



Interactive effects of seaweed and microalga extract priming as a biostimulant agent on the seed germination indices and primary growth of milkweed (*Calotropis procera* Ait.)

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Abstract

Adverse effects caused by the overuse of chemical fertilizers have led to the development of natural biostimulants like seaweed and microalga extracts being used as an alternative, environmentally-friendly approach to improve crop growth and increase agricultural yields. The current research aimed to study the interactions between seaweed and microalga extracts priming on the seed germination parameters, and the growth of *Calotropis procera*. A petri-dish trial was conducted under a controlled growth room, where the experimental seeds were treated individually with the two biostimulant primings, seaweed liquid extracts (SLEs) and microalga liquid extracts (MLEs), and combinations of SLEs + MLEs at different levels (0, 0.5, 1, and 1.5%). The results of the germination experiment indicated that most germination parameters except seed vigor were significantly increased at all levels of SLE, and 0.5% of MLE. Also, the primary growth indices such as shoot length, root length, shoot and root fresh and dry weight were remarkably enhanced in lower levels of their combined primings. Our data about combined biostimulant treatments showed that SLE 0.5 and SLE 1% + MLE 0.5% led to increasing the germination and growth of primed seeds compared to control. Ultimately, SLE 1% and MLE 0.5% priming were found to be more successful and better candidates for developing effective biostimulants for improving seed germination parameters of milkweed. Therefore, the results maintain the concept of marine algae extracts as an agricultural biocatalyst, which could be applied as alternative biostimulants in improving growth performance for some plants.

Keywords Biostimulation · Growth · Milkweed · *Sargassum* · *Spirulina* · Seed Vigor Index

Introduction

Milkweed (species: *Calotropis procera* Ait. family: *Asclepiadaceae*) is a desert shrub, which is widely distributed in arid and semiarid regions including Iran, Pakistan, India, neighboring countries of the Persian Gulf, etc. (Bahmani et al. 2018), which grows on alkaline and saline soils (Hindi 2013; Bahmani et al. 2018). This plant contains many

bioactive compounds with medicinal properties such as anticancer, insecticidal, antimicrobial, etc. (Bahmani et al. 2018).

It is noteworthy that the habitat of the milkweed plant confronts some vital threats such as the change of land use and cut-off trees, and its overexploitation in local communities (Taghvaei et al. 2012; Bahmani et al. 2018). During recent decades, although milkweed has extensively been used to afforest, this plant faces germination and establishment problems in its habitats (Bahmani et al. 2018).

To overcome the aforementioned problems, seed priming can be assumed as an efficient approach to improve physicochemical processes in the seeds before germination (Ibrahim 2016). To date, the various techniques of seed priming have been suggested to accelerate germination and promote growth of plants, including biostimulants priming (Bahmani Jafarlou et al. 2021).

Bio-stimulant priming is a technique by which the seeds absorb algae base bioactive compounds for the bootstrap of some essential metabolic processes (e.g., protein

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synthesis and, or repair of the damaged proteins using RNA and DNA), that allows early germination, but does not complete the process (Jisha et al. 2012; Ibrahim 2016).

The polysaccharides obtained from algae constitute the subject of interest in agriculture, with emphasis on its application in sustainable agriculture (Khan et al. 2009). Another important commercial application of algae is the production of healthcare products and cosmetics, as well as the biochemical industry.

Over the centuries, various types of seaweeds like *Sargassum* sp. were grown and harvested in coastal areas. Successful reports have been presented concerning algae extracts as a biostimulant in the growth regulation, crop protection, yield enhancers, and supporters to plant tolerance towards environmentally stress conditions (Bahmani Jafarlou et al. 2021).

Sargassum angustifolium (C. Agardh) is a valuable brown seaweed, from which a considerable number of the species have bloomed in the Persian Gulf and Oman Sea. This seaweed provides a desirable opportunity to assay the efficacy of extractions of *S. angustifolium* on terrestrial plants, concerning its numerous bioactive compounds, affordability, easy access, and environment-friendliness (Sohrabipour and Rabiei 1996; Salehpour et al. 2021).

Seaweed liquid extracts (SLEs) have a wide range of applications in the world. With the emphasis on the bio-stimulating activity of the seaweed, it can be used as Bio-stimulant priming (Raghunandan et al. 2019). SLEs contain macroelements and microelements, amino acids, vitamins, and phytohormones (Khan et al. 2009; Carrasco-Gil et al. 2019).

The results of many studies illustrated that SLEs could improve growth, enhance seed germination, and efficiency of horticultural crops such as *Vitis vinifera* L. (Norrie et al. 2002), *Solanum lycopersicum* L. (Hernandez-Herrera et al. 2013), and *Capsicum annuum* L. (Yildiztekin et al. 2018).

Spirulina platensis is one of the photosynthetic green microalgae. Microalgae perform nitrification under anaerobic conditions in heterocysts, include 5–10% of cells in a filament (Fleming and Haselkorn 1973).

Nowadays, microalga is produced commercially as a food source, animal feed, and bio-fertilizer with high nutritional value (Sánchez et al. 2003). *S. platensis* comprises high amino acids and the spectrum of mixed natural carotene and, xanthophyll phytopigments, which are considered a rich natural source of vitamin B₁₂ and antioxidants (Kemka et al. 2007).

Microalgae biofertilizers have been applied for various purposes, including soil enrichment and the growth and yield of crop plants. Besides, algae produce secondary metabolites that inhibit the growth of plant pathogenic microbes (Falch et al. 1995; Bahmani Jafarlou et al. 2021).

Spirulina microalga is considered an organic nitrogenous source, for example, amino acids, which have a critical function in some plants like chlorophyll synthesis (Amin et al. 2011), the optimization of the nutrient uptake, translocation and metabolism, vitamin biosynthesis, the production of dry matter of plant (Khalilzadeh et al. 2012), preserving the protein structure required for cell division, helping cells divide, enlarge, differentiation, and growth-efficient polyamines (Kakkar et al. 2000), growth promotion and the reduction of biotic and abiotic stress conditions (Souri and Hatamian 2019). Several studies have demonstrated that biological seed priming with microalgae extracts has positive effects on seed germination and plant growth of cowpea and mung bean seeds (Dmytryk et al. 2015), rice plant (Dineshkumar et al. 2018), *Zea mays* L. (Seifikalhor et al. 2020), *Allium cepa* L. (Gerles and Elsadany 2021), and *Gossypium barbadense* L. (Yanni et al. 2020).

According to the aforementioned beneficial effects, microalgae species are recommended to be used as biofertilizers instead of chemical fertilizers because of affordability and environment—friendliness.

The objectives of this study were (1) to investigate the role of SLEs priming on germination rate and seed vigor of milkweed, (2) to study the primary growth parameters of milkweed seedling after biostimulant priming with MLEs, and (3) to evaluate the effects of dual algae priming (SLEs + MLEs) at various doses on milkweed performance.

Materials and methods

Seaweed

Seaweed *Sargassum angustifolium* C. Agardh was collected at the intertidal zone of the rishehr coastal area of Bushehr, Iran, in the Persian Gulf in November 2019. Seaweed species were identified using the taxonomic key reference followed by Sohrabipour and Rabiei (1996). Collected samples were washed with seawater to remove redundant materials such as debris, shells, and sand. Samples were transported to the laboratory, washed with water, dried under shade conditions, and then ground in an electric mill (Parskhzar, CP802P, Iran). Next, 100 g of seaweed powder and 1000 ml of distilled water (DW) were mixed with constant stirring for 15 min and then boiled for 15 min. The resulting extract was passed through a filter paper and designated as a stock SLE, and then maintained at 4 °C until further use (Salehpour et al. 2021).

Microalga

Microalga *Spirulina platensis* was acquired from the marine biotechnology department of the Persian Gulf research

institute, Bushehr, Iran. This microalga was cultured on Zarrouk special medium (Zarrouk 1966). Each Erlenmeyer flask was inoculated with 10 ml stock, incubated for seven days in a growth chamber with the appropriate conditions (continuous illumination 2000 lx and the temperature 35 ± 2 °C), and then considered an inoculum for 1000 ml of the medium and transferred into 20 l white fiberglass tank, incubated for 14 days under controlled greenhouse conditions of 35 ± 2 °C and continuous illumination from 5500–6500 lx. The tank received sterile air through a pump to contentiously mix/circulate/distribute the culture during the incubation period. To prepare microalga liquid extract (MLE), *S. platensis* (100 g) was suspended in 1000 ml sterilized distilled water using a blender. Finally, the obtained solution was filtered using a filter paper (Whatman No. 42 mm) to remove debris and design. The final filtrate is considered a 100% stock (Dineshkumar et al. 2018).

Mineral assay

For the mineral analysis of the algae, samples were placed in an oven at 80 °C for two days and grinding the dried algae by a mixer, the obtained fine powder was transferred into a furnace (at 460 °C for 6 h). Then, a weight of 0.5 g of the attained ash was added by nitric acid (HNO₃, 2%) and passed through a 0.45 µm filter (glass fiber) (AOAC 1990). The absorption of minerals (P, K, Fe, Mn, and Zn) was conducted using atomic absorption spectroscopy (240FS AA, Agilent, America).

Nitrogen (total N) content in algae sample was determined after mineralization with sulfuric acid (H₂SO₄, 96%) in the presence of potassium sulfate (K₂SO₄) and a low concentration of copper (Cu) according to the Kjeldahl method (Bremner 1965). Moreover, the pH and electrical conductivity (EC) of the seaweed liquid extract and microalga liquid extract were measured by pH meter (PHS, China) and EC meter (DDS-22C, China).

Seed collection

The ripe seeds of the milkweed shrubs were randomly collected from Abad village (3,213,206 m N and 523,703 m E; 59 m a.s.l) of the Tangestan region, South of Iran, in fall 2019.

To assay purity percentage, the immature and malformed seeds were manually separated and placed in distilled water. Tetrazolium chloride was used to evaluate seed viability. The moisture percentage of seeds was calculated as follows (AOSA 1981):

$$\text{Moisture content} = \frac{\text{Seed fresh weight} - \text{Seed dry weight}}{\text{Seed dry weight}} \times 100$$

The results of seed physiological characteristics indicated 100% the purity percentage, 95% viability, and 6% moisture percentage.

Experimental Setup

Sodium hypochlorite (5%) was used to sterilize the milkweed seeds for 10 min. Next, they were rinsed three times with DW, transported to Petri dishes, and soaked in 20 ml of the algae biostimulant solutions at concentrations 0.5, 1, and 1.5% (v/v) to perform the seed priming method. After the soaking period, the seeds between two filter paper (Whatman No. 42 mm) were placed in the Petri dish (darkness at 25 °C) and allowed to dry for 24 h to reduce moisture percentage (approximately 6%). Control seeds were also soaked in a Petri dish containing distilled water.

The treatments were prepared in a completely randomized design with three replication per treatment, amounting to a total of 16 experimental units (n=48 petri dishes). All experimental seeds were transferred to sterile Petri dishes (total seed=960) containing a moist filter paper, which was soaked with 5 ml of distilled water and then transferred to a growth room (temperature 25 °C and relative humidity 65%, photoperiod 16 h light/8 h dark) (AOSA 1981). Filter papers were replaced every day to inhibit microbial infections, including fungi and bacteria, etc., in Petri dishes containing seeds.

For studying the effect of seed priming with biostimulants SLEs and MLEs, The experiment carried out by the following treatments:

1. Control, 0% extract (10 ml of DW), pH 7.0; EC 0.0 dS.m⁻¹
2. T1, 0.5% concentration (0.05 ml SLE in 9.95 ml DW), pH 7.2; EC 0.54 dS.m⁻¹
3. T2, 1% concentration (0.1 ml SLE in 9.9 ml DW), pH 7.4; EC 0.80 dS.m⁻¹
4. T3, 1.5% concentration (0.15 ml SLE in 9.85 ml DW), pH 7.6; EC 1.23 dS.m⁻¹
5. S1, 0.5% concentration (0.05 ml MLE in 9.95 ml DW), pH 7.6; EC 2.5 dS.m⁻¹
6. S2, 1% concentration (0.1 ml MLE in 9.9 ml DW), pH 8.3; EC 3.79 dS.m⁻¹
7. S3, 1.5% concentration (0.15 ml MLE in 9.85 ml DW), pH 8.6; EC 5.51 dS.m⁻¹
8. T1 + S1, 0.5% + 0.5% concentration (0.05 ml SLE in 9.95 ml DW + 0.05 ml MLE in 9.95 ml DW), pH 7.6; EC 1.36 dS.m⁻¹
9. T1 + S2, 0.5% + 1% concentration (0.05 ml SLE in 9.95 ml DW + 0.1 ml MLE in 9.9 ml DW), pH 8.1; EC 1.65 dS.m⁻¹
10. T1 + S3, 0.5% + 1.5% concentration (0.05 ml SLE in 9.95 ml DW + 0.15 ml MLE in 9.85 ml DW), pH 8.2; EC 1.97 dS.m⁻¹
11. T2 + S1, 1% + 0.5% concentration (0.1 ml SLE in 9.9 ml DW + 0.05 ml MLE in 9.95 ml DW), pH 7.9; EC 1.34 dS.m⁻¹

12. T2 + S2, 1% + 1% concentration (0.1 ml SLE in 9.9 ml DW + 0.1 ml MLE in 9.9 ml mL DW), pH 8.1; EC 2.0 dS.m⁻¹
13. T2 + S3, 1% + 1.5% concentration (0.1 ml SLE in 9.9 ml DW + 0.15 ml MLE in 9.85 ml DW), pH 8.2; EC 2.01 dS.m⁻¹
14. T3 + S1, 1.5% + 0.5% concentration (0.15 ml SLE in 9.85 ml DW + 0.05 ml MLE in 9.95 ml DW), pH 7.9; EC 2.36 dS.m⁻¹
15. T3 + S2, 1.5% + 1% concentration (0.15 ml SLE in 9.85 ml DW + 0.1 ml MLE in 9.9 ml DW), pH 8.1; EC 2.46 dS.m⁻¹
16. T3 + S3, 1.5% + 1.5% concentration (0.15 ml SLE in 9.85 ml DW + 0.15 ml MLE in 9.85 ml DW), pH 8.2; EC 2.54 dS.m⁻¹

Biometry for germination experiment

The germinated seeds were noted daily for 19 days (assessed as the length of root emergence reached 2 mm). After no seed germination was observed in three successive days, the seed trial was completed. At the end of the experiment, shoots were removed from roots, and their fresh weight was measured through digital beam balance (scale 0.01 mg), and afterward, they dried in an oven (70 °C) for 48 h to assay their dry weight. Also, their length was carefully measured by a digital ruler (scale 0.01 mm). In addition, the seed germination percentage (Panwar and Bhardwaj 2005) and mean germination time (Kulkarni et al. 2007), and Seed vigor index (Marcos Filho 2015) were determined as follows:

$$\text{Germination percentage (GP)} = \frac{n}{N} \times 100$$

$$\text{Mean germination time (MGT)} = \frac{n(\text{ni.ti})}{nn} \times 100$$

$$\text{Seed vigor index (SVI)} = \frac{\text{GP} \times \text{Mean}(\text{SL} + \text{RL})}{100}$$

where n is the number of germination per day, N is the total number of seeds, ti is the number of days that seeds germinated, ni is the number of germinated seeds among periods, SL is the shoot length, and RL is the root length.

Statistical analysis

We used a completely randomized design (three replications per treatment) through a factorial experiment (seaweed and microalga). A Two-way ANOVA test was used to analyze all data. Data were checked for normality by the Kolmogorov-Smirnov test and homogeneity of variances by the Levene test. Tukey HSD test ($p \leq 0.05$) was used to compare means among treatments (SPSS software, version 21).

Results

Multi elemental composition of seaweed and microalga:

Mineral content revealed the presence of total N (1.34 and 6.64%), P (70.3 and 26.5 ppm), K (7580.4 and 142.8 ppm), Ca (4801.6 and 5083.3 ppm), Fe (46.5 and 60.2 ppm), Mn (21.2 and 18.5 ppm) and, Zn (16.3 and 8.7 ppm) in seaweed *S. angustifolium* and microalga *S. platensis*, respectively. Among the elements, total N, K, and Ca were abundant in the samples (Table 1).

Germination percentage and germination speed

In our experiments, the use of both algae extracts priming significantly promoted the rate of seed germination and primary growth of milkweed. There was a noticeable increase in all parameters, when 0.5% of SLE and MLE were applied to the milkweed plant, as presented in Table 2.

The results of two-way ANOVA indicated that the response of the seedlings was different in SLE, and MLE primings and no significant difference ($p \geq 0.05$) was observed by increasing the concentration of SLE in GP and GS parameters. The germination percentage was (100%) in all concentrations of SLEs and exhibited an increase in GP (50%) in comparison with the control. Similarly, almost all concentrations of SLEs showed significant positive effects on the GS (11 seed.day⁻¹), and an increase of 348% over to the control. Correspondingly, the different concentrations of MLEs priming produced a significant stimulatory effect ($p \leq 0.05$) on milkweed GP and GS. The highest values of GP and GS were found in the seeds treated with 1% MLE from *S. platensis* (with an increase of 50 and 240%, respectively, compared to control). However, MLE had a negative effect on both parameters at higher concentrations (Fig. 1 a, b). The combined priming of SLE and MLE showed

Table 1 Multielemental composition in seaweed and microalga. Values are average \pm standard deviation ($n = 3$)

Species	N (%)	P (ppm)	K (ppm)	Ca (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)
<i>S. angustifolium</i>	1.34 \pm 0.2	70.3 \pm 1.6	7580.4 \pm 48	4801.6 \pm 60.1	46.5 \pm 0.7	21.2 \pm 0.4	16.3 \pm 0.8
<i>S. platensis</i>	6.64 \pm 0.9	26.5 \pm 2.4	142.8 \pm 5.2	5083.3 \pm 98.2	60.2 \pm 0.8	18.5 \pm 0.3	8.7 \pm 0.04

Table 2 Two-way analysis of variance for the rate of seed germination indices present in biostimulants primed and non-primed seeds

Sources Parameters	SLE		MLE		SLE × MLE	
	df	Mean Square	df	Mean Square	df	Mean Square
Germination Percentage	3	185.41 ns	3	11,952.08**	9	340.97 ns
Germination Speed	3	19.65**	3	137.54**	9	17.78**
Mean Germination Time	3	0.849 ns	3	26.39**	9	1.77**
Seed Vigor Index	3	80.48 *	3	2634.15**	9	95.63**
Plumule Length	3	262.15**	3	1216.24**	9	99.65 ns
Radical Length	3	52.51 ns	3	2832.09**	9	130.78**
Plumule Fresh Weight	3	26,162.56**	3	255,573.18**	9	5070.22 ns
Radical Fresh Weight	3	2846.5*	3	75,215.62**	9	2646.04**
Plumule Dry Weight	3	59.42**	3	95.61**	9	61.17**
Radical Dry Weight	3	6.94**	3	2.77**	9	1.68**

Ns non-significant, * *p*-value < 0.05, ** *p*-value < 0.01

an inhibitory effect on GP and GS parameters at higher concentrations. The highest values of GP, i.e., 100 and, 90 (%), and GS i.e. 11, 10 and 9 (seed.day⁻¹) were found in seeds that received combined primings T1 + S1, T2 + S1, and T3 + S1, respectively (Fig. 1 a, b).

Meantime of germination and seed vigor index

The results of ANOVA showed that the response of the mean time germination and seed vigor was significantly different in seaweed and microalga primings. Application of SLEs priming showed constant changes with increasing of concentrations in MGT and SVI. The minimum value MGT was 5 seeds.day⁻¹ at the concentration of SLE 0.5%, and this decrease was 97.2% compared to the control. The maximum value of SVI was (41.75) at the concentration of SLE 0.5%, and this increase was 111.92% compared to control. The 0.5% MLE from *S. platensis* showed a significant decrease of 34.4% (2 seed.day⁻¹) in MGT and an increase of 72.57% (36.05) in SVI compared to control, respectively.

Application of MLE priming in these parameters, showed a decreasing trend with the increase of concentrations. Tukey comparisons showed a decreasing trend with increasing concentration of combined treatment. The minimum value of MGT, 1, 1, and 2 seed.day⁻¹ was observed in combined primings, including T1 + S1, T2 + S1, and T3 + S1, respectively. The maximum value of SVI was observed 41.75 at T2 + S1 treatment (Fig. 2a and b).

Shoot and root length

The shoot length did not show any significant change by increasing concentrations of SLEs primings. The highest average shoot length was 43 mm at all concentrations of SLE showed an increase of 23.7% compared to the control. The highest amount of SL (39.5 mm) was observed at 0.5% SLE and increased by 19.7% compared to the control. There was

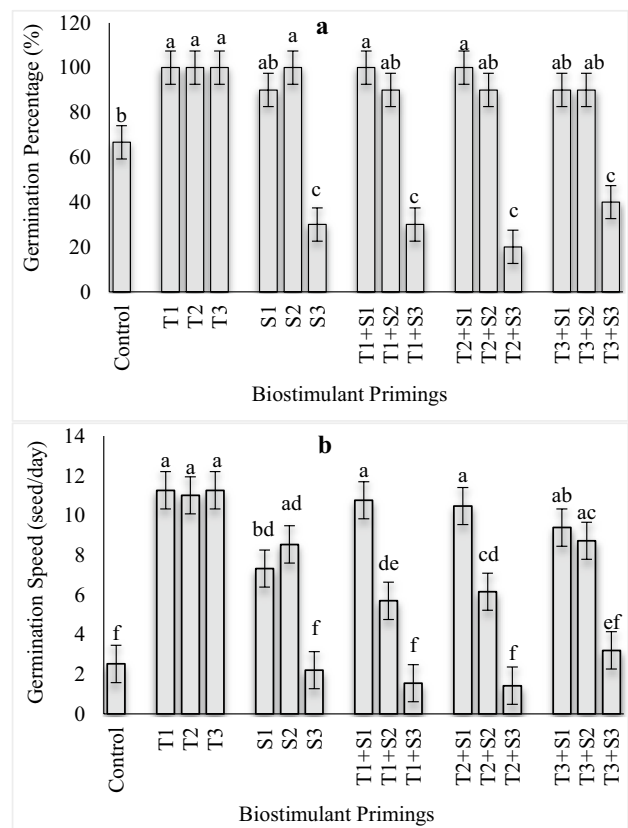


Fig. 1 Germination percentage (GP) – (a) and germination speed (GS) – (b) of milkweed seeds treated with biostimulant extracts priming at concentrations of T1 0.5%, T2 1%, T3 1.5%, S1 0.5%, S2 1% and S3 1.5% from *S. angustifolium* (T) and *S. platensis* (S). The different letters indicate significant differences among treatments based on the Tukey HSD test. The values are mean ± SE of three replicates (p value ≤ 0.05)

a noticeable decrease in shoot length parameter, when 1.5% of MLE of *S. platensis* was applied to the milkweed plant. The seaweed and microalga combined primings showed a

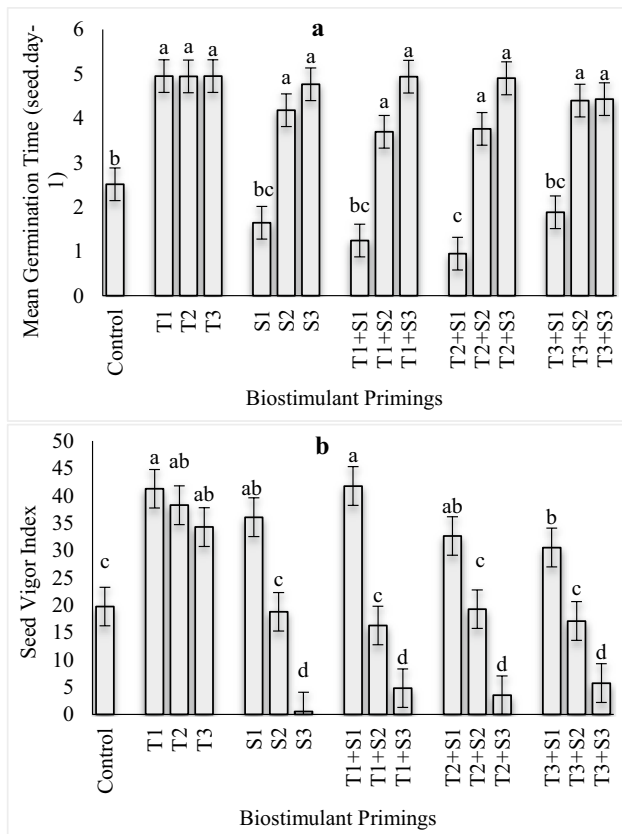


Fig. 2 Mean germination time (MGT) – (a) and seed vigor index (SVI) – (b) of milkweed seeds treated with biostimulant extracts primings at concentrations of T1 0.5%, T2 1%, T3 1.5%, S1 0.5%, S2 1% and S3 1.5% from *S.angustifolium* (T) and *S.platensis* (S). The different letters indicate significant differences among treatments based on the Tukey HSD test. The values are mean \pm SE of three replicates (p value ≤ 0.05)

reduction in SL with increasing liquid extraction concentration. The highest values of SL (40, 45, and 45.5 mm) were seen in T1 + S1, T2 + S1, and T3 + S1 combined treatments, respectively (Fig. 3a). The root length was decreased, when concentrations of SLE were increased in all treatments. The longest root length (39.5 mm) was observed at 0.5% concentration (64.5% increase compared to the control). Concurrently, a sharp decrease was seen in root length with increasing MLE concentrations. Application of 0.5% of MLE priming resulted in the highest root length (65.41 mm) with a 73.5% increment compared to the control. The longest root length enhancement was found in plants that received the blend of SLE 1% and MLE 0.5% with an increase of up to 60% (38.5 mm) compared to the control (Fig. 3a, b).

Fresh and dry weight of shoot and root

The highest value of the fresh weight of shoot and root was observed in treatment with 0.5% SLE from *S. angustifolium*,

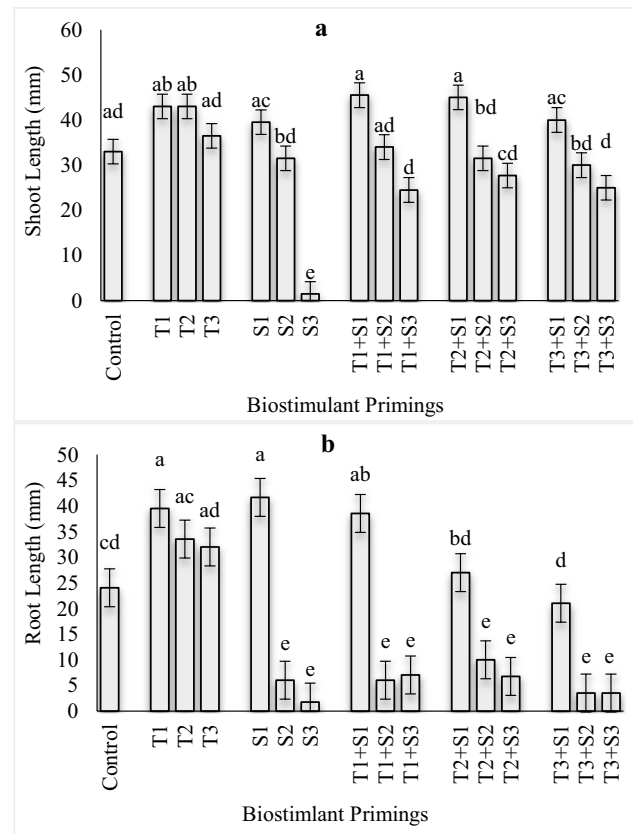


Fig. 3 Shoot length (SL) – (a) and Root length (RL) – (b) of milkweed seeds treated with biostimulant extracts primings at concentrations of T1 0.5%, T2 1%, T3 1.5%, S1 0.5%, S2 1% and S3 1.5% from *S.angustifolium* (T) and *S.platensis* (S). The different letters indicate significant differences among treatments based on the Tukey HSD test. The values are mean \pm SE of three replicates (p value ≤ 0.05)

with an increase of 55.4% (442.5 mg), and 107% (220.5 mg), respectively, compared to the control. There was a gradual decrease in the fresh weight of shoot and root parameters, when 1.5% of seaweed extract priming was applied to milkweed seeds. Increasing the concentration of MLE priming led to a sharp decrease in these parameters. In addition, SFW and RFW parameters were significantly decreased, when 1.5% concentration of MLE extract was applied.

The 0.5% MLE solution of *S. platensis* showed the highest increase in the SFW and RFW of the milkweed plant with an increase of 21.4% (318 mg), and 53% (163 mg) as compared to the control. In addition, the values of these parameters were demonstrated 318 and 163 mg, respectively.

Both concentrations 1.5 and 1% blended from SLE and MLE primings (T3 + S2, T2 + S1) showed the highest increase in fresh weight of shoot, i.e., 66.4% (436 mg), and root, i.e., 45.5% (155 mg) over the control (Fig. 4a, b). Tukey test showed a gradual decrease in dry weight of shoot and root parameters, when the milkweed plant

received seaweed priming at 1.5% concentration. The highest rate of SDW and RDW parameters was recorded 27.16 and 3.95 mg in SLE, respectively. These parameters were enhanced 196% and 197% compared to the control, when 0.5% concentration of SLE was applied. These parameters in the milkweed plant significantly decreased, when MLE priming at 1.5% concentration was applied. Also, The 0.5% MLE priming of *S. platensis* showed the highest increase in the SDW and RDW of the milkweed plants with an increase of 32.6% (12.15 mg), and 114.2% (2.85 mg) as compared to the control.

The combined treatments T1 + S1 and T2 + S1 showed the highest increase in dry weight of shoot, i.e., 22.6% (11.23 mg), and 26% (11.55 mg) over the control (Fig. 4a, b). Also, an increase of root dry weight (134.5%) was observed in plants treated with the 0.5% blend from SLE and MLE priming (T1 + S1) compared to the control (Fig. 5a, b).

Discussion

The present study indicates that the priming of *S. angustifolium*, *S. platensis* extracts and *S. angustifolium* + *S. platensis* have significant potential as biostimulants to enhance the growth and yield of milkweed plants. These results support earlier works (Hernandez-Herrera et al. 2013; Dineshkumar et al. 2018).

This positive effect can be attributed to some growth regulators present in marine algal extracts like ethylene, kinetin, and gibberellic acid, which play an essential role in accelerating germination time, boosting growth, and development (Raghunandan et al. 2019). The amplifying effect of algae extracts may be due to phytohormones, which cause the synthesis of hydrolytic enzymes (Bahmani Jafarlou et al. 2021). The researchers have demonstrated that seaweed extracts may reduce the likely inhibitors of abscisic acid in the seeds, which improves

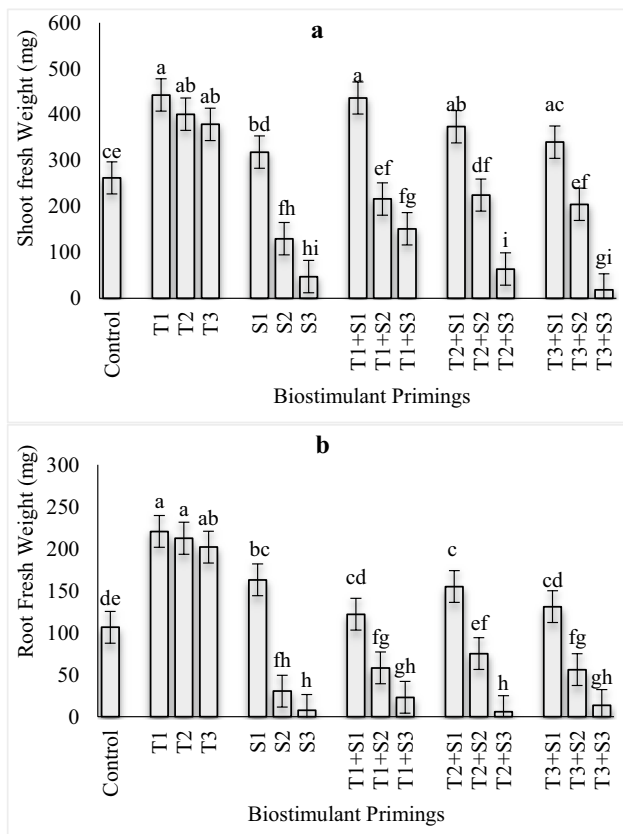


Fig. 4 Shoot fresh weight (SFW) – (a) and root fresh weight (RFW) – (b) of milkweed seeds treated with biostimulant extracts primings at concentrations of T1 0.5%, T2 1%, T3 1.5%, S1 0.5%, S2 1% and S3 1.5% from *S.angustifolium* (T) and *S.platensis* (S). The different letters indicate significant differences among treatments based on the Tukey HSD test. The values are mean ± SE of three replicates (p value ≤ 0.05)

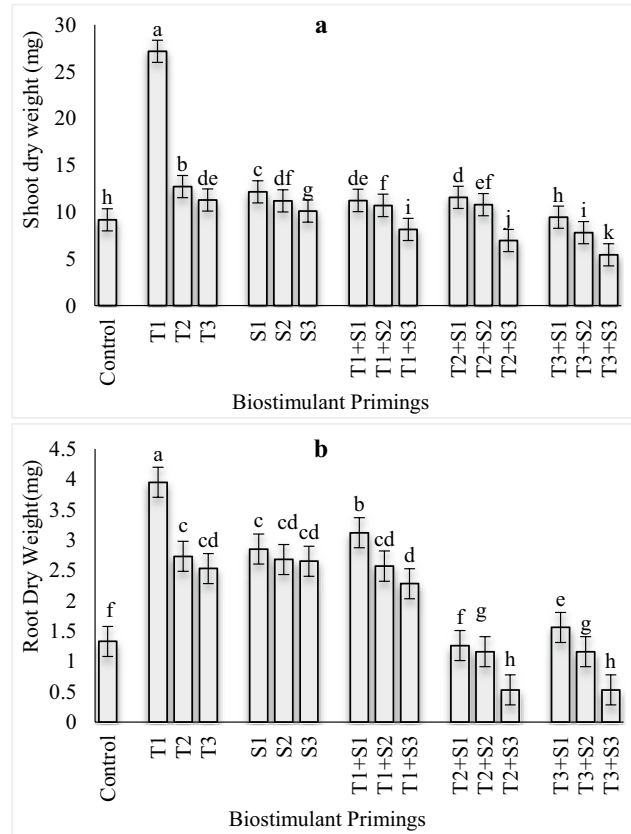


Fig. 5 Shoot dry weight (SDW) – (a) and root dry weight (RDW) – (b) of milkweed seeds treated with biostimulant extracts primings at concentrations of T1 0.5%, T2 1%, T3 1.5%, S1 0.5%, S2 1% and S3 1.5% from *S.angustifolium* (T) and *S.platensis* (S). The different letters indicate significant differences among treatments based on the Tukey HSD test. The values are mean ± SE of three replicates (p value ≤ 0.05)

the germination indices (Battacharyya et al. 2015; Hussein et al. 2021).

Furthermore, there are other possible mechanisms, which can demonstrate the valuable performance of algae priming on the early emergence of milkweed seeds, including the activation of metabolic functions of seeds before germination and the seeds preparation for more rapid germination.

In addition, the brown algal extract contains compatible betaines and betaine-like compounds (Yildiztekin et al. 2018) in plants to reduce the osmotic stress caused by salt stress (Yousefi et al. 2017; Liu et al. 2018). Khan et al (2009) illustrated that the bioactive compounds had a compelling performance in the successfully forming of somatic embryos seeds.

In contrast, the extracts priming of seaweed and microalga and combined of them (EC from 1.23 to 5.51 dS.m⁻¹) showed a significant negative effect on the germination of milkweed seeds at high concentration (1.5%), by inhibiting the ability of seeds to absorb water. Basher et al (2012) studied the effect of seaweed, and saline water on the germination rate, and growth of tomato seedlings, that their results were similar to our data.

The low concentrations of SLEs and MLEs enhanced GP, GS, SL, and RL, whereas they decreased MGT led to greater seed vigor in treated seeds. The high concentration of both algae extracts priming as well as their combined extracts priming (especially, 1.5%) decreased the parameters as mentioned earlier in treated milkweed seeds. High concentrations of salt present in algal extracts significantly enhanced the germination time and reduced the germination indices in tomatoes (Hernandez-Herrera et al. 2013; Rehmat et al. 2021). In the present study, SLE of *S. angustifolium* and MLE of *S. platensis*, and their combined extracts (at higher concentrations), decreased germination and growth that this reduction could be caused by high salt in extracts.

Algal extracts contain the amount of salt, which causes to reduce the absorption of water by seeds of milkweed at high levels, thereby decreasing growth parameters, like shoot and root (Woodell 1985). Increasing germination indices at low concentrations of algal extracts can be due to ameliorating the metabolic activities during the priming stages (Bahmani Jafarlou et al. 2021). Pretreatment priming may regulate signaling pathways during early growth stages and cause a faster defense response by trees (Jisha et al. 2012). Therefore, biostimulants served in our experiment may be triggered essential phytochemical changes like hydrolysis, enzyme activation, and breaking physiological dormancy in milkweed seeds to germinate and grow under undesirable conditions (Jisha et al. 2012).

In our study, the high concentrations of algal extracts, i.e., 1.5% (individually and combined), had toxic and negative effects on roots and shoots of milkweed plants. In line

with our findings, Hernandez-Herrera et al. (2013) also indicated the negative effects of algae extracts (at high levels) on the growth of tomato seedlings, which these effects may be caused by a high electrical conductivity in the growth medium. In agreement with our results, Kumari et al. (2011) observed the detrimental effects of marine algal extracts at high concentrations (from 2.0 to 10%) on *Vigna mungo* seedlings. Workers have previously reported with respect to algal extracts on seed germination of plant species such as pine tree (Atzmon and Van Staden 1994) and soybean (Rathore et al. 2009) and tomato (Hernandez-Herrera et al. 2013; Garcia-Gonzalez and Sommerfeld 2016; Carrasco-Gil et al. 2019).

There are several possible reasons for the adverse responses is associated with the effects of high dosages of algal extracts on the early growth of milkweed, such as shoot, root length, and their FW can be mentioned some factors like the existence of regulator hormones or high levels of minerals, including Na⁺ and Cl⁻ (Garcia-Gonzalez and Sommerfeld 2016; Latique et al. 2017).

The enhancement of germination and early growth indices of milkweeds can be due to secondary metabolites present in seaweed like macro- and microelement nutrients, amino acid, vitamins, cytokinins, auxins, and abscisic acid, which effectively stimulate cellular metabolisms in plants and finally lead to raised growth parameters and yield (Ragunandan et al. 2019). The increment in shoots indices can be attributed to some phytohormones, including the auxins content in the algal extracts, which play an influential role in cell division and elongation of plants resulting in the betterment of the shoot and root growth and biomass (Rouphael et al. 2017; Yildiztekin et al. 2018).

Besides, the capability of microalgae to increase milkweed plant growth can be associated with their high nitrogen contents, amino acids, vitamins, and phytohormones (Priyadarshani and Rath 2012). There were similar reports about the beneficial efficiencies of microalgae extracts like *Dunaliella salina*, *Phaeodactylum tricornutum*, *S. maxima*, *Chlorella ellipsoidea*, and *S. platensis* on seedlings of pepper, wheat, and rice, which improved early growth during the germination process (Abd El-Baky et al. 2010; Guzman-Murillo et al. 2013; Dineshkumar et al. 2018).

The high concentrations of the SLE and MLE primings increased some primary growth parameters (shoot and root weight and length) in treated milkweed caused by the mineral elements present in extracts. Another positive effect of algal extract use at different concentrations may be due to its various components working synergistically (Fornes et al. 2002). Similar studies performed about algae extractions lead to increase growth and improve phytochemical contents in cowpea, soybean, and tomato plants (Hernandez-Herrera et al. 2013; Garcia-Gonzalez and Sommerfeld

2016; Vasantharaja et al. 2019; Kocira et al. 2019; Rehmat et al. 2021).

Our data showed a high concentration of macro elements such as Ca, K, and total N in both seaweed and microalga. This finding has also been confirmed by Dineshkumar et al (2018) and Hussein et al (2021) on *S. platensis* and *Cystoseira compressa* for the growth and yield of *Zea mays* and *Vigna sinensis* seedlings, respectively. These extracts of algae are a suitable source of K⁺ so that it helps in regulating the relative water content, controls stomatal conductivity, and the gas exchange rate and increase meristematic growth in plants. The presence of Ca²⁺ in extracts improves some activities and functions of the cell, including enzyme activation, elongation, and stability of the cells.

There are several possible reasons for the opposite results that can be associated with the different values of mineral contents present in studied algae can highly be related to various factors, like the season, location, and analytical methods (Salehpour et al. 2021).

Conclusions

The plant growth-promoting ability of liquid extracts from seaweed *S. angustifolium* and microalga *S. platensis* as biostimulants priming was studied in *C. procera*. The biostimulants extract showed promising results in *C. procera* at lower doses ($\leq 1\%$). Combined algal treatments showed an increment in seed germination percentage, seed germination speed, shoot and root fresh and dry weight, and decreased mean time germination. Our results confirmed that the biostimulants priming increased the seed vigour indices by increasing the number of germinated seed, shoot, and root length in milkweed. Therefore, this study suggests that the application of *S. angustifolium* and *S. platensis* extracts (individual and combined) as seed primings would be highly potent, eco-friendly, and cost-effective for improving the crop biomass and yield. Hence, we suggest that field experiments need to be conducted to evaluate the effects of algae biostimulants on plants growth to pave a new path towards commercialization.

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Declarations

Conflict of interest The authors declare that we have no competing interests.

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