance was comparable to foliar spraying. Root-based delivery during early growth stages enhances carbohydrate metabolism and accumulation in fruits, while foliar spraying supports later-stage fruit development by directly supplying bioactive compounds to leaves, potentially accelerating photosynthesis and maximizing yield. These findings align with [21]) and [22], confirming that cyanobacterial biostimulants

improve crop yield and fruit biomass under physiological stress, and with [23]emphasizing the role of increased leaf area in enhancing photosynthetic capacity and productivity. Overall, 75% *A. variabilis* with optimal application (soaking or foliar spraying) was the most efficient treatment for achieving maximum total fruit weight per 10 plants. The similar outcomes between the first and second harvests indicate that the extract provides sustained yield benefits across production stages.

Conclusion

We conclude from the current study that the application of *Anabaena variabilis* extract effectively enhances the growth and yield of cucumber plants under CGMMV infection. The results indicate that the response of plants is strongly dependent on both extract concentration and application method. The intermediate concentration (75%) was identified as the most efficient treatment, suggesting that moderate levels optimize plant physiological

performance, whereas higher concentrations do not necessarily lead to further improvement. In addition, seed soaking and foliar spraying proved to be the most effective application methods for promoting plant growth and fruit production. Overall, these findings confirm the potential of *A. variabilis* extract as a promising and sustainable biostimulant for mitigating the adverse effects of viral infection and improving cucumber productivity under stress conditions.

References

- [1] Rubatzky, V. E., & Yamaguchi, M. World Vegetables: Principles, Production, and Nutritive Values. 2nd ed. New York: Springer; 1997. <https://doi.org/10.1007/978-1-4615-6015-9>
- [2] Youssef, S.M., et al. 2022. Foliar spray or soil drench: Microalgae application impacts on soil microbiology, morpho-physiological and biochemical responses, oil and fatty acid profiles of chia plants under alkaline stress. Biology, 11(12): 1844. <https://doi.org/10.3390/biology11121844>
- [3] Central Statistical Organization (CSO). 2022. Cultivated area, average yield and production of vegetable crops in Iraq for 2022. <http://www.cosit.gov.iq>. Accessed 10 Jan 2026.
- [4] Maynard, D. N. & Hochmuth, G. J. Knott's Handbook for Vegetable Growers. 5th ed. Hoboken, NJ: Wiley- Interscience; 2007. <https://doi.org/10.1002/9780470121474>
- [5] ASTA. 2018. Cucumber green mottle mosaic virus (CGMMV). <https://www.betterseed.org/wp-content/uploads/US6708-ASTA-CGMMV-Brochure-Addendum-ENG-January-2018.pdf>. Accessed 10 Jan 2026.

- [6] Singh JS, Singh DP, Prabha R, Gupta VK. 2016. Cyanobacteria: a precious bio-resource in agriculture, ecosystem, and environmental sustainability. *Front Microbiol* 7: 529. <https://doi.org/10.3389/fmicb.2016.00529>
- [7] Al-Dulami AKE, Al-Fahd MAW. 2023. Bio-mass production of *Spirulina platensis* and its use in inducing hostility resistance against Potyvirus Potato virus Y. *IOP Conf Ser Earth Environ Sci* 1158: 072018. <https://doi.org/10.1088/1755-1315/1158/7/072018>
- [8] Fu, Z.Q. & Dong, X. (2013). Systemic acquired resistance: turning local infection into global defense. *Annual Review of Plant Biology*, 64: 839–863. <https://doi.org/10.1146/annurev-arplant-042811-105606>
- [9] Chanda B, Shamimuzzaman M, Gilliard A, Ling KS. 2021. Effectiveness of disinfectants against the spread of tobamoviruses: Tomato brown rugose fruit virus and Cucumber green mottle mosaic virus. *Virol J* 18: 7. <https://doi.org/10.1186/s12985-020-01479-8>
- [10] Agdia Inc. 2021. CGMMV insights – guest article. <https://www.agdia.com/company/new/1803406/cgmmv-insights-guest-article>. Accessed 10 Jan 2026.
- [11] Farhan DA, Al-Fahad MAW. 2021. Immune diagnosis of Potyvirus bean common mosaic virus (BCMV) and its biological resistance with *Spirulina platensis* and some medicinal plants on two bean cultivars under open cultivation conditions. *Nat Volatiles Essent Oils* 8(6): 1843–1857. <https://www.nveo.org/index.php/journal/article/view/3847>
- [12] Jalal, A., Oliveira Junior, J.C., Ribeiro, J.S., Fernandes, G.C., Mariano, G.G., Trindade, V.D.R., & Reis, A.R.D. (2021). Hormesis in plants: Physiological and biochemical responses. *Ecotoxicology and Environmental Safety*, 207: 111225. <https://doi.org/10.1016/j.ecoenv.2020.111225>
- [13] Canellas, L.P. & Olivares, F.L. (2014). Physiological responses to humic substances as plant growth promoter. *Chemical and Biological Technologies in Agriculture*, 1:3. <https://doi.org/10.1186/2196-5641-1-3>
- [14] Pelizer, L.H., & Moraes, I. de O. (2014). A method to estimate the biomass of *Spirulina platensis* cultivated on a solid medium. *Brazilian Journal of Microbiology*, 45(3), 933–936.

<https://doi.org/10.1590/s1517-83822014000300024>

[15] Roupahel, Y. & Colla, G. (2020). Editorial: Biostimulants in Agriculture. *Frontiers in Plant Science*, 11:40. <https://doi.org/10.3389/fpls.2020.00040>

[16] Craigie, J.S. (2011). Seaweed extract stimuli in plant science and agriculture. *Journal of Applied Phycology*, 23(3):371–393. <https://doi.org/10.1007/s10811-010-9560-4>

[17] Battacharyya, D., Babgohari, M.Z., Rathor, P., & Prithiviraj, B. (2015). Seaweed extracts as biostimulants in horticulture. *Scientia Horticulturae*, 196:39–48. <https://doi.org/10.1016/j.scienta.2015.09.012>

[18] Macias-Benitez, S., Garcia-Martinez, A. M., Caballero-Jimenez, P., Gonzalez, J. M., Tejada, M., & Parrado, J. (2020). Rhizospheric organic acids as biostimulants: Monitoring feedbacks on soil microorganisms and biochemical properties. *Frontiers in Plant Science*, 11, 633. <https://doi.org/10.3389/fpls.2020.00633>

[19] Li, J., Van Gerrewey, T., & Geelen, D. (2022). A Meta-Analysis

of Biostimulant Yield Effectiveness in Field Trials. *Frontiers in plant science*, 13, 836702. <https://doi.org/10.3389/fpls.2022.836702>

[20] El-Nakhel, C., Petropoulos, S. A., Di Mola, I., Ottaiano, L., Cozzolino, E., Roupahel, Y., & Mori, M. (2023). Biostimulants of Different Origins Increase Mineral Content and Yield of Wild Rocket While Reducing Nitrate Content through Successive Harvests. *Horticulturae*, 9(5), 580. <https://doi.org/10.3390/horticulturae9050580>

[21] Chabili, A., Minaoui, F., Hakkoum, Z., Douma, M., Meddich, A., & Loudiki, M. (2024). A Comprehensive Review of Microalgae and Cyanobacteria-Based Biostimulants for Agriculture Uses. *Plants*, 13(2), 159. <https://doi.org/10.3390/plants13020159>

[22] Lu, Q., Jin, L., Tong, C., Liu, F., Huang, B., & Zhang, D. (2024). Research Progress on the Growth-Promoting Effect of Plant Biostimulants on Crops. *Phyton- International Journal of Experimental Botany*, 93(4), 661–679. <https://doi.org/10.32604/phyton.2024.049733>

[23] Senousy, H. H., Alhaj Hamoud, Y., Abu- Elsaoud, A. M., &

Al- Zoubi, O. M. (2023). Algal bio- stimulants enhance salt tolerance in common bean: Dissecting morphological, physiological, and genetic mechanisms for stress adaptation. *Plants*, 12(21), 3714. <https://doi.org/10.3390/plants12213714>

[24] Skoog, D. A., West, D. M., Holler, F. J., & Crouch, S. R. (2014). Fundamentals of analytical chemistry (9th ed.). Cengage Learning. <https://doi.org/10.1007/s00216-013-7242-1>