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EFFECT OF FERMENTED *SARGASSUM*-BASED BIO-FERTILIZER ON GERMINATION' GROWTH AND PHOTOSYNTHESIS IN CUCUMBER AND TOMATO SEEDLINGS: A COMPARATIVE STUDY

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Abstract

In recent years, the adverse effects of excessive use of chemical fertilizers in agricultural production have become increasingly evident, and environmental problems have also been caused worldwide. Cucumbers (*Cucumis sativus* L.) and tomatoes (*Solanum lycopersicum* L.) are crops with high output and consumption worldwide. Seaweed fertilizer is a kind of fermentation product obtained by processing seaweed. However, on which dilution factor of seaweed fertilizer has the best effect on the development of crop seedlings is yet to be determined. In this study, we investigated various dilutions of the seaweed fertilizer to determine the most appropriate dilution that has the most potent effect on the growth of cucumber and tomato seedlings. Studies have shown that when the dilution factor of the seaweed fertilizer dilution is 0.6%-1.2%, it substantially improves the seed germination indices, including Germination energy and Germination rate of cucumber and Tomato seedlings. Similarly, moderate seaweed fertilizer concentrations (0.8%) enhanced both crops' leaf and cotyledon areas. Stem thickness and height were also increased after applying seaweed fertilizers (from 0.6 to 1%). Photosynthetic pigments, including Chlorophyll 'a', chlorophyll "b", total chlorophyll and carotenoid contents, were also higher after moderate applications of seaweed fertilizer. The research results can be used as a reference for applying seaweed fertilizer dilution in cucumber and tomato seedling breeding.

Key words: *Cucumis sativus* L.; Seaweed fertilizer; *Solanum lycopersicum* L.; Breeding

Introduction

Cucumbers (*Cucumis sativus* L.) and tomatoes (*Solanum lycopersicum* L.) are two of the world's most produced and consumed vegetables. In recent years, the area and total yield of cucumber and tomato have steadily increased. The output of cucumbers was from 354.7 M tons in 2013 to 465.9 M tons in 2022, and the production of tomatoes was from 766.4 M tons in 2013 to 871.5 M tons in 2022 (Anon., 2022). The quality of crops at the seedling stage has a profound impact on the subsequent growth and yield. Especially in modern agricultural technology production, which widely adopts factory seedlings, higher germination rate, lower mean germination time (MGT), and higher seedling growth rate are the basis for achieving higher economic benefits (Pablo *et al*., 2020). Shorter germination time means higher yield; more vigorous seedlings will bring better growing plants, produce more high-quality fruits, and get higher yields (Businelli *et al*., 2015). Based on the existing land planting environmental conditions, increase in the production of cucumbers and tomatoes will bring substantial economic and environmental benefits (Yinkun *et al*., 2019).

Seaweed is a rich and renewable natural resource, including green, brown, and red macroalgae. These seaweeds are easy to collect as raw materials and have great potential in developing processed products. Seaweed fertilizer is a kind of organic fertilizer produced by pre-processing the collected seaweed and then processing it through a series of steps (Lopez-Mosquera *et al*., 2011). Currently, the standard processing methods mainly include stirring macerated seaweed in vats containing hot water, acidic or alkaline hydrolysis with or without steam, pressure burst techniques or the cell burst method, and others (Rengasamy *et al*., 2016). Seaweed fertilizers can affect the growth of crops because they contain various elements necessary for plant growth, different types of hormones, and higher oxygen content (Battacharyya *et al*., 2015; Chanthini *et al*., 2019; Pablo *et al*., 2020). During the growth of crops, the appropriate concentration of dilute seaweed fertilizers has positive effects (Selvam & Sivakumar, 2014; Vijayakumar, 2019). At the same time, the seaweed fertilizer also improves soil fungal communities and soil enzyme activities (Wang *et al*., 2017). In addition, the application of seaweed fertilizer also promotes composting processes (Guixiang *et al*., 2019). Recent studies have confirmed that seaweed fertilizer has a promising impact on crop growth and yield and has been used as an environmentally friendly biofertilizer (Ashok-Kumar *et. al*., 2012; Battacharyya *et al*., 2015). However, currently, there are no clear standards for the development, production, and application of seaweed biofertilizers.

The overuse of chemical fertilizers has caused many problems (Głąb & Gondek, 2014; Wei *et al*., 2018;). Food safety and environmental issues also urge researchers worldwide to find new ways to increase crop yields. Although seaweed fertilizer has received wide attention as a new organic fertilizer, researchers still need to explore its standardization and use. The research on the effect of

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seaweed fertilizer on the growth of cucumber seedlings and tomato seedlings by different dilution times in this study is part of the standardization of seaweed fertilizer application. Further, there is a need to investigate various kinds of seaweed and processing techniques to develop more effective seaweed-based biofertilizers at minimum applied concentrations. The initial phases of seed germination and seedling growth are crucial for the development and productivity of crops. The objective of the study was to investigate the indicators of germination and seedling growth rates of cucumber and tomato after the seaweed fertilizer diluent with various concentration gradients to standardize when the seaweed fertilizer production process using *Sargassum horneri* as a raw material. It will provide a practical basis for the production process of cucumber and tomato breeding and seedling cultivation when using seaweed fertilizer.

Material and Methods

Collection of seaweeds: The seaweed used in the present study was *Sargassum horneri*, belonging to the Phaeophyta (group of brown algae). Material of this species was collected from Nan Huang Cheng island, Peng Lai, Shandong Province (Latitude 38°21N; Longitude 120° 54 E). Seaweed material was collected carefully by hand and washed with seawater to remove debris, shells, and sand. The seaweed was placed in polythene bags, kept in an ice box containing slush ice, and transferred to the laboratory. Then, the material was washed thoroughly using tap water, and maximum care was taken to remove the salt and epiphytes from the surface of the samples.

Preparation of seaweed liquid fertilizers: The seaweed was cut into small pieces separately, oven-dried for 72 h at 60°C, and then ground in an electric mill to less than 0.15 mm. Seaweed powder was mixed with distilled water in a ratio of 1:40 (w/v). It was sterilized at 121℃ for 20 min. After the temperature of the seaweed mixture was lowered to room temperature, *Bacillus halosaccharovorans* was added to the mixture and fermented at 45°C for 8 to 14 h. This strain of *Bacillus halosaccharovorans* was purchased from China General Microbiological Culture Collection Center and cultured in LB medium at 45°C for 12 h before being added to the seaweed mixture. The fermentation liquid was treated with a 100% concentration of seaweed liquid fertilizer. Various concentrations of dilution of seaweed fertilizer were prepared using sterilized distilled water in the following amounts: 0%(control), 0.2%, 0.4%, 0.6%, 0.8%, 1.0%, 1.2%, 1.4%, 1.6%, and 2%). Later, they were refrigerated at 4°C for future application.

Collection of seeds: Cucumber and tomato seeds were purchased from Yantai Seed Company. All the seeds used in this study were carefully chosen having similar dimensions and colour. The surface of the seeds was sterilized with 0.1% mercuric chloride for 10 minutes, then washed in sterile distilled water three times and absorbed surface moisture with filter paper. The experiment was conducted in the Biochemical Laboratory of the Yantai Institute of China Agricultural University from October to November 2023.

Seed germination test: Cucumber and tomato seeds, after surface sterilization treatment, were treated at different seaweed fertilizer concentrations and divided into ten groups of 100 seeds each. The 100 seeds were divided into five groups (5 parallel controls), 20 seeds each; each group of seeds was immersed in 20 mL of the seaweed liquid fertilizer of each concentration in sealed conical flasks, 25°C (16/8, Light/Dark) for 12 h. After the immersion, the seeds were taken out of the treatment solution and evenly spread in Petri dishes (9 cm) with filter paper at the bottom (20 seeds per dish). Each treatment group was given the seaweed liquid fertilizer solution corresponding to the concentration, incubated at 25±0.5°C. From the beginning, the germination of the seeds in the petri dishes was observed every 12 hours, and the number of germinated seeds (radius extension> 2 mm) was recorded.

Different seed germination indices were observed, including.

1) $$\text{Germination energy} = \frac{\text{Number of germinated seeds within 3d}}{\text{Number of tested seeds}} \times 100$$

2) $$\text{Germination energy} = \frac{\text{Number of germinated seeds within 7d}}{\text{Number of tested seeds}} \times 100$$

Seedling preparation: The cucumber seeds and the tomato seeds were cultured for 72 and 96 hours, respectively, 30 seedlings of the same size and growth status of the same group were selected from each group for the seedling stage test. The seedling stage test was carried out in a seedling tray (size), one per hole, and the transplanting substrate was a mixture of peat, vermiculite, and perlite (2:1:1). After the transplantation, each plant was irrigated with 30 mL of the dilution liquid of seaweed liquid fertilizer having the same concentration as the seedlings at their germination. The seedlings were cultivated at 22/17°C (Day /Night), with a humidity of 50% and 12 /12h (Light/Dark). The spraying and watering of the seaweed liquid fertilizer diluent were performed at 17:00 every day. After every 48 hours, different concentrations of seaweed liquid were sprayed thoroughly on the upper and lower surfaces of the leaves or cotyledons of seedlings. The dilutions (about 30 mL) of each concentration gradient were irrigated every 72 hours. Cucumbers were tested on the 25th day, while tomatoes were on the 30th day from the start of germination.

Analysis at the seedling stage

Measurement of seedling leaf area: The area of leaves and cotyledons was measured by calculating the number of grids. Shape of the leaf and cotyledon was drawn on transparent paper, and then the number of grids in the leaf outline was counted. During the grids' calculation, when the blade's edge exceeded half of the grid, it was counted as 1, and in case of less than half of the grid, it was not counted. The length and width of the grids on the graph paper were 1mm, hence the data was given in mm^2.

In measuring the cotyledon area of cucumber, ten plants from each group having the same growth potential were randomly taken and the area of two cotyledons from one plant was calculated. The average was calculated as cotyledon area of the group. While measuring the leaf area of the first true leaf of cucumber and tomato, plants were randomly chosen (10 plants/ treatment group) and area was measured. The average of these parameters were taken.

Seedling rhizome indicators: Ten representative plants of each growing group were taken from each treatment group, and the thickness of the stem of the plant (first neck section) was measured with a Vernier calliper. The seedlings' height (from the stem's base to the growth point) and root length (from the base of the stem to the root tip) were measured. Fresh and dry weights of the shoots and roots were also recorded and shown as the mean of each treatment group.

Chlorophyll content: For each treatment group, 10 representative plants were randomly selected to determine chlorophyll contents. Each leaf was weighed and then put into a stoppered triangle bottle containing chilled ethanol-acetone (1:1). After that, extraction was performed in the dark, the extracts were centrifuged twice. The absorbance of the supernatant was recorded at a wavelength of 663 and 645 nm using an ultraviolet-visible spectrophotometer, while for carotenoid contents, absorbance was recorded at 480 and 510 nm. The chlorophyll and carotene contents were calculated as mg/g fresh leaf weight (Arnon, 1949).

Analysis of data: Treatments were compared for significant differences by One-way ANOVA. Duncan Multiple Range Test (DMRT) was performed as a follow-up of ANOVA.

Results

The effect of seaweed fertilizer on the germination of cucumber and tomato seeds: According to Table 1, at 1.2% concentration of seaweed fertilizer, the germination of cucumber seeds was improved. However, the promotion effect gradually declined in lower concentrations of seaweed fertilizer. A higher concentration (1.6% or more) inhibits the seed germination, and at 2% seed showed no germination.

When the concentration of the seaweed fertilizer was lower than 1.2% for tomato seeds, it showed the best promotion effect on accelerated germination. The promotion effect was gradually decreased at 1.4% or lower concentration of seaweed fertilizer. However, at higher concentration (more than 1.4%) the germination of seeds was inhibited.

The effect of seaweed fertilizer on cucumber and tomato seedlings

The effect of seaweed fertilizer on the area of cotyledon and true leaf of cucumber and tomato seedlings: The cotyledon area of cucumber seedlings was inhibitory when treated with higher concentrations (greater than 1.6%) of seaweed fertilizer. The highest cotyledon area in cucumber was observed at 0.6 and 0.8% concentrations of seaweed fertilizers (Fig. 1a). Continuous decline in cotyledon areas was observed in concentrations higher than 0.8% of seaweed fertilizer. Similarly, concentrations lower than 0.6% showed lower cotyledon areas in cucumber seedlings. Similarly, the concentration of 0.8% of the seaweed fertilizer in tomatoes displayed the largest cotyledon area of seedlings. The diluent of seaweed fertilizer promoted an increase in the cotyledon area of tomato seedlings. When the dilution factor of sea weed fertilizer was reduced from 0.2% to 0.8%, the promoting effect on cotyledons' area was decreased. Also, it was observed that with the increase in concentrations from 0.8 to 1.6%, the promoting effect was declined significantly in the seedlings (Fig. 1a).

Leaf areas of both seedlings i.e. cucumber and tomato, showed similar results when tested at various concentrations of seaweed fertilizers. When the concentration of seaweed fertilizer dilution was 0.8%, the effect of seaweed fertilizer on the growth of true leaves of cucumber and tomato seedlings was most obvious. The concentrations lower than 0.8% showed a significant reduction of leaf areas of cucumber and tomato seedlings. Further, when we applied concentrations higher than 1%, the inhibition of leaf areas was apparent in both crops (Fig. 1b).

The effect of seaweed fertilizer on cucumber and tomato seedling stem thickness and stem height: At concentrations between 0.2-1.4%, seaweed fertilizer promoted the stem length and stem thickness of cucumbers and tomatoes (Fig. 2a and b). When the dilution factor exceeded 1.4%, the seaweed fertilizer inhibited the growth of cucumber and tomato stem length and thickness. For cucumber seedlings, at the dilution factor of 0.6%, the seaweed fertilizer showed the highest increase in seedling stem thickness and length. In tomatoes, the promotion effect was attenuated with the concentration decrease when the dilution was lower than 0.6%. The promotion effect was increased when concentration were between 0.8 to 1.4% (Fig. 2a). The dilution factor of 0.8% for tomato seedlings had the most apparent impact on the stem length. However, below than 0.8%, the promotion effect was attenuated. Similarly, the promotion effect was decreased when the dilution ratio was above 1.4% (Fig. 2b).

Table 1. The effect of seaweed fertilizer on the germination energy and germination rate of cucumber and tomato seeds.

Concentrations of seaweed fertilizer (%)	Cucumber		Tomato	
	Germination energy (%)	Germination rate (%)	Germination energy (%)	Germination rate (%)
*CK	81.4 ± 3.3d	91.2 ± 6.3c	82.5 ± 3.9a	91.5 ± 2.9bc
0.2	82.2 ± 2.1c	92.2 ± 2.3bc	84.2 ± 2.6a	92.7 ± 1.3bc
0.4	82.4 ± 3.2c	92.4 ± 4.2bc	85.5 ± 2.4a	94.5 ± 3.2ab
0.6	85.6 ± 4.5bc	93.2 ± 6.3b	88.8 ± 5.2a	93.8 ± 4.2b
0.8	86.9 ± 3.3bc	93.9 ± 2.3b	89.2 ± 4.7a	96.2 ± 3.7ab
1.0	89.4 ± 4.5b	96.2 ± 8.5ab	91.2 ± 3.1a	98.8 ± 2.5a
1.2	92.1 ± 2.4a	98.5 ± 6.4a	92.0 ± 4.1ab	95.0 ± 7.1ab
1.4	92.2 ± 3.3a	91.2 ± 6.3c	88.2 ± 2.8b	93.8 ± 4.8b
1.6	87.8 ± 6.6b	88.8 ± 10.3cd	83.8 ± 2.2b	93.8 ± 2.2b
2.0	85.5 ± 3.7bc	87.5 ± 9.6d	83.2 ± 1.8b	90.2 ± 1.8c

Note: Different lowercase letters (a, b, c, d) indicate significant (p<0.05) differences between different treatments in the effect of seaweed fertilizer dilution on the germination energy and germination rate of cucumber and tomato seeds.

* Controls, with distilled water on filter papers in Petri plates

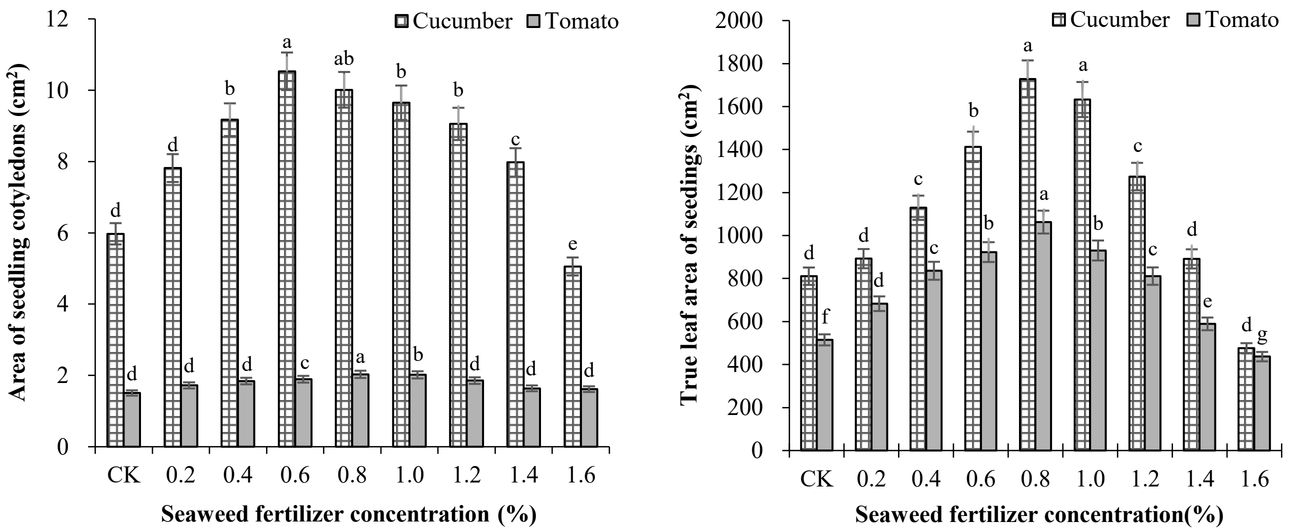

Fig. 1. The effect of seaweed fertilizer on cotyledons (a) and leaf (b) area of cucumber and tomato seedlings. Bar graphs were drawn using the average data (mean ± standard error of mean as error bars) from 10 randomly selected seedlings of tomato and cucumber. According to Duncan's multiple range test (DMRT), Varied alphabets indicate significant differences between the bars. CK=controls.

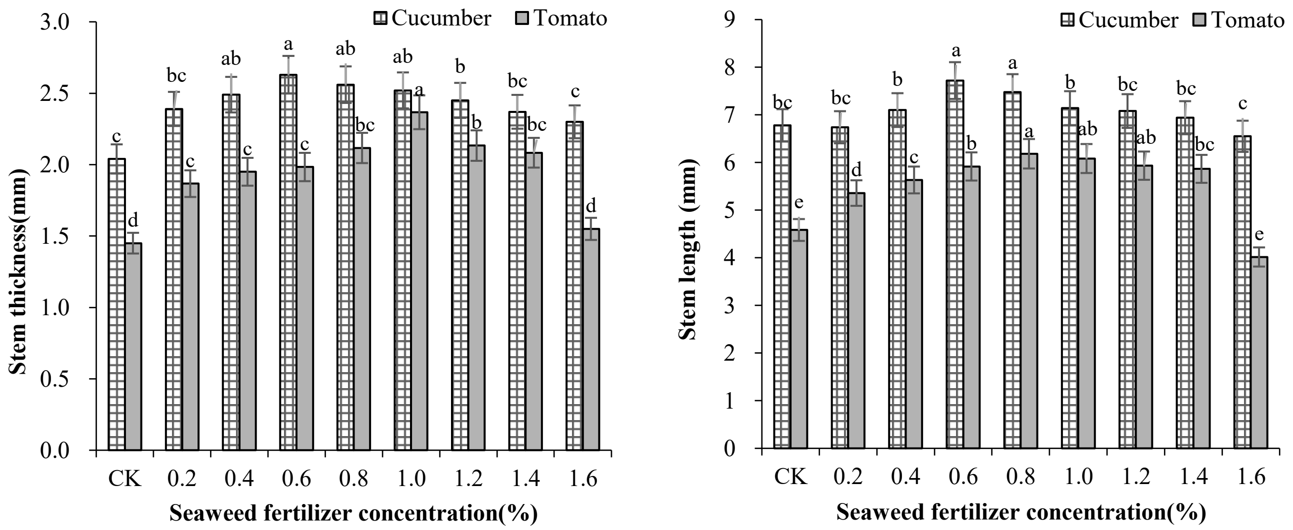

Fig. 2. The effect of different concentrations of seaweed fertilizer on the stem thickness (a) and stem lengths (b) of cucumber and tomato seedlings.

The effect of seaweed fertilizer on the water content of cucumber and tomato seedlings

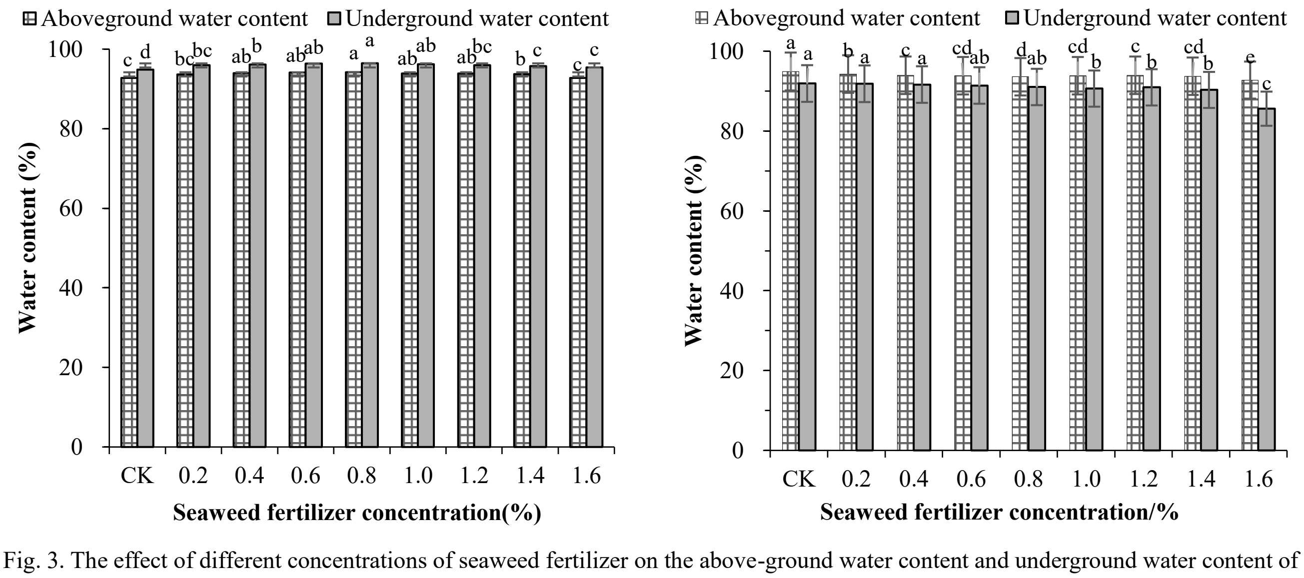

Fig. 3. The effect of different concentrations of seaweed fertilizer on the above-ground water content and underground water content of Cucumber (a) and tomato (b) seedlings.

The effect of seaweed fertilizer dilution on the root-shoot ratio of cucumber and tomato seedlings

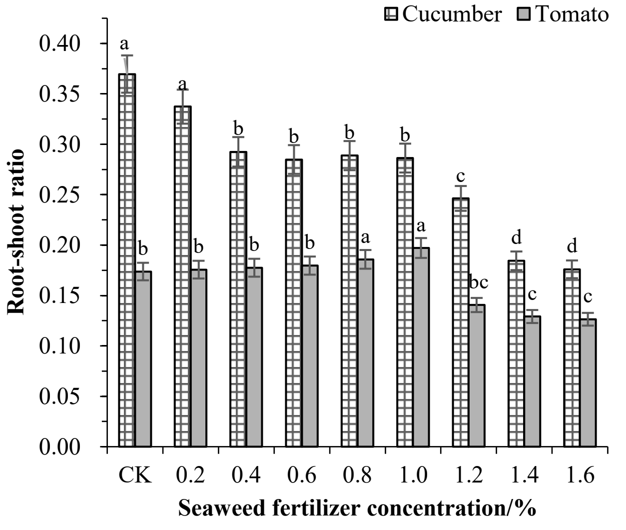

Fig. 4. The effect of different concentrations of seaweed fertilizer on the root-shoot ratio of cucumber and tomato seedlings.

For cucumber seedlings, the dilutions between 0.2-1.4% of the seaweed fertilizer increased the water content of the above-ground- and underground parts. At a dilution of 0.6%, the water content on the above-ground part was reached the highest. Whereas at 0.8% of dilution, the water content of the underground part was found the highest (Fig. 3a).

The seaweed fertilizer dilution reduced above-ground and underground water contents of tomato seedlings. At the 0.8% dilution, the water content of the above-ground part of tomato seedlings was the lowest. With the decrease of the dilution of the seaweed fertilizer, the effect of the dilution on the lowering of the water content of the tomato seedlings in the ground was increased gradually (Fig. 3b).

For cucumber seedlings, the higher the dilution factor of the seaweed fertilizer, the lower was the root-to-shoot ratio of the seedlings. For tomato seedlings, when the dilution ratio of seaweed fertilizer was 0.2-1%, promoted the seedling root-shoot ratio. When the dilution ratio of seaweed fertilizer was 1%, the root-shoot ratio of the seedling reached the maximum. As the dilution ratio of seaweed fertilizer was increased from 1%, the seedling root-shoot ratio was gradually decreased in cucumber and tomato. When the seaweed fertilizer dilution factor was 1%-1.6%, the seaweed fertilizer had an inhibitory effect on the root-to-shoot ratio of both crops (Fig. 4).

The effect of seaweed fertilizer dilution on the photosynthetic pigments of cucumber and tomato seedling leaves: In cucumber seedlings, when the dilution of the seaweed fertilizer was between 0.8-1.2%, the chlorophyll and carotene contents of cucumber seedlings were increased. At the dilution of seaweed fertilizer was 1 to 1.2%%, the content of chlorophyll a in seedlings and cotyledons and chlorophyll b in cotyledons were the highest. When the dilution factor of seaweed fertilizer was 1.6%, chlorophyll contents in true leaves and cotyledons of seedlings and carotenes were found to be highest. (Fig. 5).

When the seaweed fertilizer dilution was between 0.2- to 0.6% for tomato seedlings, an increase in the chlorophyll and carotene contents was observed. At the lower (<0.06%) dilution factor, there was no inhibitory effect on the chlorophyll and carotene content of tomato seedlings. The dilution factor rose from 0.8%, and the contents of true leaf chlorophyll a and chlorophyll b, cotyledon carotene, and total chlorophylls were declined continuously (Fig. 6).

Discussion

This study explored the impact of seaweed fertilizer with different dilutions on the growth of cucumber and tomato seedlings at the initial stages. It was evident that the dilution ratio of seaweed fertilizer between 0.6 and 1.2% has the most apparent promoting effects on the growth of cucumber and tomato seedlings. Germination indices were found to be higher in these concentrations. Comparing the development of cucumber and tomato seedlings under the action of seaweed fertilizer, it was observed that the cucumber seedlings showed a higher response than tomato seedlings. It is known from previous research reports that seaweed fertilizer has a universal growth-promoting effect on many horticultural crops (Battacharyya *et al.*, 2015) and can change the diversity and community structure of soil bacteria in the rhizosphere of plant roots (Chen *et al.*, 2020). Further, recently, it was reported that seaweed fertilizers derived from brown algae, when inoculated in the soil or applied on foliar parts, showed sound stimulating effects on crop productivity and development due to oligo carrageenans (Mukherjee & Patel, 2020). These oligo carrageenans stimulate cell division, nitrogen assimilation, and the process of photosynthesis (Bi *et al.*, 2011). Seed germination indices and seedling growth were improved in cucumber and tomato after applying sargassum-based fertilizer. However, our results demonstrate that this promoting effect on seed germination and seedling vigor is concentration-dependent. Further, the specific concentrations vary when comparing the two crops. Our results suggest that for better results, seaweed fertilizer should be screened before application to a particular plant species. Both crops also demonstrated that the application of higher concentrations may be inhibitory. The effect of seaweed fertilizer on seed germination and seedling growth is caused by plant growth regulators (auxin, cytokinin, and gibberellin), amino acids, and minerals (Qingyu *et al.*, 2020; Vasuki *et al.*, 2020).

The area of leaves and cotyledons in both tested crops has improved significantly in concentrations between 0.6 to 1.2% of seaweed fertilizers. Higher and lower than this range significantly reduced these parameters in both crops. Leaf and cotyledon areas are two very important factors for growth and development in the initial stages. Both parameters provide photosynthetic area to comparatively large photosynthetic dependent organs of the seedlings. The ratio between the photosynthetic and non-photosynthetic parts is higher in the early stages. An increase in leaf and cotyledon areas lowers this ratio for faster growth and strength of seedlings. Removal or smaller size of cotyledons reduces growth parameters, including stem diameter, shoot and root lengths, and number of leaves (Nunes *et al.*, 2024). Water contents in the above-ground and below-ground parts of cucumber and tomato change slightly after applying seaweed fertilizer. This slight increase in water contents may be correlated with the area of cotyledons and leaves of seedlings. An increase in the area of cotyledons and leaves enhanced the transpiration rate, ultimately increasing the water absorption from the soil. This improvement in water uptake may result from a slight improvement of water contents in the seedlings of cucumber and tomato.

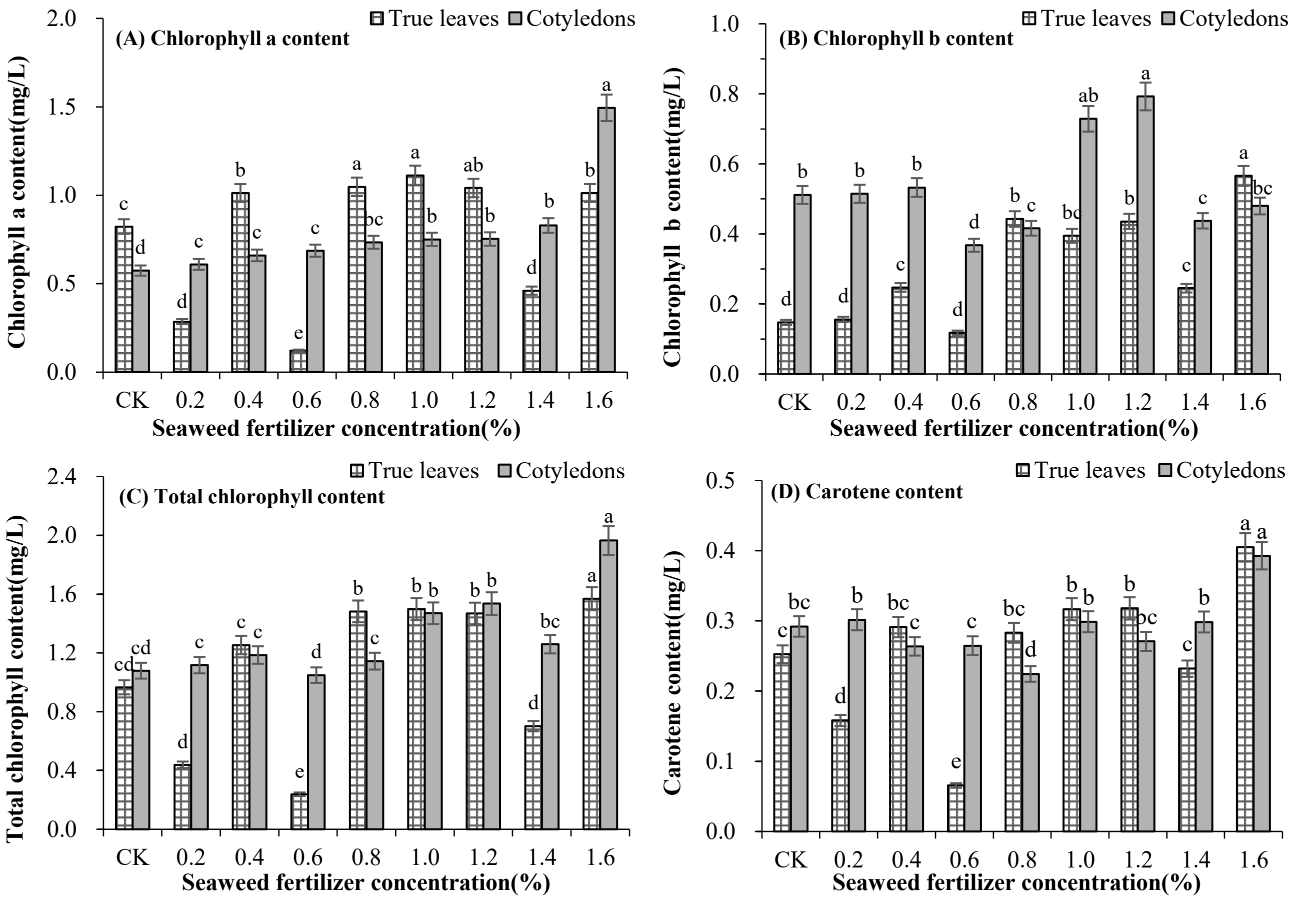

Fig. 5. The effect of different concentrations of seaweed fertilizer on the pigment concentration of cucumber seedlings.

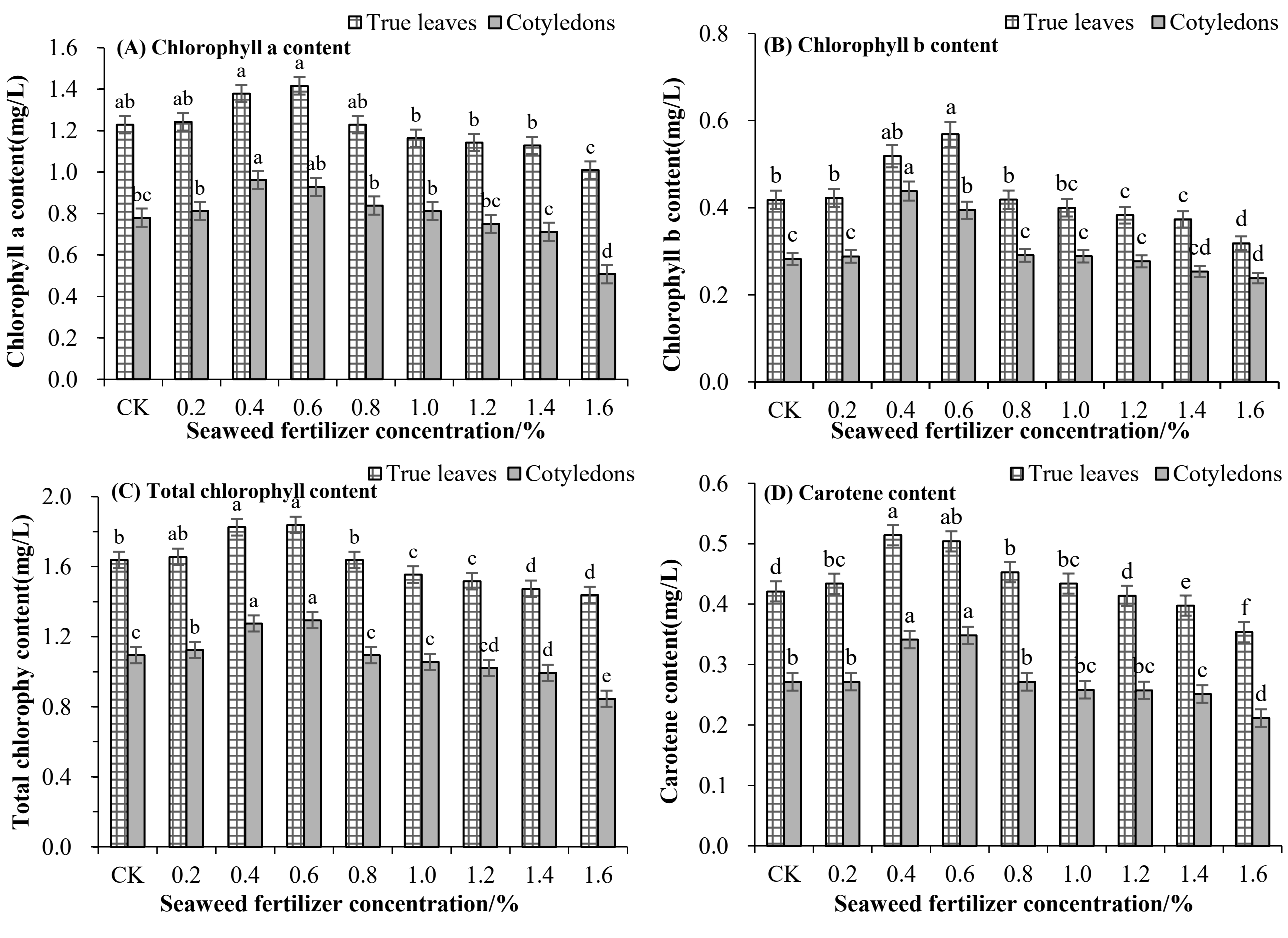

Fig. 6. The effect of different concentrations of seaweed fertilizer on the pigment concentration of tomato seedlings.

Different photosynthetic pigments also revealed an improvement in these optimum ranges of seaweed fertilizers, indicating overall enhancement in photosynthetic machinery's capacity for higher metabolic reactions. In recent studies, Sargassum-based fertilizer improved tomatoes' yield, ripening time, and photosynthetic capacity (Yao *et al*., 2020). Different concentrations of plant growth regulators have dissimilar effects on various crops at different growth stages. It appeared that even in a species, the application concentrations should be known according to the growth stages of the plants to achieve higher productivity. Therefore, nursery work is an essential step in the cultivation process of horticultural crops. It is indispensable to use environmental friendly organic agricultural methods to promote the healthy growth of horticultural crop seedlings and help them lay the foundation for future high-quality products.

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