

# Effect of *Trichormus variabilis* biomass concentration and application method on cucumber growth under Cucumber Green Mottle Mosaic Virus (CGMMV) infection

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**Abstract.** Alangood ZT, Al-Fahad MA. 2026. Effect of *Trichormus variabilis* biomass concentration and application method on cucumber growth under Cucumber Green Mottle Mosaic Virus (CGMMV) infection. *Asian J Agric* 10 (1): g100154. <https://doi.org/10.13057/asianjagric/g100154>. This study evaluated the effect of cyanobacterium *Trichormus variabilis* biomass on cucumber (*Cucumis sativus*) performance under Cucumber Green Mottle Mosaic Virus (CGMMV) infection in open-field conditions. Viral infection was confirmed using CGMMV-specific ImmunoStrip assays prior to treatment application. The experiment was arranged in a factorial design including three concentrations of *T. variabilis* dried biomass suspension (50%, 75%, and 100%) and three application methods (seed soaking, foliar spraying, and soil drenching), in addition to healthy and infected controls. Biomass concentrations significantly influenced disease severity, disease incidence, chlorophyll content (SPAD), fruit weight, and fruit firmness. The 75% concentration consistently reduced disease severity and incidence and improved physiological and yield-related traits compared with the 50% concentration. For several parameters, no significant differences were detected between the 75% and 100% concentrations, indicating a plateau response at higher biomass levels. Application method had limited influence on most measured traits. Differences in hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) accumulation were observed using a potassium iodide (KI)-based qualitative assay, indicating relative variation in oxidative status among treatments. The study was conducted during a single growing season, at one field location, and on a single cucumber cultivar, further multi-environment validation and standardized biomass characterization are required before making broader recommendations can be made. Overall, *T. variabilis* biomass at an intermediate concentration mitigated CGMMV-associated disease symptoms and improved cucumber productivity under the tested conditions.

**Keywords:** Biostimulants, Cucumber Green Mottle Mosaic Virus (CGMMV), cyanobacteria, disease severity, *Trichormus variabilis*

## INTRODUCTION

Cucumber (*Cucumis sativus*) is an economically and nutritionally important vegetable crop cultivated widely under both open-field and protected production systems. In Iraq, cucumber production represents a substantial component of vegetable agriculture, covering approximately 81,524 donums and yielding 195,924 tons annually (Central Statistical Organization (CSO) 2022). However, expansion of cultivation areas and intensified production systems have increased crop exposure to plant pathogens, particularly viral diseases, which significantly reduce yield and fruit quality (Youssef et al. 2022).

Viral infections in cucurbits are especially problematic because they spread rapidly, limited curative options, and persistence in agricultural environments. Among cucurbit-infecting viruses, Cucumber Green Mottle Mosaic Virus (CGMMV) is recognized as one of the most destructive pathogens. It induces leaf mottling, deformation, plant stunting, fruit hardening, and internal discoloration, leading to considerable economic 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 management strategies (American Seed Trade Association (ASTA) 2018).

Once infection is established, no direct antiviral treatment is available; therefore, disease management relies largely on preventive practices, sanitation measures, resistant cultivars when available, and integrated crop management strategies.

In recent years, cyanobacterial biomass has attracted attention as a plant biostimulant due to its content of diverse bioactive compounds, including amino acids, vitamins, phytohormone-like substances, polysaccharides, and antioxidant-related molecules that may influence plant physiological performance under stress conditions (Singh et al. 2016). Algal- and cyanobacterial-based products have been reported to enhance nutrient uptake, stimulate vegetative growth, and improve plant tolerance to abiotic and biotic stresses. Several studies have documented reductions in viral symptom severity and improvements in growth parameters following algal biomass applications (Soosaar et al. 2005; Fu and Dong 2013). Biomass preparations of *Limnospira platensis* (syn. *Spirulina platensis*) have been associated with decreased symptom expression and improved plant vigor in different crops under viral challenge (Al-Qaisi and Al-Fahad 2023). Similarly, Al-Dulami and Al-Fahad (2023) reported reductions in Potato virus Y symptom severity in potato plants treated with algal biomass-based formulations, suggesting potential

roles of such treatments in sustainable disease management strategies.

Among cyanobacteria, *Trichormus variabilis* has received increasing attention due to its ability to fix atmospheric nitrogen and produce a wide range of bioactive compounds, including phytohormone-like substances, amino acids, and antioxidants. These characteristics contribute to improved plant growth and physiological stability under stress conditions. In addition, species of *T. variabilis* have been associated with enhanced nutrient availability and modulation of plant metabolic responses, suggesting their potential role in improving plant performance under biotic stress conditions (Ismail and Abo-Hamad 2017; Refaay et al. 2022; Morsi et al. 2023).

The cyanobacterial species *T. variabilis* (formerly *Anabaena variabilis*) has been reclassified based on modern cyanobacterial taxonomy (Komárek and Anagnostidis 1989). This taxonomic revision was further supported by molecular phylogenetic studies confirming the separation of *Trichormus* from *Anabaena* (Rajaniemi et al. 2005).

Despite these findings, field-based evaluations of *T. variabilis* biomass specifically targeting CGMMV-associated disease parameters in cucumber under open-field conditions remain limited. In addition, systematic comparison of different biomass concentrations within a factorial experimental framework has not been thoroughly investigated. Determining whether intermediate or higher biomass levels provide optimal mitigation of disease symptoms and improvement of physiological traits is important for practical field application. Furthermore, comparative assessment of different application methods—seed soaking, foliar spraying, and soil drenching—under uniform environmental conditions is necessary to identify efficient delivery strategies.

Therefore, the present study aimed to evaluate the effect of different concentrations of *T. variabilis* dried biomass suspension applied through three treatment methods (seed soaking, foliar spraying, and soil drenching) on disease incidence, disease severity, chlorophyll content, fruit weight, and fruit firmness of cucumber plants under CGMMV infection in open-field conditions. The study focused on measurable disease and productivity outcomes rather than mechanistic confirmation of resistance.

## MATERIALS AND METHODS

### Experimental site and period

The field experiment was conducted during the spring growing season of 2025 in 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 (blocks). The treatment structure consisted of nine factorial combinations derived from three concentrations of *T. variabilis* dried biomass suspension (50%, 75%, and 100%) and three application methods (seed soaking, soil drenching, and foliar spraying), in addition to healthy and infected control treatments. Within each block, all treatments (11 total treatments) were

randomly assigned to experimental plots using simple randomization to minimize spatial field variability. Each experimental unit consisted of 10 plants, and the plot mean (average of 10 plants) was considered the experimental unit for statistical analysis. Plants were spaced 20 cm apart within rows, and buffer zones were maintained between adjacent plots and blocks to prevent potential treatment interference and cross-contamination. Irrigation was supplied through a drip irrigation system throughout the growing season. No chemical fertilizers or insect control measures were applied during the experimental period to avoid confounding treatment effects. The soil at the experimental site was classified as loamy sandy soil typical of the region. Average daily temperatures during the spring season ranged from approximately 30°C to 40°C, with relative humidity between 40% and 55%. No extreme weather events were recorded during the experimental period. All other agronomic practices followed the technical recommendations of the Iraqi Ministry of Agriculture.

### Serological confirmation of CGMMV infection

Cucumber Green Mottle Mosaic Virus (CGMMV) was diagnosed based on characteristic symptom observation supported by serological confirmation using a virus-specific ImmunoStrip assay (Agdia, France). Symptomatology included leaf mottling with alternating light and dark green areas, leaf deformation, plant stunting, and overall growth reduction, consistent with previously reported descriptions of CGMMV infection. Serological detection was performed using ImmunoStrip kits containing monoclonal antibodies specific to the CGMMV coat protein. Prior to experimental inoculation, five representative leaf samples were tested to confirm the identity of the inoculum source; four samples yielded positive reactions and one sample tested negative. Following mechanical inoculation, five additional randomly selected leaf samples from inoculated plants were tested during the post-inoculation period to verify infection establishment. Positive reactions were confirmed by the appearance of both control and test lines, whereas samples displaying only the control line were considered negative. Young, newly developed leaves were selected for analysis due to their higher expected viral accumulation. Leaf tissues were homogenized in extraction bags, and the lower end of the ImmunoStrip was immersed in the extract and incubated at room temperature for approximately 30 min according to the manufacturer's instructions. ImmunoStrip assay was used as a qualitative serological tool to confirm the presence or absence of CGMMV. Although this method provides rapid field-level detection, it did not quantify viral load and may exhibit lower analytical sensitivity compared with molecular techniques.

### Preparation and standardization of *Trichormus variabilis* biomass

The cyanobacterium *T. variabilis* was obtained from the Virology Laboratory, Plant Protection Department, College of Agriculture, Tikrit University, Iraq, and cultured in BG-11 broth (HiMedia Laboratories, India) under controlled laboratory conditions to obtain sufficient biomass. At the exponential growth phase, biomass was harvested and

thoroughly washed with distilled water to remove residual medium components. The collected material was then dried in a forced-air oven at 45°C for 24 h until constant dry weight was achieved. The dried biomass was weighed using an analytical balance and subsequently re-suspended in distilled water to prepare standardized biomass suspensions on a dry weight basis (weight/volume, W/V). The 100% concentration was defined as 1 g dry biomass L<sup>-1</sup> distilled water. The 75% and 50% concentrations corresponded to 0.75 g L<sup>-1</sup> and 0.5 g L<sup>-1</sup>, respectively. All suspensions were freshly prepared using distilled water only, without residual BG-11 medium, to avoid nutrient-related confounding effects. Therefore, treatments in the present study were defined as dried biomass suspensions rather than live cultures or chemically characterized extracts. Concentrations were standardized solely on a dry weight basis to ensure consistent biomass dosage across treatments.

### Experimental design

The experiment was conducted using A Randomized Complete Block Design (RCBD) with three replicates (blocks). The study included nine factorial treatment combinations (three biomass concentrations × three application methods of *T. variabilis*), in addition to two non-factorial controls (healthy control and infected control), resulting in a total of eleven treatments. Within each block, all eleven treatments were randomly assigned to experimental plots to ensure full randomization at the plot level and to minimize the influence of field variability. No hierarchical or split-plot structure was used; each treatment combination was considered an independent experimental unit within each block. Each experimental unit consisted of 10 plants. Measurements were recorded from individual plants within each plot and averaged prior to statistical analysis. The plot mean was considered the experimental unit for all statistical analysis.

### Methods of biomass suspension application

#### Seed soaking

Cucumber seeds were soaked in the respective *T. variabilis* dried biomass suspensions (50%, 75%, and 100%) for 12 h immediately prior to sowing. A standardized ratio of approximately 100 mL of suspension per 100 seeds was used to ensure complete immersion and uniform exposure. After soaking, seeds were air-dried at room temperature under shade conditions to remove surface moisture and then sown directly in the field. This treatment was applied once as a preventive measure prior to mechanical viral inoculation.

#### Soil drenching

Soil drenching was performed by applying 200 mL of the respective biomass suspension per plant directly to the rhizosphere zone at the base of each plant. Applications were conducted twice: the first at the established vegetative growth stage (four-true-leaf stage) and the second after the appearance of initial CGMMV symptoms. The applied volume was precisely measured using a graduated cylinder

to ensure consistent biomass delivery per plant across all treatments.

#### Foliar spraying

Foliar applications were carried out using a calibrated hand sprayer, delivering 200 mL of biomass suspension per plant per application to achieve uniform canopy coverage without runoff. Spraying was performed twice: the first at the four-true-leaf stage (preventive application) and the second following the appearance of initial CGMMV symptoms (curative application). No surfactant was added to avoid potential confounding effects on leaf permeability. Application was conducted during early morning hours to minimize evaporation and ensure uniform absorption.

#### Mechanical inoculation with CGMMV

Mechanical inoculation with Cucumber Green Mottle Mosaic Virus (CGMMV) was performed under open-field conditions. Infected leaf samples were collected from cucumber plants exhibiting 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), composed of KH<sub>2</sub>PO<sub>4</sub> (1.36 g L<sup>-1</sup>) and NaHPO<sub>4</sub> (1.42 g L<sup>-1</sup>). The homogenate was filtered through two layers of cheesecloth followed by filter paper to obtain a clarified extract. Mechanical inoculation was conducted at the four-true-leaf stage by gently rubbing the upper surface of one fully expanded leaf per plant with the viral extract using a sterile cotton swab. No abrasive material was used to avoid excessive mechanical injury and to maintain uniform tissue disturbance across all treatments under field conditions. Although abrasive agents are commonly employed to increase infection efficiency, preliminary observations indicated that consistent symptom development could be achieved without abrasives under the prevailing environmental conditions. All plants within each experimental unit were subjected to mechanical inoculation to ensure uniform infection pressure across treatments. All inoculations were performed on the same day and at a uniform 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 viral extract and were maintained in a spatially separated area under the same field conditions to prevent unintended virus transmission while preserving experimental comparability. 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 values 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 Chanda et al. (2021).

### Assessment of disease incidence and severity

#### Assessment of disease incidence

Disease incidence of Cucumber Green Mottle Mosaic Virus (CGMMV) was assessed 14 days after mechanical inoculation, when typical mosaic symptoms were fully developed and stable. All plants within each experimental unit (10 plants per plot) were visually examined at the same physiological growth stage to ensure uniform evaluation. Disease incidence was calculated as the percentage of symptomatic plants relative to the total number of examined plants per experimental unit, following Bock et al. (2022), according to the equation [1].

$$DI(\%) = \frac{n}{N} \times 100 \quad [1]$$

Where, *DI*: Disease incidence, *n*: Number of diseased plants, *N*: Total number of assessed plants

Incidence values were calculated separately for each replicate and treatment prior to statistical analysis. The calculated incidence represented the plot-level value used for subsequent ANOVA.

#### Assessment of disease severity

Disease severity was evaluated concurrently with disease incidence (14 days post-inoculation) using a standardized six-grade rating scale (0-5) (Chiang et al. 2017), commonly adopted in plant pathology studies for qualitative symptom assessment of viral diseases in cucurbits. Each plant was individually scored based on visible symptoms as follows:

0: Healthy plant with no visible symptoms, 1: Mild mosaic symptoms, 2: Moderate mosaic with slight leaf deformation, 3: Severe mosaic with vein banding, 4: Generalized mosaic with pronounced deformation and initial tissue necrosis, 5: Extensive tissue necrosis, severe leaf curling, and marked growth reduction

Disease ratings were performed independently by two trained evaluators following the same standardized criteria to reduce subjectivity. Prior to evaluation, both assessors were calibrated using representative symptomatic plants to ensure consistent interpretation of severity categories and minimize scoring bias.

The Disease Severity Index (DSI) was calculated according to the standard formula of McKinney (1923), as shown in equation [2]:

$$DSI(\%) = \left[ \frac{\sum(d \times n)}{D \times N} \right] \times 100 \quad [2]$$

Where, *d*: Disease rating score; *n*: Number of plants in each rating category, *D*: Maximum disease score (5), *N*: Total number of evaluated plants per experimental unit

The calculated DSI represented the plot-level severity value used for statistical analysis.

#### Determination of chlorophyll content (SPAD Values)

Leaf chlorophyll content was estimated using a portable SPAD meter (FT-YD Plant Nutrient Analyzer, Fengtu, China), which provides relative chlorophyll index readings expressed as SPAD values. Measurements were conducted at the full vegetative growth stage, 14 days after mechanical inoculation, coinciding with disease incidence and severity assessments to ensure uniform developmental comparison among treatments. Within each experimental unit (10 plants per treatment per replicate), three plants were randomly selected for chlorophyll assessment. From each selected plant, three SPAD readings were taken from fully expanded leaves of similar physiological age. Measurements were obtained from three canopy positions (upper, middle, and lower leaves) to ensure representative sampling of chlorophyll distribution within the plant. Severely necrotic or heavily damaged tissues were excluded to avoid biased readings. All measurements were performed between 09:00 and 11:00 a.m. under consistent environmental conditions to minimize diurnal variation effects. The mean of the three readings per plant was calculated, followed by the mean per experimental unit, and these values were used for subsequent statistical analysis. The procedure for SPAD-based chlorophyll estimation was performed according to Zhang et al. (2022).

#### Determination of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) accumulation

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) accumulation in cucumber leaves was evaluated using a potassium iodide (KI)-based qualitative method adapted from Junglee et al. (2014). Leaf samples were collected after the appearance of viral symptoms at the same developmental stage used for disease assessment. Fully expanded leaves of similar physiological age were selected, while severely necrotic tissues were avoided to ensure comparability among treatments. Three plants per replicate were sampled. Fresh leaf tissue (0.5 g) was homogenized in 5 mL of distilled water using a mortar and pestle. The homogenate was filtered through double-layered cheesecloth to obtain a clear extract. One milliliter of plant extract was transferred into a test tube, followed by the addition of 1 mL of 1% (w/v) KI solution and 1 mL of 5% acetic acid. The reaction mixture (final volume 3 mL) was mixed thoroughly and incubated for 10 min at room temperature in darkness to prevent photodegradation. H<sub>2</sub>O<sub>2</sub> accumulation was assessed qualitatively based on the intensity of yellow-to-brown coloration formed as a result of the reaction. Color intensity was visually compared among treatments and classified into descriptive categories (light, moderate, or dark brown) to indicate relative oxidative status. The assay was conducted in duplicate to confirm consistency of observations.

#### Assessment of yield-related traits

Fruit weight per plant: Fruit weight per plant was determined by harvesting all marketable fruits from each experimental unit (10 plants per plot) at physiological maturity. Fruits were harvested at uniform commercial maturity based on size, color, and firmness criteria to ensure standardization across treatments. Harvesting was conducted twice during the production period at

comparable developmental stages. The total fruit weight per plot was recorded using a digital balance, and the mean fruit weight per plant ( $\text{g plant}^{-1}$ ) was calculated by dividing total plot yield by the number of plants within the experimental unit.

**Fruit firmness:** Fruit firmness was measured at harvest using a handheld fruit penetrometer (FT-327). Three readings were taken from equidistant points around the equatorial region of each fruit after removal of a small portion of peel to ensure consistent penetration depth. The average firmness per fruit was calculated, followed by averaging across fruits within each experimental unit. Results were expressed in  $\text{kg cm}^{-2}$  according to the method described by Li et al. (2016). All yield-related data were analyzed using ANOVA under the factorial RCBD model described previously.

#### Statistical analysis

Data from the  $3 \times 3$  factorial treatments were analyzed using Analysis of Variance (ANOVA) appropriate for a factorial Randomized Complete Block Design (RCBD), with biomass concentration and application method considered fixed factors and block treated as a random factor. The experimental unit for statistical analysis was the plot (10 plants), and treatment means were calculated as plot averages prior to analysis. Model assumptions of normality and homogeneity of variance were evaluated using residual diagnostics. When the F-test indicated significant treatment effects ( $P \leq 0.05$ ), mean separation among factorial treatment combinations was conducted using Tukey's Honestly Significant Difference (HSD) test at the 5% significance level to control the family-wise error rate. Healthy and infected controls were included in the overall experimental layout to allow direct comparison within the same environmental context. However, factorial main effects (concentration and method) and their interaction were interpreted exclusively within the  $3 \times 3$  factorial treatment structure to preserve the correct error term and avoid confounding treatment hierarchy. Standard Error (SE) values were calculated based on block-level replication ( $n = 3$ ) and were presented alongside treatment means.

## RESULTS AND DISCUSSION

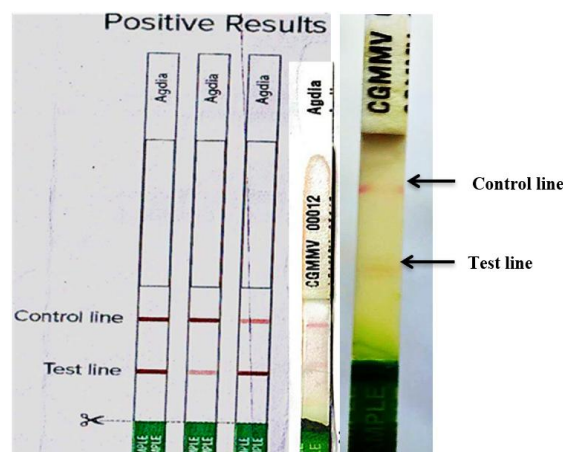
### Immunological confirmation of CGMMV infection

The 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). 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. Therefore,

viral presence was verified based on combined symptom expression and serological detection.

### Effect of *Trichormus variabilis* concentration and application method on CGMMV severity

As presented in Table 1, biomass concentration significantly affected CGMMV disease severity ( $P \leq 0.05$ ), whereas application method did not exert a significant main effect. However, the interaction between concentration and application method was statistically significant, indicating that the magnitude of concentration response varied slightly among application approaches. Regarding the main effect of concentration, 75% (33.55) and 100% (35.33) treatments recorded significantly lower severity values compared with the 50% concentration (42.88). No significant difference was detected between 75% and 100%, suggesting that disease suppression plateaued beyond the intermediate concentration level. This pattern indicates that increasing biomass dose from 50% to 75% substantially improved symptom mitigation, whereas further increase to 100% did not provide proportional additional benefit under the present field conditions. Although application method alone did not significantly influence severity, the interaction data demonstrate that certain concentration-method combinations (e.g., 75% foliar spraying and 100% soil drenching) contributed to relatively lower severity values. The healthy control exhibited minimal severity (3.00), whereas the infected control recorded the highest severity (88.00), confirming successful infection establishment and clear treatment differentiation. While mechanical inoculation was standardized across treatments, minor biological variability in infection intensity under open-field conditions cannot be entirely excluded. However, the consistent elevation of severity in infected controls relative to treated plants supports the reliability of observed treatment effects. Overall, the data indicate that intermediate biomass concentration (75%) was sufficient to reduce symptom severity under CGMMV pressure. However, these findings demonstrate symptom mitigation rather than confirmed mechanistic resistance, as viral accumulation was not quantitatively assessed.



**Figure 1.** ImmunoStrip detection of CGMMV on cucumber leaves

**Table 1.** Effect of *Trichormus variabilis* concentrations, application methods, and their interaction on CGMMV severity in cucumber plants

Treatments	Concentrations			Mean of treatments
	50%	75%	100%	
Seed soaking	39.33 ± 1.45 CD	33.67 ± 1.45 EF	38.67 ± 2.40 C-E	37.22 ± 1.77 A
Foliar spraying	47.00 ± 1.53 B	31.67 ± 1.45 F	34.33 ± 2.40 D-F	36.88 ± 1.79 A
Soil drenching	42.33 ± 1.20 BC	35.33 ± 1.45 D-F	33.00 ± 2.89 EF	37.66 ± 1.85 A
Healthy control			3.00 ± 1.15 G	
Infected control			88.00 ± 2.08 A	
Mean of concentrations	42.88 ± 1.39 A	33.55 ± 1.45 B	35.33 ± 2.56 B	-

Note: Values represent Mean ± SE ( $n = 3$ ). Different letters indicate significant differences according to Tukey's HSD test at  $P \leq 0.05$

**Table 2.** Effect of *Trichormus variabilis* concentrations, application methods, and their interaction on CGMMV disease incidence in cucumber plants

Treatments	Concentrations			Mean of treatments
	50%	75%	100%	
Seed soaking	67.50 ± 1.20 BC	35.00 ± 1.15 EF	44.50 ± 1.30 C-E	49.00 ± 1.22 A
Foliar spraying	75.00 ± 1.25 B	32.50 ± 1.10 F	42.50 ± 1.35 D-F	50.00 ± 1.23 A
Soil drenching	70.00 ± 1.18 BC	37.50 ± 1.20 D-F	43.00 ± 1.40 EF	50.17 ± 1.26 A
Healthy control			5.00 ± 0.90 G	
Infected control			100.00 ± 0.00 A	
Mean of concentrations	70.83 ± 1.21 A	35.00 ± 1.15 B	43.33 ± 1.35 B	

Note: Values represent Mean ± SE ( $n = 3$ ). Different letters indicate significant differences according to Tukey's HSD test at  $P \leq 0.05$

**Table 3.** Effect of *Trichormus variabilis* concentrations, application methods, and their interaction on CGMMV infection percentage in cucumber plants

Treatments	Concentrations			Mean of treatments
	50%	75%	100%	
Seed soaking	63.66 ± 2.02 C	31.33 ± 0.88 F	42.65 ± 1.45 D	45.88 ± 1.64 A
Foliar spraying	65.66 ± 2.03 B	35.33 ± 1.45 E	45.52 ± 1.15 D	48.83 ± 1.54 A
Soil drenching	69.00 ± 1.53 B	36.81 ± 1.45 E	50.23 ± 1.15 C	52.01 ± 1.37 B
Healthy control			4.33 ± 1.45 G	
Infected control			97.00 ± 1.73 A	
Mean of concentrations	75.10 ± 1.86 A	34.49 ± 1.26 C	46.13 ± 1.25 B	-

Note: Values represent Mean ± SE ( $n = 3$ ). Different letters indicate significant differences according to Tukey's HSD test at  $P \leq 0.05$

#### Effect of *Trichormus variabilis* concentration and application method on CGMMV disease incidence

As presented in Table 2, biomass concentration significantly affected Cucumber Green Mottle Mosaic Virus (CGMMV) disease incidence ( $P \leq 0.05$ ), whereas application method did not exert a significant main effect. However, the interaction between concentration and application method was statistically significant, indicating that the magnitude of concentration response varied slightly among application approaches. Regarding the main effect of concentration, 75% (35.00%) treatment recorded significantly lower disease incidence compared with 50% concentration (70.83%). 100% concentration (43.33%) also showed a reduction in disease incidence compared with 50%, but did not differ significantly from the 75% treatment, suggesting that disease suppression plateaued beyond the intermediate concentration level. This pattern indicates that increasing biomass dose from 50% to 75% substantially improved disease reduction, whereas further increase to 100% did not provide proportional additional benefit under the present field conditions. Although application method alone did not significantly influence disease incidence, the interaction data demonstrate that

certain concentration-method combinations (e.g., 75% foliar spraying and 75% seed soaking) contributed to relatively lower disease incidence values. The healthy control exhibited minimal incidence (5.00%), whereas the infected control recorded the highest incidence (100.00%), confirming successful infection establishment and clear treatment differentiation. While mechanical inoculation was standardized across treatments, minor biological variability in infection intensity under open-field conditions cannot be entirely excluded. However, the consistent elevation of disease incidence in infected controls relative to treated plants supports the reliability of observed treatment effects. Overall, the data indicate that intermediate biomass concentration (75%) was sufficient to reduce CGMMV disease incidence under field conditions.

#### Effect of *Trichormus variabilis* concentration and application method on CGMMV infection percentage

As shown in Table 3, both biomass concentration and application method significantly influenced CGMMV infection percentage, and a significant interaction between the two factors was detected. Regarding the main effect of concentration, 75% treatment recorded the lowest infection

percentage (34.49%), followed by the 100% concentration (46.13%), whereas 50% concentration exhibited the highest infection percentage (75.10%). All concentration levels differed significantly from one another, indicating a concentration-dependent response. The superior performance of 75% concentration suggests that intermediate biomass levels were sufficient to reduce the proportion of symptomatic plants under the experimental conditions, whereas lower biomass concentration (50%) was insufficient and higher concentration (100%) did not provide proportional additional benefit. Concerning application methods, soil drenching recorded the highest mean infection percentage (52.01%), which differed significantly from seed soaking (45.88%) and foliar spraying (48.83%). No significant difference was observed between seed soaking and foliar spraying, suggesting comparable effectiveness between these two approaches in limiting infection incidence. The healthy control showed a very low infection percentage (4.33%), while the infected control recorded the highest value (97.00%), confirming successful infection establishment and adequate differentiation among treatments. Although infection percentage differed significantly among treatments, it should be noted that mechanical inoculation under field conditions may inherently introduce some degree of biological variability in infection establishment. However, the consistently high infection level in the infected control supports the reliability of inoculation pressure across experimental units.

#### Effect of *Trichormus variabilis* concentration and application method on chlorophyll content (SPAD)

Analysis of variance indicated that biomass concentration, application method, and their interaction significantly affected relative chlorophyll content (SPAD values) ( $P \leq 0.05$ ) (Table 4). Regarding the main effect of concentration, plants treated with 75% (34.83 SPAD units) and 100% (34.23 SPAD units) concentrations exhibited significantly higher chlorophyll content compared with the 50% concentration (30.91 SPAD units). No significant difference was detected between 75% and 100%, indicating a plateau response beyond the intermediate biomass level. This pattern suggests that moderate biomass supply was sufficient to mitigate chlorophyll degradation associated with CGMMV infection, whereas further increases in concentration did not result in proportional enhancement. For application methods, seed soaking resulted in the highest mean SPAD value (34.62), significantly exceeding soil drenching (32.19), while foliar spraying (33.15) did not differ significantly from either method. The interaction effect revealed that the highest SPAD value among infected treatments was obtained with 75% concentration applied via seed soaking (36.33), whereas the lowest value was recorded at 50% concentration combined with soil drenching (30.04). The healthy control exhibited the highest chlorophyll content (43.20), whereas the infected control showed the lowest value (24.71), confirming the strong negative impact of CGMMV infection on leaf greenness. It should be noted that SPAD measurements provide an indirect estimation of relative chlorophyll concentration based on leaf optical properties.

#### Effect of *Trichormus variabilis* concentration and application method on $H_2O_2$ accumulation

Visual evaluation of hydrogen peroxide ( $H_2O_2$ ) accumulation using the potassium iodide (KI) colorimetric assay showed observable variation in color intensity among treatments (Figure 2 and Table 5). Healthy control plants exhibited transparent to pale yellow coloration, whereas infected control plants displayed dark brown coloration, indicating comparatively higher oxidative reaction intensity under CGMMV infection. Among *Trichormus variabilis* treatments, color intensity ranged from dark yellow to light brown. The 75% concentration generally produced lighter coloration across application methods compared with the 50% concentration, while 100% concentration showed intermediate responses depending on application method. However, these observations are based solely on visual comparison of color intensity.  $H_2O_2$  accumulation was assessed strictly as a qualitative indicator using visual classification of KI reaction color.

#### Effect of *Trichormus variabilis* concentration and application method on fruit weight per plant ( $g\ plant^{-1}$ )

The results presented in Table 6 indicated significant effects of biomass concentration, application method, and their interaction on fruit weight per plant ( $P \leq 0.05$ ). Regarding the main effect of concentration, 75% treatment produced the highest mean fruit weight ( $287.67\ g\ plant^{-1}$ ), followed by 100% concentration ( $256.67\ g\ plant^{-1}$ ), with no significant difference between these two levels. In contrast, 50% concentration resulted in a significantly lower mean fruit weight ( $164.22\ g\ plant^{-1}$ ). At the interaction level, seed soaking at the 75% concentration recorded the highest fruit weight per plant ( $343.67\ g$ ), significantly exceeding the other concentration  $\times$  method combinations. Foliar spraying at 75% ranked second, whereas soil drenching treatments generally produced comparatively lower values within the same concentration levels. Considering the main effect of application method, seed soaking resulted in the highest overall fruit weight ( $248.33\ g\ plant^{-1}$ ), followed closely by foliar spraying ( $244.00\ g\ plant^{-1}$ ), while soil drenching recorded the lowest mean value ( $216.22\ g\ plant^{-1}$ ). The healthy control exhibited the highest fruit weight ( $422.33\ g\ plant^{-1}$ ), whereas the infected control showed a markedly reduced value ( $82.67\ g\ plant^{-1}$ ), confirming the substantial negative impact of CGMMV infection on productivity. It should be noted that fruit weight per plant reflects total yield and did not distinguish between potential increases in fruit number and/or fruit size.



Figure 2. Hydrogen peroxide ( $H_2O_2$ ) accumulation in cucumber plants under CGMMV infection

### Effect of *Trichormus variabilis* concentration and application method on fruit firmness (kg cm<sup>-2</sup>)

The results presented in Table 7 demonstrated significant effects of biomass concentration, application method, and their interaction on fruit firmness under CGMMV infection ( $P \leq 0.05$ ). Regarding the main effect of concentration, the 75% treatment recorded the highest mean firmness (8.01 kg cm<sup>-2</sup>), followed closely by 100% concentration (7.99 kg cm<sup>-2</sup>), with no significant difference between these two levels. In contrast, 50% concentration produced a significantly lower mean firmness (7.27 kg cm<sup>-2</sup>). At the interaction level, foliar spraying at the 75% concentration resulted in the highest fruit firmness (8.61 kg cm<sup>-2</sup>), significantly exceeding most other interaction treatments. Soil drenching at 100% ranked among the higher values (8.11 kg cm<sup>-2</sup>), whereas foliar spraying at 50% recorded the lowest firmness (6.43 kg cm<sup>-2</sup>). Concerning the main effect of application method, no significant differences were observed among seed soaking (7.91 kg cm<sup>-2</sup>), foliar spraying (7.63 kg cm<sup>-2</sup>), and soil drenching (7.72 kg cm<sup>-2</sup>), as these treatments shared the same statistical grouping according to Tukey's HSD test. The healthy control

exhibited the highest firmness value (9.91 kg cm<sup>-2</sup>), whereas the infected control showed a markedly reduced firmness (4.67 kg cm<sup>-2</sup>), reflecting the detrimental impact of CGMMV infection on fruit structural integrity.

**Table 5.** Visual estimation of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) accumulation in cucumber leaves under different *Trichormus variabilis* treatments

Treatment methods	Algal concentrations (%)	Number of samples	Observed color
Seed soaking	50	3	Dark yellow
Seed soaking	75	3	Light brown
Seed soaking	100	3	Light brown
Foliar spraying	50	3	Dark brown
Foliar spraying	75	3	Light brown
Foliar spraying	100	3	Dark yellow
Soil drenching	50	3	Light brown
Soil drenching	75	3	Light brown
Soil drenching	100	3	Dark yellow
Healthy control	Transparent / pale yellow		
Infected control	Dark brown		

**Table 4.** Effect of *Trichormus variabilis* concentrations, application methods, and their interaction on relative chlorophyll content (SPAD values) in cucumber plants

Treatments	Concentrations			Mean of treatments
	50%	75%	100%	
Seed soaking	32.21 ± 0.92 CD	36.33 ± 1.04 B	35.34 ± 0.98 B	34.62 ± 0.98 A
Foliar spraying	30.47 ± 0.85 D	33.83 ± 0.91 BC	35.14 ± 1.02 B	33.15 ± 0.93 AB
Soil drenching	30.04 ± 0.88 D	34.34 ± 0.96 BC	32.21 ± 0.89 CD	32.19 ± 0.91 B
Healthy control	43.20 ± 1.21 A			
Infected control	24.71 ± 0.74 F			
Mean of concentrations	30.91 ± 0.88 B	34.83 ± 0.97 A	34.23 ± 0.96 A	-

Note: Values represent Mean ± SE ( $n = 3$ ). Different letters indicate significant differences according to Tukey's HSD test at  $P \leq 0.05$

**Table 6.** Effect of *Trichormus variabilis* concentrations, application methods, and their interaction on fruit weight per plant in cucumber

Treatments	Concentrations			Mean of treatments
	50%	75%	100%	
Seed soaking	160.00 ± 2.13 E	343.67 ± 6.84 B	241.33 ± 4.92 CD	248.33 ± 4.63 A
Foliar spraying	170.67 ± 3.18 E	287.67 ± 5.21 C	273.67 ± 5.74 CD	244.00 ± 4.71 AB
Soil drenching	162.00 ± 2.52 E	231.67 ± 4.86 D	255.00 ± 5.10 CD	216.22 ± 4.16 B
Healthy control	422.33 ± 8.94 A			
Infected control	82.67 ± 1.97 F			
Mean of concentrations	164.22 ± 2.61 B	287.67 ± 5.64 A	256.67 ± 5.25 A	-

Note: Values represent Mean ± SE ( $n = 3$ ). Different letters indicate significant differences according to Tukey's HSD test at  $P \leq 0.05$

**Table 7.** Effect of *Trichormus variabilis* concentrations, application methods, and their interaction on fruit firmness in cucumber

Treatments	Concentrations			Mean of treatments
	50%	75%	100%	
Seed soaking	7.96 ± 0.31 CB	7.78 ± 0.18 CB	8.00 ± 0.21 CB	7.91 ± 0.23 A
Foliar spraying	6.43 ± 0.22 D	8.61 ± 0.14 B	7.87 ± 0.20 CB	7.63 ± 0.19 A
Soil drenching	7.42 ± 0.21 C	7.63 ± 0.20 C	8.11 ± 0.18 CB	7.72 ± 0.20 A
Healthy control	9.91 ± 0.33 A			
Infected control	4.67 ± 0.19 E			
Mean of concentrations	7.27 ± 0.26 B	8.01 ± 0.17 A	7.99 ± 0.19 A	-

Note: Values represent Mean ± SE ( $n = 3$ ). Different letters indicate significant differences according to Tukey's HSD test at  $P \leq 0.05$

## Discussion

### *Disease severity*

The intermediate biomass concentration (75%) resulted in a significant reduction in Disease Severity Index (DSI) compared with the 50% treatment, whereas no significant difference was detected between 75% and 100%. This pattern suggests the presence of a threshold response, in which increasing biomass concentration beyond an optimal intermediate level did not confer additional symptom suppression under the prevailing field conditions. Such a plateau effect may reflect saturation of plant responsiveness to the applied biomass rather than a linear dose-response relationship. Although application method did not exert a significant main effect, the significant concentration  $\times$  method interaction indicates that treatment performance was partially dependent on delivery approach. Because the overall method effect was statistically non-significant, these interaction differences should be interpreted with caution and not overemphasized. The observed reduction in symptom severity is consistent with previous studies reporting that algal-based preparations can mitigate viral symptom expression and improve plant performance under biotic stress (El-Boukhari et al. 2020; Di Sario et al. 2025). Reductions in disease severity under viral infection have been associated with the application of cyanobacterial and algal-derived extracts that can enhance plant defense activation and reduce symptom development through elicitation of resistance-related physiological responses under pathogen pressure (Singh et al. 2016; Righini et al. 2022).

### *Disease incidence*

The observed reduction in disease incidence following treatment with *T. variabilis* extract suggests its potential role in reducing disease development under Cucumber green mottle mosaic virus pressure. This is in agreement with previous reports indicating that CGMMV is a highly destructive cucurbit virus with limited control options (Webster and Jones 2018; Ruiz et al. 2021). Cyanobacteria are known to produce a wide range of bioactive secondary metabolites that may influence plant physiological responses and contribute to improved tolerance under biotic stress conditions (Abo-Shady et al. 2023; Nawaz et al. 2024). These compounds are generally considered to function as elicitors that may activate defense-related responses in plants. The higher effectiveness observed at 75% concentration suggests an optimal dose-response relationship, where moderate levels of bioactive compounds were sufficient to reduce disease incidence, whereas higher concentration did not provide additional reduction. Similar trends have been reported in other studies involving natural elicitors, where intermediate concentrations resulted in the most consistent biological responses (Calvo et al. 2014; du Jardin 2015). Moreover, cyanobacterial treatments have been reported to improve overall plant growth and physiological performance, which may indirectly contribute to reduced disease incidence (Righini et al. 2022). Overall, these results indicate that *T. variabilis* extract can contribute to reducing disease incidence in cucumber plants under CGMMV infection pressure.

### *Infection percentage*

A significant reduction in infection percentage was observed at 75% concentration compared with 50% treatment, whereas 100% concentration did not provide additional improvement over the intermediate level. This response pattern reinforces the non-linear dose relationship observed for disease severity and suggests that maximal protective benefit under the present field conditions was achieved at the intermediate biomass level. Increasing concentration beyond this point did not further reduce infection establishment, indicating a potential biological saturation effect rather than a proportional dose response. Soil drenching exhibited slightly higher infection percentages compared with seed soaking and foliar spraying. However, because no consistent statistical superiority was detected between seed soaking and foliar spraying, the practical differences between these two application methods appear limited. The variation observed among methods may partly reflect differences in spatial distribution of biomass relative to infection sites rather than inherent differences in treatment efficacy. It is also important to acknowledge that mechanical inoculation efficiency can vary under field conditions. Nevertheless, the high infection level observed in the infected control confirms that inoculation pressure was sufficient to ensure reliable disease establishment. The reduction in infection incidence represents a statistically supported outcome. While previous studies have suggested that algal-derived biostimulants may contribute to enhanced stress tolerance (Walters et al. 2013; Ronga et al. 2019; Fahad and Mohamed 2025)

### *Chlorophyll content*

SPAD values were significantly higher at 75% and 100% concentrations compared with 50%, indicating improved chlorophyll retention under CGMMV infection. The absence of a significant difference between 75% and 100% further supports the plateau response observed across several measured parameters, suggesting that the intermediate biomass level was sufficient to maintain leaf pigment stability under viral stress. Increasing concentration beyond this level did not result in additional chlorophyll preservation, which may reflect a biological saturation threshold in the plant response. Seed soaking resulted in slightly higher SPAD values compared with soil drenching. This may indicate that early exposure to the biomass suspension contributed to greater physiological stability during subsequent viral challenge. However, since foliar spraying did not differ significantly from seed soaking, consistent practical superiority among application methods cannot be established based on the present data. It is important to emphasize that SPAD readings provide an indirect estimate of relative chlorophyll content rather than a direct measurement of photosynthetic rate, carbon assimilation efficiency, or chlorophyll fluorescence parameters. Previous studies have reported that algal-derived products may enhance chlorophyll stability under stress conditions (Hernández-Herrera et al. 2014; Al-Fahad et al. 2020). The observed increase in SPAD values is in agreement with reports indicating that algal and microalgal extracts can enhance chlorophyll content and delay pigment degradation

by improving photosynthetic efficiency and protecting chloroplast integrity under stress conditions (El-Nakhel et al. 2022; Arenas et al. 2025).

#### *H<sub>2</sub>O<sub>2</sub> accumulation*

Qualitative variation in hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) accumulation was observed among treatments based on potassium iodide (KI) staining intensity. Leaf extracts from infected control plants exhibited the darkest brown coloration, whereas healthy plants showed very light or pale yellow coloration. Treatments with *T. variabilis*, particularly at 75% concentration, generally showed intermediate staining intensity. The KI assay provides only visual and qualitative assessment; the observed differences represent relative comparisons rather than quantitative measurements of H<sub>2</sub>O<sub>2</sub> concentration. The stronger staining observed in infected controls is consistent with previously reported associations between viral infection and altered oxidative status (Apel and Hirt 2004; Waszczak et al. 2018). KI staining has been widely used as a qualitative indicator of H<sub>2</sub>O<sub>2</sub> presence in plant tissues (Junglee et al. 2014), and early histochemical visualization of H<sub>2</sub>O<sub>2</sub> in plant tissues was described by Peter Schopfer (1994).

#### *Fruit weight per plant (g plant<sup>-1</sup>)*

Fruit weight per plant was significantly higher at 75% compared with 50%, while no significant difference was detected between 75% and 100%. This indicates that the intermediate concentration was sufficient to enhance yield under CGMMV stress conditions without requiring maximal extract concentration. The interaction analysis showed that seed soaking at 75% produced the highest mean value; however, because treatment method alone was not consistently significant across comparisons, the overall response appears primarily concentration-dependent. It should be noted that total fruit weight per plant was recorded as a cumulative yield parameter, and fruit number per plant was not separately quantified. Therefore, it cannot be determined whether yield improvement resulted from increased fruit number, increased individual fruit size, or a combination of both. Although algal extracts have been associated with improved crop productivity in previous studies (Bulgari et al. 2019; Ronga et al. 2019).

#### *Fruit firmness (kg/cm<sup>2</sup>)*

Fruit firmness was significantly improved at 75% and 100% compared with 50%, with no significant difference between the two higher concentrations. This plateau response suggests that increasing extract concentration beyond the intermediate level did not provide additional firmness enhancement under the experimental conditions. Although foliar spraying at 75% yielded the highest interaction value, treatment means did not differ consistently, indicating that biomass concentration was the dominant factor influencing firmness. Previous reports have shown that algal-based biostimulants can influence fruit quality parameters (Yakhin et al. 2017; Shahrajabian et al. 2021).

#### *Limitations of the study*

This study had several limitations first, viral infection was confirmed using symptom observation and ImmunoStrip serological assays; however, no quantitative molecular confirmation (e.g., RT-PCR or ELISA quantification) was performed to determine viral load across treatments. Therefore, differences among treatments reflect disease expression rather than quantified viral accumulation. Second, the algal extract concentration was standardized on a volumetric basis (50%, 75%, and 100%) without biochemical characterization of specific bioactive compounds. Consequently, the exact composition and concentration of active metabolites were not analytically verified. Third, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) accumulation was assessed using a qualitative KI staining method, which provides visual comparison but does not allow precise quantification of oxidative status or antioxidant activity. Finally, the experiment was conducted during a single growing season and under a single field location and cultivar. Therefore, extrapolation of the results to other environmental conditions or genetic backgrounds should be made cautiously.

In conclusion, the present study demonstrated that application of *T. variabilis* extract significantly reduced disease expression and improved selected physiological and yield-related traits in cucumber under CGMMV infection under open-field conditions. Among the tested concentrations, 75% concentration consistently showed the most favorable overall performance, resulting in lower disease incidence and severity, as well as improved chlorophyll content, fruit weight, and fruit firmness compared with the 50% concentration. No significant differences were observed between 75% and 100% concentrations for some parameters, indicating that the response plateaued beyond the intermediate level. Regarding treatment methods, differences among seed soaking, foliar spraying, and soil drenching were generally not statistically significant for most measured traits, suggesting that concentration exerted a stronger influence than application method under the experimental conditions. The observed variation in hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) accumulation among treatments, indicates differences in relative oxidative status. Overall, the findings indicate that *T. variabilis* extract, particularly at intermediate concentration, represents a promising biostimulant candidate for integration into sustainable CGMMV management strategies and environmentally friendly cucumber production systems. Future studies incorporating multi-season validation, quantitative viral diagnostics, biochemical profiling of extracts, and quantitative oxidative stress measurements are recommended.

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