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Physiological Response of Fenugreek (*Trigonella foenum-graecum* L.) Seeds and Seedlings to *Dasycladus vermicularis* and *Jania rubens* Seaweed Extracts

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Abstract

This study evaluates the biostimulatory efficacy of aqueous macroalgal extracts derived from *Dasycladus vermicularis* (DVAE) and *Jania rubens* (JRAE) as sustainable biofertilizers. The physiological impacts of these extracts were assessed across a gradient of concentrations (0.1%, 0.5%, 1.0%, 1.5%, and 2.0%, alongside an untreated control) on the germination dynamics and early seedling ontogeny of *Trigonella foenum-graecum*. Empirical observations revealed that exogenous application of both donor species significantly enhanced key germination indices, including germination percentage (GP), seed germination index (SGI), and germination energy (GE). Furthermore, vegetative vigor was positively influenced, as evidenced by a marked elongation in both plumule (PL) and radicle (RL) lengths. This morpho-physiological promotion was strongly coupled with an elevation in the total photosynthetic pigment profile of *T. foenum-graecum* seedlings, driven by the simultaneous upregulation of chlorophyll a and chlorophyll b contents. Given the pronounced biofertilizing potency exhibited by *D. vermicularis* and *J. rubens, particularly at lower concentrations, further field-scale investigations* are warranted to validate their selective agronomic performance under diverse environmental conditions.

Keywords: Biofertilizers, Germination Bioassay, Extracts, *Dasycladus vermicularis*, *Jania rubens*, *Trigonella foenum-graecum.*

INTRODUCTION

Throughout human history, plants have played a fundamental role as a major source of medicinal remedies and therapeutic compounds (Klebe, 2025). Although allopathic medicine has undergone significant advancements over the past century, plants continue to play a vital role as a source of therapeutic compounds in both conventional and traditional medical practices across the globe. Plant alkaloids and other naturally occurring substances from plants, fungi, and bacteria are sources of many pharmaceutical medications (Adejoke et al., 2019). It has been estimated that more than 60% of medicines are plant-based (Allkin, 2017). The high content of bioactive compounds in medicinal plants highlights their importance as a source of novel drug candidates. Among these metabolites are alkaloids, glycosides, coumarins, flavonoids, steroids, and numerous other biologically active compounds with therapeutic potential. Because of its high nutritional value and availability of bioactive components, including flavonoids, saponins, and alkaloids, fenugreek *(Trigonella foenum-graecum* L.), a widely known leguminous species, has been used extensively in traditional medicine and as a dietary supplement (Petropoulos, 2002). Because fenugreek seeds have anti-inflammatory, antioxidant, and antidiabetic qualities (Wani, 2025; Basch et al., 2003), this plant is crucial for both agricultural and medical research. When planted in the right soil, fenugreek seeds sprout in about three days. According to Guiry and Vequist (2011), the seedlings grow into upright or semi-erect plants that can reach heights of 30 to 60 cm. By fixing nitrogen, fenugreek, an annual leguminous plant that self-pollinates, improves soil fertility. The green, thin bushes have yellow-brown pods that hold ten to twenty seeds. The seeds have dimensions of 3–6 mm in length, 2–5 mm in breadth, and roughly 2 mm in thickness (Im & Maliakel, 2008). They are brownish-yellow, hard, tiny, and rhomboidal in shape (Guiry et al., 2011).

Fenugreek seeds are characterized by a pleasant bitter taste and a distinctive maple-like flavor. They are biologically endospermic and possess a gelatinous, fibrous, and adhesive texture. The seeds consist of a thick, corneous layer of white, translucent endosperm that surrounds a yellow embryo and a hard central core. Fenugreek seeds possess a rich chemical composition characterized by high levels of dietary fiber, phytonutrients, and diverse phytochemicals (Giridhar et al., 2023). About 23–26% of the seeds are protein, 6–7% are lipids, and 58% are carbs, with dietary fiber making up almost 25% of the carbohydrate fraction. With a rich profile of essential nutrients and therapeutic properties, fenugreek serves as a highly valuable dietary resource. In its dried form, the plant is an excellent source of iron, yielding approximately 33 mg per 100 g of dry weight. Meanwhile, fresh fenugreek leaves are predominantly composed of moisture at 86.1%, supplemented by 6% carbohydrates, 4.4% protein, 1.1% fiber, 0.9% fat, and 1.5% essential minerals. Beyond these macronutrients, the leaves are packed with vital micronutrients, including calcium, phosphorus, thiamine, riboflavin, niacin, and carotene. Notably, a 100 g serving of fresh leaves delivers impressive antioxidant support with roughly 220.97 mg of ascorbic acid (vitamin C) and 19 mg of beta-carotene. Furthermore, fenugreek is deeply enriched with potent bioactive phytochemicals, specifically flavonoids, alkaloids, and saponins, which act as the primary drivers behind the plant's diverse medicinal and pharmacological benefits. Likewise, marine algae constitute an important natural reservoir of bioactive compounds and have attracted considerable attention as a complementary source of therapeutic and nutritionally valuable metabolites alongside terrestrial medicinal plants. Seaweeds are increasingly recognized as valuable natural resources for sustainable agriculture due to their high content of plant growth regulators, macro- and micronutrients, and potassium salts, all of which contribute to improved crop growth and productivity (Kumawat & Kumawat, 2023).

The utilization of seaweeds as biofertilizers and soil conditioners has garnered significant interest due to their capacity to enhance soil fertility and serve as effective green manure (Battah et al., 2021). They supply crucial macronutrients such as nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur alongside essential trace elements like zinc and copper. Additionally, marine algae naturally synthesize phytohormones, including auxins, gibberellins, and cytokinins, which play a fundamental role in regulating plant growth and development (Rengasamy, 2004). Commercial fertilizers leveraging these benefits are manufactured from diverse brown seaweed genera such as *Ascophyllum*, *Macrocystis*, *Laminaria*, *Ecklonia*, *Durvillaea*, *Cabophyllum*, *Himanthalia*, *Sargassum*, and *Turbinaria* as well as red seaweeds, including *Pachymenia*, *Lithothamnion*, and *Phymatolithon* (Rao et al., 2018).

A substantial body of research confirms that seaweed-derived products stimulate plant development, boost antioxidant capacity, and elevate tolerance to environmental stresses, underscoring their value as eco-friendly biostimulants in contemporary agriculture (Craigie, 2011; Zhang & Schmidt, 2000; Zhang et al., 2003; Briceño-Domínguez et al., 2014; Hernández-Herrera et al., 2014). Consequently, these traits have fueled a growing interest in marine algae within agricultural and biotechnological research. In particular, pairing these natural marine biostimulants with medicinal herbs like fenugreek has attracted considerable scientific focus, offering a promising strategy to optimize plant growth, crop productivity, and phytochemical composition.

MATERIALS AND METHODS

Study Site

The study was conducted in March 2025 on the Al-Hamama coast, located on the northeastern Mediterranean shoreline, north of Al-Bayda, Libya. This coastal area served as the sampling site for the collection of marine macroalgae used in the present investigation. The study aimed to evaluate the agricultural potential of two marine seaweed species as biostimulants by assessing their effects on seed germination and selected physiological growth parameters of fenugreek (*Trigonella foenum-graecum* L.; family Fabaceae), which was used as the experimental model crop.

Collection and Processing of Seaweed Samples

Manual harvesting of seaweeds has been practiced for centuries and remains the most common method for collecting naturally growing species from coastal habitats (Van Poelgeest et al., 2013). Fresh specimens of two marine macroalgal species were collected manually during low tide from the intertidal zone of the Al-Hamama coast in March 2025. The collected species included the green alga *Dasycladus vermicularis* (Scopoli) Krasser (Phylum Chlorophyta, Family Dasycladaceae) and the red alga *Jania rubens* (Linnaeus) J.V. Lamouroux (Phylum Rhodophyta, Family Corallinaceae) (Figure 1).

Dasycladus vermicularis *Jania rubens*

Figure (1). Marine seaweed species used in the present study

The collected samples were thoroughly washed with seawater to remove sand, epiphytes, and other debris, followed by rinsing with distilled water. The cleaned seaweeds were shade-dried at room temperature and then oven-dried at 60°C until a constant weight was achieved. The dried materials were ground into a fine powder and stored in airtight containers for subsequent preparation of aqueous extracts (Lobban & Harrison, 1994; Lee, 2008).

Taxonomic Identification

Taxonomic identification of the collected specimens was performed at the Department of Botany, Faculty of Science, Omar Al-Mukhtar University. The macroalgae were identified using standard taxonomic keys and classification methodologies outlined by (Oswald et al., 1960; Abbott & Hollenberg ., 1976; Al-Ahmad & Aleem .,1993; Jha ., 2009). Species nomenclature adhered to the classification framework proposed by (Guiry., 2011), and all scientific names were formally verified using the AlgaeBase database.

Preparation of Aqueous Seaweed Extracts

To prepare the biostimulatory treatments, the freshly harvested seaweed samples were first meticulously rinsed with water to eliminate any remaining debris and impurities. The cleaned biomass was initially shade-dried at ambient temperature for 2–4 days, followed by oven-drying at 60°C for 12 hours to achieve a constant dry weight. Once thoroughly dehydrated, the material was manually broken down and finely pulverized using a mechanized laboratory grinder. The aqueous stock extracts were prepared by mixing 100 g of the resulting seaweed powder with 1000 mL of distilled water, allowing the mixture to infuse for 24 hours. The crude suspension was then filtered through a double layer of muslin cloth; the obtained filtrate was designated as the 100% stock aqueous extract (Ramarajan, 2012). From this primary solution, a series of working concentrations, specifically 0.1%, 0.5%, 1.0%, 1.5%, and 2.0% were prepared by dilution with distilled water, while pure distilled water was retained as the control treatment. These prepared aqueous extracts of *Dasycladus vermicularis* (DVAE) and *Jania rubens* (JRAE) were stored at 4°C to preserve their integrity prior to evaluating their effects on the germination performance of *Trigonella foenum-graecum* seeds.

Germination Bioassay

A Petri dish bioassay was conducted to evaluate the biostimulatory effects of the aqueous seaweed extracts on the germination dynamics and early seedling development of *Trigonella foenum-graecum* L. The measured parameters included germination percentage (GP), seed germination index (SGI), germination energy (GE), plumule length (PL), and radicle length (RL). Prior to experimental setup, the seeds were surface-sterilized by immersion in a 2% Clorox solution for 2 minutes, rinsed four times with distilled water, and subsequently soaked in aerated distilled water for 24 hours to initiate imbibition. Following this, ten uniform seeds were placed into each 9-cm diameter Petri dish, which had been lined with two layers of Whatman No. 1 filter paper. The trial was carried out in a controlled laboratory environment with diurnal temperatures maintained between 19–22°C during the day and 12–14°C at night (Bewley et al., 2013).

The experimental layout followed a randomized complete block design with two replicates per treatment. Each Petri dish was initially supplied with 10 mL of its respective treatment, either the control (distilled water) or one of the aqueous seaweed extract concentrations (0.1%, 0.5%, 1.0%, 1.5%, and 2.0%). The corresponding solutions were replenished daily throughout the course of the experiment. After a 7-day incubation period, the final germination rates, plumule lengths, and radicle lengths were recorded for subsequent data analysis.

Quantitative Evaluation of Germination Parameters

To evaluate the dynamics of seed germination, several quantitative metrics were determined using the following mathematical formulations:

1. Germination Percentage (GP)

The final germination percentage was calculated to reflect the overall viability of the seeds using the formula:

$$GP = Ni / S$$

Where:
Ni represents the cumulative number of seeds that successfully germinated by the final day i
S represents the total number of seeds originally sown in the Petri dish.

2. Seed Germination Index (SGI)

To assess the rate and uniformity of the germination process over time, the seed germination index was calculated in accordance with the methodology described by Scott *et al*. (1984):

$$SGI = \Sigma\ Ti\ Ni/S$$

Where:
Ti represents the specific number of days elapsed after sowing.
Ni represents the number of seeds that germinated on that specific day i
S represents the total number of seeds originally sown.

3. Germination Energy (GE)

Germination energy was recorded on the fourth day after sowing following the protocol outlined by (Farooq et al., 2005). This index measures the speed of germination by evaluating the proportion of seeds that germinated early relative to the total population:

$$GE = GP\ (4th\ day)/TNST$$

Where:

- GP (4th day) represents the number of germinated seeds recorded on the fourth day after sowing.
- TNST represents the total number of seeds tested within the experimental unit.

Quantification of Photosynthetic Pigments

The concentrations of key photosynthetic pigments, specifically chlorophyll a, chlorophyll b, and total carotenoids, were determined spectrophotometrically following the experimental framework established by (Metzner et al., 1965).

To extract the pigments, a known fresh weight of leaf tissue was homogenized in 85% aqueous acetone for 5 minutes. The resulting homogenate was centrifuged, and the supernatant was collected and diluted to a standardized final volume using additional 85% aqueous acetone.

The absorbance (E) of the clarified extract was measured against an 85% aqueous acetone blank at specific wavelengths of 452.5 nm, 644 nm, and 663 nm using a spectrophotometer. Accounting for the appropriate dilution factors, the pigment concentrations were calculated using the mathematical equations derived by (Metzner et al., 1965):

The final calculated concentrations were converted and expressed as milligrams per gram of leaf fresh weight (mg/g fresh wt.).
Chlorophyll a = $10.3\ E_{663} - 0.918\ E_{644}$
Chlorophyll b = $19.7\ E_{644} - 3.87\ E_{663}$
Carotenoids = $4.2\ E_{452.5}$ - (0.0264 chlorophyll a + 0.426 chl. b)
The values were then expressed in mg/g fresh wt.

Statistical Analysis

Statistical analyses were performed using Minitab statistical software (Version 19.0, Minitab Inc., USA). Data were analyzed by one-way analysis of variance (ANOVA) to evaluate differences among treatment groups, followed by Tukey's post hoc test for multiple comparisons. The results are presented as mean ± standard deviation (SD). Statistical significance was accepted at $P < 0.05$.

RESULTS

Germination Dynamics and Early Seedling Development

The biostimulatory effects of aqueous extracts derived from *Dasycladus vermicularis* (DVAE) and *Jania rubens* (JRAE) were evaluated based on the germination performance and initial seedling growth of *Trigonella foenum-graecum* L. (Family: Fabaceae). The key metrics analyzed included germination percentage (GP), seed germination index (SGI), germination energy (GE), alongside plumule length (PL) and radicle length (RL).

The experimental findings indicated that both DVAE and JRAE exerted a positive influence on the germination process of *T. foenum-graecum* seeds, with the germination percentage displaying a dose-dependent increase corresponding to higher extract concentrations (Figure 2). Following a 7-day incubation period, all extract-treated groups achieved a maximum germination percentage of 100%. In contrast, the untreated control group exhibited a lower germination rate of 90%. These outcomes demonstrate that both marine macroalgae extracts successfully optimized seed germination under the evaluated laboratory conditions.

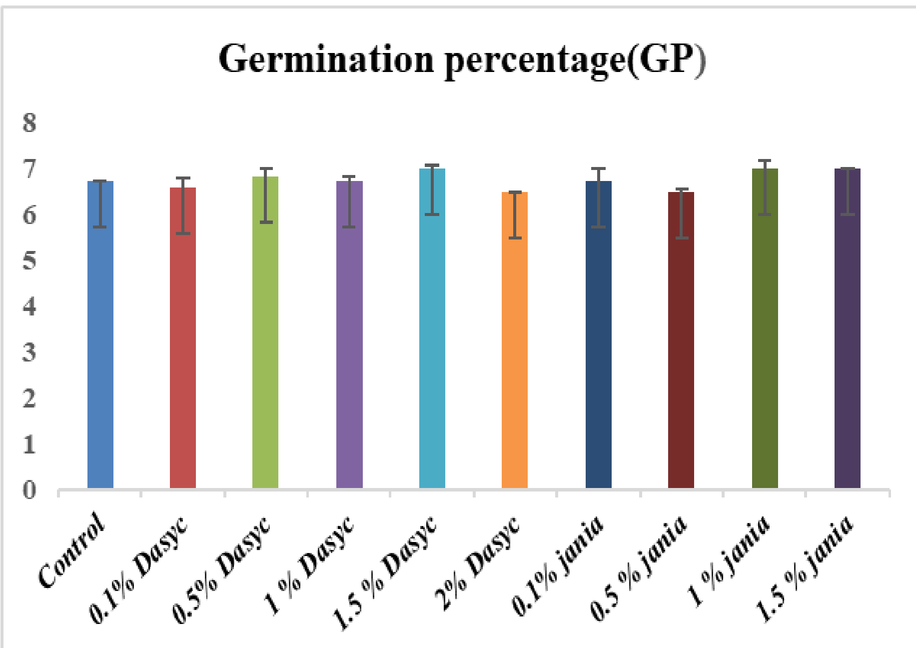

Figure (2). Effect of Various Concentrations of *Dasycladus vermicularis* and *Jania rubens* Aqueous Extracts on the Germination of *Trigonella foenum-graecum* L.

The quantitative metrics for the seed germination index (SGI) and germination energy (GE) of *Trigonella foenum-graecum* are illustrated in Figures 3 and 4, respectively. Both indicators demonstrated a highly positive response following exposure to the aqueous extracts of *Dasycladus vermicularis* (DVAE) and *Jania rubens* (JRAE). Notably, the SGI experienced a significant, dose-dependent escalation as the concentrations of both macroalgal extracts increased. This clear upward trend signifies a substantial improvement in both the overall rate and physiological vigor of seed germination when compared to the untreated control group.

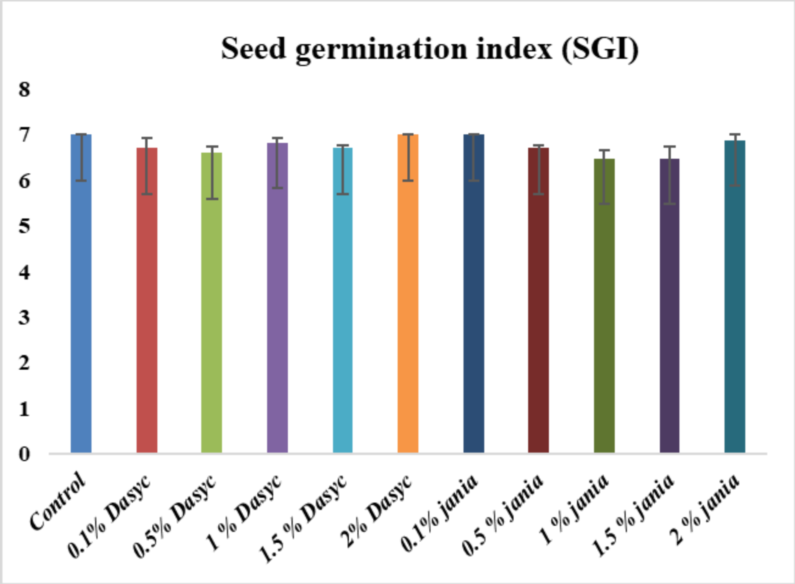

Figure (3). Effect of Different Concentrations of *Dasycladus vermicularis* and *Jania rubens* Aqueous Extracts on the Seed Germination Index of *Trigonella foenum-graecum* L.

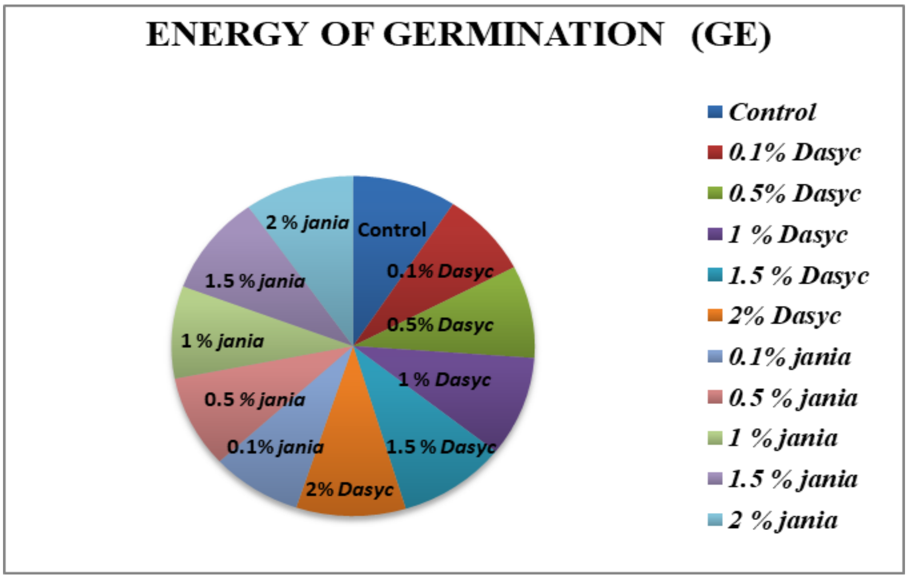

Figure (4). Effect of different concentrations of *Dasycladus vermicularis* (DVAE) and *Jania rubens* (JRAE) aqueous extracts on the germination energy of *Trigonella foenum-graecum* L.

Seedling Elongation and Early Growth Dynamics

The biometric responses of *Trigonella foenum-graecum* seedlings, specifically plumule length (PL), radicle length (RL), and overall seedling length (SL), following exposure to varying concentrations of *Dasycladus vermicularis* aqueous extract (DVAE) and *Jania rubens* aqueous extract (JRAE) are detailed in Figures 5, 6, and 7, respectively. On the whole, both macroalgal treatments stimulated early seedling development in a clear, concentration-dependent manner. Plumule elongation showed a marked increase as the concentrations of DVAE and JRAE rose. After a 7-day incubation period, plumule lengths peaked at 13.6 cm and 11.6 cm under the maximum 2% treatment of DVAE and JRAE, respectively, representing a substantial increase compared to the 3.4 cm recorded in the untreated control (Figure 5). A similar positive trend was observed in radicle development (Figure 6). For the DVAE-treated cohorts, radicle length extended from a baseline control value of 2.2 cm up to 3.1 cm at the 2% concentration. Correspondingly, JRAE application enhanced radicle length from the 2.2 cm control baseline to 2.9 cm at the highest concentration. Reflecting these individual tissue gains, total seedling length experienced a significant boost under both experimental treatments (Figure 7). With the application of DVAE, seedling length expanded from 5.6 cm in the control group to 16.7 cm at the 2% threshold. Similarly, the 2% JRAE treatment elevated total seedling length from 5.6 cm to 14.5 cm. Collectively, these data demonstrate that both marine extracts serve as highly effective biostimulants during early vegetative development, with *Dasycladus vermicularis* exhibiting a marginally higher promotive efficacy than *Jania rubens* at the maximum evaluated concentration.

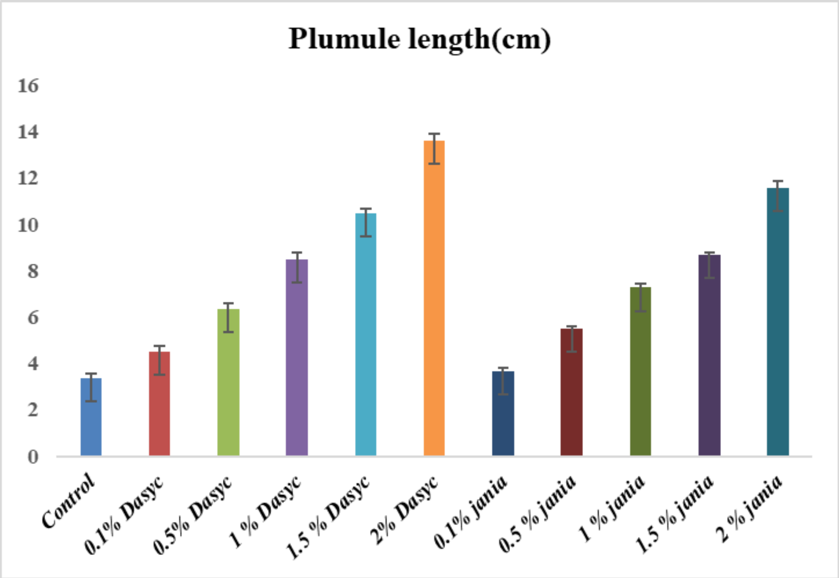

Figure (5). Effect of different concentrations of *Dasycladus vermicularis* (DVAE) and *Jania rubens* (JRAE) aqueous extracts on the plumule length of *Trigonella foenum-graecum* L

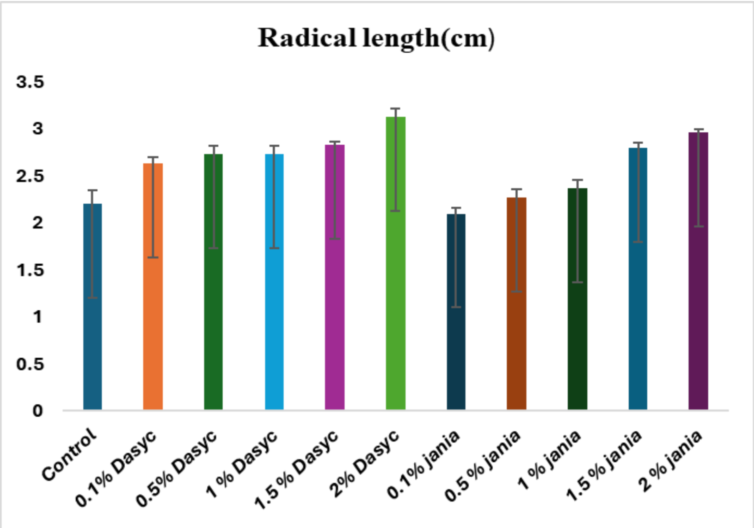

Figure (6). Effect of different concentrations of *Dasycladus vermicularis* (DVAE) and *Jania rubens* (JRAE) aqueous extracts on the radicle length of *Trigonella foenum-graecum* L.

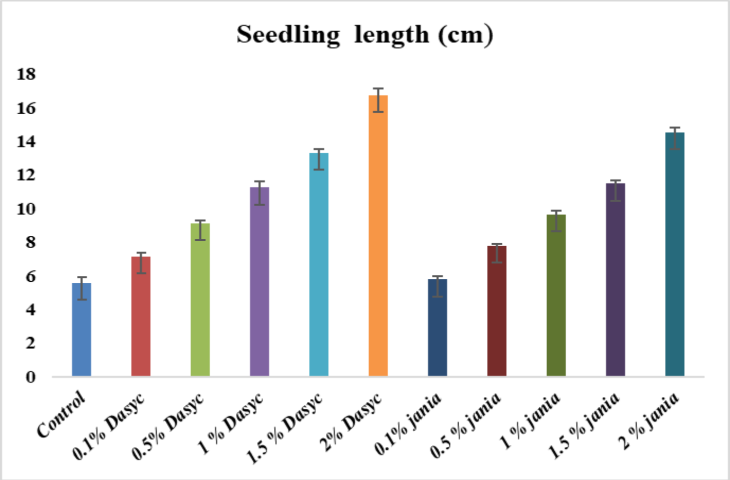

Figure (7). Effect of different concentrations of *Dasycladus vermicularis* (DVAE) and *Jania rubens* (JRAE) aqueous extracts on the seedling length of *Trigonella foenum-graecum* L.

Variation in Photosynthetic Pigment Accumulation

The physiological impact of the macroalgal treatments on the photosynthetic apparatus of *Trigonella foenum-graecum* seedlings is illustrated in Figures 8, 9, and 10. In the untreated control seedlings, baseline concentrations of chlorophyll a and chlorophyll b were recorded at 6.5 mg/g and 6.7 mg/g fresh weight, respectively. Application of the 2% seaweed extracts markedly elevated these parameters; specifically, DVAE treatment drove concentrations up to 17.8 mg/g for chlorophyll a and 17.5 mg/g for chlorophyll b, while JRAE application resulted in 14.7 mg/g and 15.1 mg/g, respectively. Furthermore, total carotenoid levels exhibited a parallel upward trend, expanding from a control baseline of 7.6 mg/g to peak values of 12.1 mg/g under DVAE treatment and 13.6 mg/g under JRAE treatment.

As a reflection of these individual component gains, the total photosynthetic pigment content was significantly enhanced by the biofertilizer treatments (Figure 11). From a baseline pool of 20.7 mg/g in the control seedlings, the 2% aqueous extracts of *Dasycladus vermicularis* (DVAE) and *Jania rubens* (JRAE) successfully elevated the total pigment content to 44.8 mg/g and 46.3 mg/g, respectively. These values represent substantial net increases of 116.4% and 123.7% over the control group, underscoring a powerful biostimulatory effect on the accumulation of the plant's light-harvesting pigments.

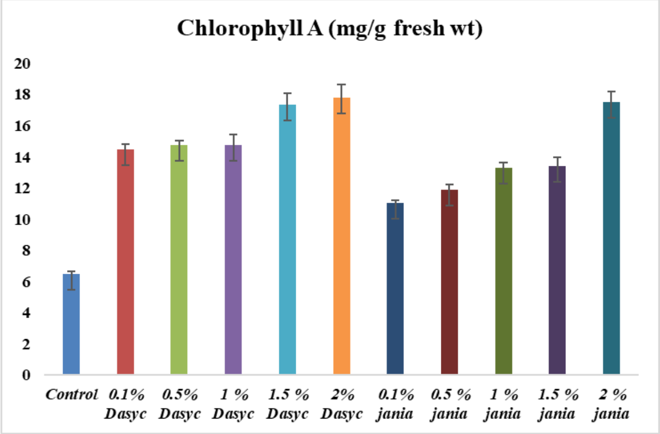

Figure (8). Effect of different concentrations of *Dasycladus vermicularis* (DVAE) and *Jania rubens* (JRAE) aqueous extracts on the chlorophyll a content of *Trigonella foenum-graecum* L.

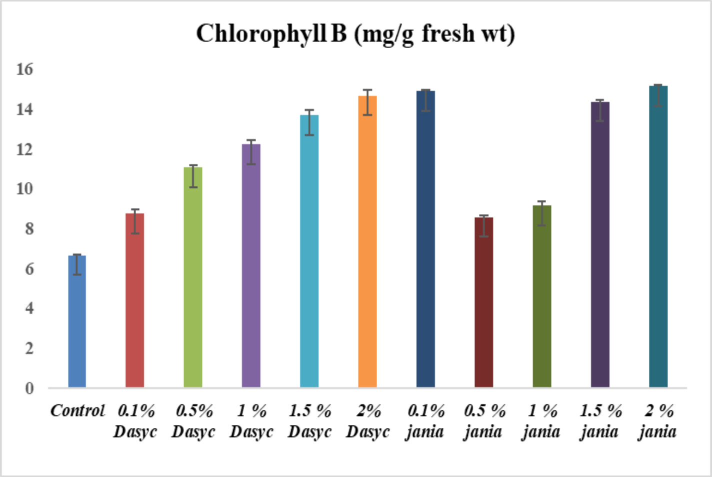

Figure (9). Effect of different concentrations of *Dasycladus vermicularis* (DVAE) and *Jania rubens* (JRAE) aqueous extracts on the chlorophyll *b* content of *Trigonella foenum-graecum* L.

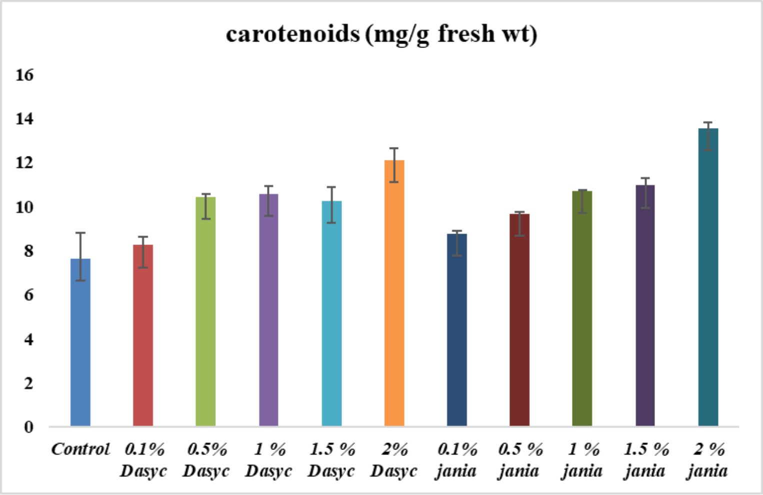

Figure (10). Effect of different concentrations of *Dasycladus vermicularis* (DVAE) and *Jania rubens* (JRAE) aqueous extracts on the carotenoid content of *Trigonella foenum-graecum* L

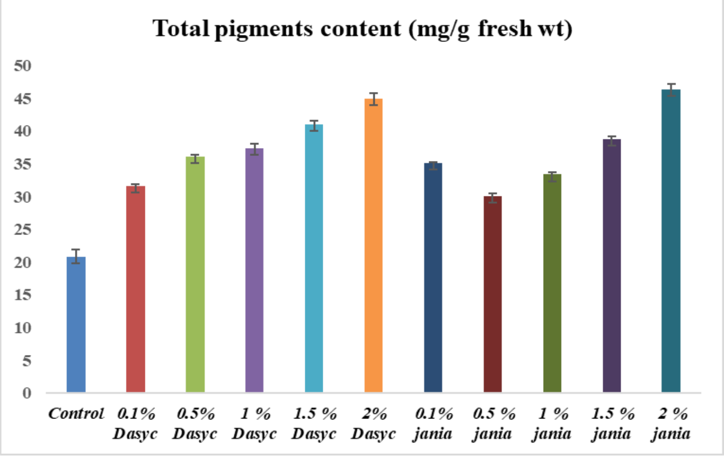

Figure (11). Effect of different concentrations of *Dasycladus vermicularis* (DVAE) and *Jania rubens* (JRAE) aqueous extracts on the total pigment content of *Trigonella foenum-graecum* L.

DISCUSSION

In the present study, the application of seaweed extracts (SWE) significantly enhanced the vegetative performance and seedling vigor of *Trigonella foenum-graecum* L. These findings align with previous literature documenting the growth-promoting efficacy of SWE across various crops, including barley, wheat, and cabbage (Alaila *et al*., 2022; Elsalhin *et al*., 2022; Kannan *et al*., 2016). This stimulatory effect is likely driven by synergistic interactions between endogenous plant growth regulators and the diverse bioactive compounds inherent to seaweed extracts (Teixeira da Silva et al., 2013). Furthermore, the observed improvements in plant height and overall growth characteristics can be attributed to the combined supply of essential macronutrients (NPK) and nitrogenous growth regulators, such as auxins and cytokinins, provided by the extracts. Furthermore, organic constituents within SWE, including amino acids, methionine, and organic acids, are known to act as natural chelators, thereby enhancing nutrient uptake and increasing the bioavailability of essential minerals in the rhizosphere (Papenfus et al., 2013).

The exogenous supply of key phytohormones, particularly auxins and gibberellic acid, may further modulate plant development by triggering the endogenous synthesis of vitamins and vital growth hormones in treated tissues (O'Dell, 2003). The macro- and micronutrient profile of SWE plays a fundamental role in optimizing shoot development, given that nitrogen, phosphorus, and potassium are indispensable for fundamental cellular processes and structural growth (Attememe, 2009). Parallel responses have been recorded in *Arachis hypogaea*, where accelerated growth and biochemical enhancements were directly linked to the nutrient richness and hormone content of applied extracts (Ganapathy-Selvam & Sivakumar, 2014). Similarly, Rathore *et al*. (2009) reported substantial boosts in the vegetative traits of soybean (*Glycine max*) following SWE treatment. The vegetative improvements observed in our study are also consistent with recent findings on *Trigonella foenum-graecum* (Mafakheri & Asghari, 2025), as well as earlier reports on *Abelmoschus esculentus* (Thirumaran *et al*., 2009) and *Vigna sinensis* L. (Sivasankari *et al*., 2006) under foliar or localized SWE applications. Additionally, the presence of cytokinins in seaweed extracts acts as a physiological stimulant, upregulating metabolic activities and elevating total chlorophyll synthesis. This enhancement in photosynthetic pigment accumulation directly improves photosynthetic efficiency, leading to higher assimilate production and storage, which ultimately manifests as superior shoot and seedling biomass allocation (Janick & Whipkey, 2002).

CONCLUSION

The findings of this study indicate that liquid extracts obtained from *Dasycladus vermicularis* and *Jania rubens* are rich in essential micro- and macronutrients. Their application positively influenced seed germination and plant growth, demonstrating their effectiveness as natural organic fertilizers. Consequently, the use of eco-friendly seaweed liquid extracts may represent a sustainable strategy for enhancing crop performance and increasing agricultural yield.

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ETHICS

No ethical issues are associated with this manuscript, and the authors are not aware of any ethical concerns that may arise following its publication.

Duality of interest: The authors declare that they have no duality of interest associated with this manuscript.

Author contributions: Ahlam K. Alaila conceived and designed the study, conducted the experimental work, analyzed the data, and prepared the initial draft of the manuscript. Hamida E. Elsalhin supervised the research, contributed to data interpretation, critically revised the manuscript, and approved the final version. Both authors read and approved the final manuscript.

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