INSTM Bulletin
Marine and Freshwater Sciences

Vol. 49, 2024, 30-43

Research Article

Effect of the green macroalgae *Chaetomorpha linum* liquid extract on the germination, growth and pigments concentration of the chickpea *Cicer arietinum* L., 1753

Leila CHEBIL AJJABI*, Leila KTARI, Imen BEN DOUAOUA, & Marwa MELKI

University of Carthage. National Institute of Marine Sciences and Technologies, Tunis 2025, Tunisia

***Correspondence**: leila.chebil@instm.rnrt.tn

Received: 12/10/2023; **Accepted**: 02/05/2024; **Published**: 04/06/2024

Abstract: Fertilizer production and application are growing excessively with increased agricultural expenses and environmental damage. As alternatives, organic fertilizers issued from natural resources such as seaweed liquid extracts (SLE) are considered as potential plant biostimulant agents. In this study, the impact of the green algae *Chaetomorpha linum* SLE on seed germination, yield and pigmenting characteristics of the chickpea *Cicer arietinum* was investigated in laboratory settings and in pots. Several SLE concentrations varying from 1%, 3%, 5%, 8% to 10% were prepared using distilled water.

The application of SLE significantly affected all assessed parameters over the control (0%). Use of SLE at the lowest concentration (1%) showed maximum seed germination, root and shoot length and contents of total chlorophyll, chl (a), and chl (b). Accordingly, the results suggest that *C. linum* SLE could be used as an alternative organic fertilizer because it is environmentally friendly and profitable. This extract, rich in plants growth components, could find its future use in various sectors.

Keywords: *Chaetomorpha linum*; *Cicer arietinum*; Seaweeds; Biofertilizers; Plant growth; Pigments.

1. Introduction

The use of algal extracts has become increasingly popular because of their potential use in sustainable and organic farming (Nabti et al., 2016). Seaweed liquid extracts (SLEs) are more commonly used than fresh seaweed owing to their marketability and ease of implementation (Verkleij, 1992; Hernández-Herrera et al., 2014). Besides, seaweed extracts have been shown to be more effective than chemical fertilizers owing to their important organic matter content which facilitates holding moisture and minerals in the soil top layer accessible to the roots (Zodape et

al. 2010). These organic fertilizers derived from renewable marine resources are the most effective complement to chemical fertilizers. Thus, they can be applied at different levels to crops for soil or root soaking or foliar spraying (Spagnuolo and Domenico, 2021).

Additionally, the seaweed extracts are used to boost seed germination, to provide plants with resistance to biotic-abiotic stressors and to increase nutrient uptake into the soil (Ali et al., 2021). These positive findings are credited to SLE high levels of micro and macro elements, vitamins, polysaccharides as well as a multiple plant growth regulators including cytokinins and auxins at varying levels and other substances that support plant growth (Karthikeyan and Shanmugam 2016). Thus, SLE growth stimulating effects on seed germination (Venkataraman et al., 1993, Mostafa et al., 1999), production (Sekar et al., 1995) and biochemical properties (Thirumalthangam et al., 2003) of agricultural culture have been extensively described.

SLEs are efficient biostimulant in several crops such as potato (Lopez-Mosquera 1997), olives (Chouliaras et al. 2009), watermelons (Abdel-Mawgoud et al., 2010), soybean (Rathore et al., 2009), tomato (Campobenedetto et al., 2021, Cozzolino et al., 2021), wheat plants (Latique et al. 2017, Chebil Ajjabi et al. 2019, Latique 2021), strawberries (Kapur et al., 2018), spinach (Rouphael et al., 2018), grapevines (Frioni et al., 2018), cucumber (Hassan et al., 2021) and raddish (Spain et al., 2022).

Seaweeds which are considered as source of natural products are described with plant biostimulating potentials in numerous research works (Godlewska et al., 2016, Gibilisco et al., 2020). Seaweeds present a considerable performance in agriculture, particularly in developing countries where unreasonable utilization of pesticides and chemical fertilizers is a matter of consideration.

Increasing number of liquid fertilizers and biostimulants based on seaweed products gained the global bio-fertilizer markets as they enclose several plant growth regulators like gibberillin and auxin (Zodape et al., 2010).

The brown alga *Ascophyllum nodosum* is widely used to produce biofertilizers in land cultivation (Crouch and Van Staden, 1993). Besides this species, more brown seaweeds belonging to *Turbinaria*, *Sargassum*, *Fucu*s and *Laminaria*, genuses are tested (Hong et al., 2007). Recently, an increased interest in using green algae such as *Ulva reticulata* (Ganapathy et al., 2013), *Codium tomentosum* (Mohy El-Din, 2015), *Chaetomorpha linum* (Chebil Ajjabi et al., 2019), and *Chaetomorpha antennina* (Chanthini et al., 2019) for growing vine, wheat and tomatoes respectively.

C. linum flourish amply in nutrient rich environments as anthropogenic coastal areas. Such seaweed material interferes with human activity, disrupting, for instance, navigation, aquaculture, and tourism. Environment protection agencies are thus compelled to clean algal thalli and dispose them as waste. Each profitableuse of this waste material is much desired from the standpoint of environmentally sustainable development.

The chickpea *Cicer arietinum* L., 1753, a dicotyledonous of Fabaceae family, is among the important food leguminous plants cultivated in Tunisia. Chickpeas are grown in Tunisia on 30,000 hectares, a third of the total area (110,000 ha) of pulses, with an average yield of 300 thousand tons per year and an average productivity of 630 Kg/ha (Sleïmi et al., 2001).

In keeping with these aspects, the present work was undertaken to study the biostimulating potential of the local green alga *C. linum* (O.F.Müller) Kützing 1845

extract with regard to growth, yield parameters and seed germination of the chickpea *C. arietinum*.

2. Materials and Methods

2.1. Collection of seaweeds

C. linum, is a thread-like green macroalga, very abundant on the Tunisian coasts, and especially in lagoon environments (Ksouri et al., 1997). Sampling was carried out on the southern shore of the Northern Lake of Tunis (36°49'N, 10°14'E) where salinity was 37-38 PSU and water temperature varied between 15-20°C.The seaweed was hand-picked and washed rigorously with seawater and lastly, with fresh water to eliminate epiphytes and sticking sand particles.

2.2. Preparation of seaweed liquid extract

Seaweeds were left to dry in the shade for four days, then dried in the oven for 24 hours at 60°C. Afterwards, materials were ground by hand and made into fine powder with an electrical crusher. Seaweed powder was added using distilled water in a 1:20 (w/v) ratio and was then incubated for 30 minutes in a 40°C water bath (Mohanty and Adhikary, 2018). The contents were filtered into a double-layer cheese fabric and cooled at room temperature. Filtrate was centrifuged at 10000 rpm during 10 minutes and kept in the fridge at 4°C until later use. The resulting supernatant was considered as 100 % seaweed liquid extract and from which five different concentrations 1%, 3%, 5%, 8% and 10%, were prepared using distilled water.

The physical observations such as color and pH were determined by Konica minolta colorimeter and Eutech instruments pHmeter model pH 2700, respectively. The chemical elements such as total nitrogen, magnesium, potassium, calcium, phosphate, iron, zinc, copper and chloride were quantified using Inductively Coupled Plasma Spectrometry (ICP-OES Optima 8000 Perkin Elmer).

2.3. Experimental design and treatments

2.3.1. Germination assays

The chickpea *Cicer arietinum* seeds were provided to us from the laboratory of food legumes at the National Institute of Agronomic research of Tunis. Seeds of consistent size, color and weight were chosen. Chickpea seeds were disinfected with ethanol (80%) for 30 seconds and sodium hypochlorite (5%), successively, and then washed three to five times with sterilized distilled water. The healthy selected seeds with about the same size (0.9 cm), were divided into batches of 10 each and put to germinate in Petri dishes between two layers of cotton moistened with 5 mL of the varied concentration of SLE (1%, 3%, 5%, 8% or 10%) depending on the treatment.

The Petri dishes were maintained in incubator at 25°C ±2°C. In control (0%), seeds were treated with distilled water and maintained under the same conditions.

The germination is marked by the exit of the radicle out of the seed tegument. The experiment is conducted in triplicate and germination is followed for 7 days.

2.3.2. Field trial

In order to stimulate their growth, the seeds already germinated in the Petri dishes and became seedlings at the end of the first stage (7 days) were transplanted three germinated seeds per pot filled with 100 g of fine soil. The seedlings were planted in earth pots to a depth of 2.5 cm below ground level and were left to grow. The seeds were then watered in their pots with algal extract at a rate of 5 mL per day per pot to avoid mineral deficiencies. The treatments were implemented according to

the different concentrations of SLE (1%, 3%, 5%, 8% or 10%). At the same time, the control pots (0%) were watered only with tap water. The pots were placed outside and at ambient temperature 22-25°C. Seedlings were allowed to grow for 30 days.

2.4. Biostimulant assays

Growing parameters including seed germination percentage, root length, shoot length and biochemical parameters were regularly monitored.

2.4.1. Germination assays

Germination was registered daily by counting the emergent radicle. Each species has specific germination precocity according to its nature. This parameter is expressed by the rate of the first seeds germinated after 24 h from sowing.

To better understand the physiological significance of germination kinetics, the germinated seeds were counted daily until day 7 of the experiment.

The germination speed is evaluated by the coefficient of velocity and the average time T50 which corresponds to the germination of 50% of the seed lot.

The time taken to achieve 50% germination (T50) was determined using the formula of Coolbear et al. (1984) as amended by Farooq et al. (2005):

$$\mathbf{T50\ (day)=t_i + [(N / 2 - n_i) (t_i - t_j)] / n_i – n_j}$$

Where, N is the final number of emergence and n_i, n_j are the cumulative number of seeds germinated by adjacent counts at times t_i and t_j, respectively when $n_i < N / 2 < n_j$

The coefficient of velocity (CV) and the percentage of seed germination (GP) were measured using the following equations:

$$\mathbf{CV(\%)=(\sum N_i)/(\sum [\![N_i*t_i]\!])*100}$$ (Kotowski, 1926)

$$\mathbf{GP(\%)=N_i/N_t*100}$$ (Nichols & Heydecker, 1968)

Where, N_i is the number of seeds germinated at time t_i, N_t is the initial total seed number, t is the days following the start of the germination assay and $\sum N_i$= total number of newly germinated seeds at time t_i. After 7 days, growth parameters like length of radicle (future root) and epicotyls (future shoot) were analyzed (Fig.1).

Figure 1. Germinated chickpea seed
a: Radicle ; **b**: Epicotyl (scaling x10)

2.4.2. Pigment Contents in the cultivated plants

After 30 days, plants were uprooted and washed with tap water. Only the aerial parts were subjected to analysis. The quality parameters for treated chickpea plants were calculated in terms of pigment contents such as total chlorophyll, chlorophyll "*a"* and chlorophyll "*b"* based on the methodology described in Lichtenthaler and Wellburn (1983). The resulting color solution was analyzed with a UV spectrophotometer type LLG Labware. Measurements have been conducted at wavelengths of λ=663and 645nm.

The concentrations of total chlorophyll (Total Chl), chlorophyll a (Chl(a)), and chlorophyll b (Chl(b)) were calculated based on the equations below (Arnon,1949):

Total Chl (mg/g) = 20.2A645 +8.02 A663
Chl (a) (mg/g) = 12.7 A663 − 2.69 A645
Ch (b) (mg/g) = 22.9 A645 − 4.68 A663

2.5. Statistical Analysis

All measures were carried out in triplicate. All reported data are the mean ± standard deviation and were analyzed using a one-way variance analysis (ANOVA). Statistically significant differences in means ($p \leq 0.05$) were estimated by the Duncan test with Sigma Stat 3.1.

3. Results

3.1. Characteristics of the seaweed liquid extract

The physico-chemical properties of *C. linum* SLE before preparation of the different concentrations were shown in Table 1. The extract is rich in mineral elements, particularly with nutrients such as Nitrogen, Potassium, Phosphorus, Sodium, Magnesium, Calcium,Manganese and Zinc.

Table 1. Physico-chemical properties of *Chaetomorpha linum* liquid extract

CONSTITUENT	VALUES
Color	Light brown
pH	6.7
Salinity (psu)	19
Nitrogen Kjeldahl (mg N/L)	193.7
Potassium (mg/L)	3135.6
Phosphorus (mg/L)	51.2
Sodium (mg/L)	747.6
Calcium (mg/L)	434.5
Iron (mg/L)	96.1
Manganese (mg/L)	2.4
Magnesium (mg/L)	1.51
Aluminium (mg/L)	0.87
Zinc (mg/L)	0.08
Nickel (mg/L)	0.05
Cobalt (mg/L)	0.04
Cadmium (mg/L)	0.005
Chrome (mg/L)	<0.041
Copper (mg/L)	<0.026
Lead (mg/L)	<0.019

3.2. Seed Germination

The chickpea seeds treated with the lowest SLE concentrations showed higher significantly different rates of germination (at 95% confidence level), while higher concentrations slow down the germination.

Figure 2 shows the variations in the percentages of the early germinated SLE-treated seeds. Indeed, from the first day, most of seeds germinated with the different percentages of extract.

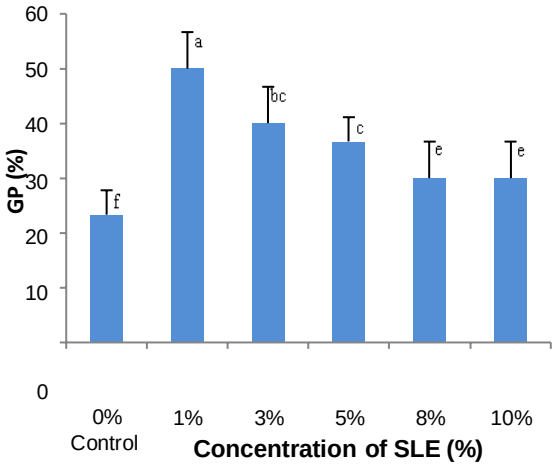

Figure 2. Germination precocity percentage (GP) of chickpea seeds under control and different concentrations of seaweed liquid extract (values are mean ± SD, n = 10). Values with the same letter are not significantly different at P<0.05

The findings showed that the germination percentage of the seeds after 24 hours was increased in SLE-treated seeds when compared to control. The increased seed germination was recorded at lower concentrations. The highest germination precocity percentage (50%) was recorded with 1% SLE. This percentage is 2 times higher than that obtained in control.

The evolution of the germination speed expressed by the velocity coefficient (CV) and the average germination time (T50) showed that control and treated seeds have different T50 values (Fig. 3). On the other hand, by increasing the SLE concentration, the germination speed is reduced and its duration is prolonged. In fact, the highest velocity coefficient was found in seeds having received the 1%

treatment (53.39%) with the shortest T50 (1.87 days) (Fig.3). Above this concentration, a decreased germination speed is recorded with an increased T50.

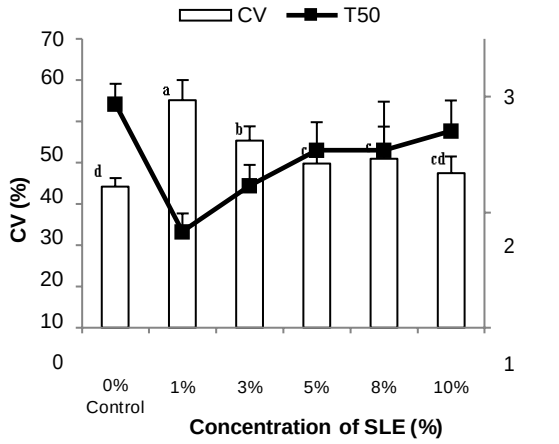

Figure 3. Coefficient of velocity (CV) and mean time (T50) of germinated chickpea seeds under control and different concentrations of seaweed liquid extract SLE (values are mean ± Sd, n=10). Values with the same letter are not significantly different at P<0.05

The daily chickpea seeds monitoring demonstrated that all seeds germinated in all treatments as well as in the control (Fig. 3). Moreover, the rate of 100% germinated seeds was obtained at different times. Indeed, the seeds of the control crop reached 100% germination on the 5th day, whereas the SLE treated seeds totally germinated on the 4th days (Fig.4).

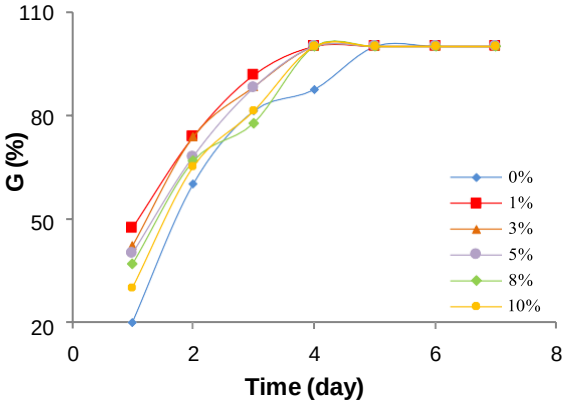

Figure 4. Effect of different concentrations of seaweed liquid extract (0%, 1%, 3%, 5%, 8% or 10%) on germination (G) kinetics of chickpea seeds (values are mean ± Sd, n=10)

3.3. Morphological Parameters

The SLE application enhanced the plant growth that was visible in the seeds enriched by *C. linum* SLE when compared to the control. The effect of SLE on growth parameters such as radicle and epicotyl lengths is shown in Table 2.

The maximum radicle length (3.41±0.97cm) and epicotyl length (3.42±0.74 cm) were observed at 1% concentration of SLE at significantly different level (at 95% confidence level) (Table 2). Beyond this concentration, these variables decreased substantially. The lowest radicle length (2.04±0.35 cm) and epicotyl length (1.76±0.52cm) were obtained at 10% concentration of SLE (Table 2).

Application of 1% SLE showed that radicle and epicotyl lengths were increased 3.2 and 2.3 times, respectively as compared to control.

Major differences at a significance level of 5% were observed for radical and epicotyls lengths at 1% SLE treatment. Furthermore, no significant difference was observed between values obtained at 3%, 5%, 8% and 10% SLE treatments (Table 2).

3.4. Photosynthetic Pigments

Chlorophyll levels in crops after 30 days are reported in Table 3. In most cases, total concentrations of Chl, Chl(a) and Chl(b) were higher in all treatments compared to the control group. The highest contents of total Chl, Chl(a) and Chl(b) were recorded in 1% SLE treatment. Application of 1% SLE showed an increase of 5.8, 7.1 and 2.4 times of total Chl, Chl(a) and Chl(b) concentrations, respectively as compared to those recorded in control. It can be seen that as SLE concentrations increased, the chlorophyll content of plants declined (Table 3).

Table 2. Effect of different concentrations of *C. linum* liquid extract on radicle and epicotyls lengths of chickpea seeds after 7 days of germination (values are mean ± Sd, n = 10).

Length (cm)	Control	Seaweed Liquid Extract (SLE)				
	0%	1%	3%	5%	8%	10%
Radicle	1.04±0.14 ^{d}	3.41±0.97 ^{a}	2.43±0.65 ^{b}	2.27±0.46 ^{b}	2.06±0.31^{bc}	2.04±0.35 ^{c}
Epicotyl	1.51±0.39^{d}	3.42±0.74^{a}	2.30±0.90^{b}	1.94±0.32^{bc}	1.84±0.43^{c}	1.76±0.52^{c}

Values with the same letter are not significantly different at P<0.05.

Table 3. Effect of different concentrations of *C. linum* liquid extract on total chlorophyll, chlorophyll (a) and chlorophyll (b) concentrations of chickpea seedlings after 30 days of pots growth (values are mean ± Sd, n= 10)

Content (mg/g DW)	Control	Seaweed Liquid Extract (SLE)				
	0%	1%	3%	5%	8%	10%
Total Chl	3.96±0.04^{e}	23.08±0.27^{a}	14.23±0.69^{b}	12.59±0.66^{c}	8.49±1.31^{d}	7.14±0.39^{d}
Chl (a)	2.69±0.29^{e}	19.20±0.14^{a}	11.27±0.09^{b}	9.72±0.12^{c}	7.78±0.43^{d}	6.39±0.25^{d}
Chl (b)	1.62±0.06^{c}	3.88±0.18^{a}	2.97±0.27^{b}	2.86±0.32^{b}	0.93±0.03^{d}	0.75±0.09^{d}

Values with the same letter are not significantly different at P<0.05.

4. Discussion

Seaweed and their extracts are known for boosting the growth in fruits, vegetablesand other crops since they are rich in carbohydrates (Karthik & Jayasri, 2023), macro and micro elements (Blunden et al. 1997), phenylacetic acid (Taylor and Wilkinson 1997), vitamins and phytohormones like gibberellins, cytokinins, auxins, and betaines (El Sheekh and El Saied, 2000). Thus, marine seaweeds are considered effective biofertilizers in agriculture since they contain all the plant growth components and hormones needed by plants. The present study indicated that utilization of *Chaetomorpha linium* liquid extract enhanced germination and growth of chickpea seed.

These results are in agreement with earlier results reporting thatgrowth enhancement may be attributable tothe existence of growth-promoting substance like hormones (auxins and gibberellins), micronutrients (Fe, Zn,Co, Cu, Mo and Mn), amino acids andvitamins in SLE (Ramarajan et al. 2012,Ganapathy Selvam and Sivakumar 2013,Layek et al. 2018, Chantini et al. 2019).In this study, seaweed extract application inchickpea seed at the lowest concentration of SLE (1%) showed the highest seed germination while minimum seed germination was observed at the highest concentration (10%). In addition, the treated seeds exhibited a higher germination rate (100%) and emerging 1 day earlier than untreated seeds (control). This can be explained by the salt sensitivity of chickpea Studies conducted by Sleïmiet al. (1999) on salt tolerance of several chickpea species (Amdoun 1, Chetoui, Kesseb, ILC482...etc.) have confirmed this sensitivity and showed

that most salt tolerant species to high concentrations (35 mM of Na Cl) were Amdoun 1 and ILC482. The chickpea variety used in this study is “Rebha” and seems to be salt sensitive since optimum germination and growth rates were recorded in the treatment settled with the lowest SLE concentration (1%).

Similar findings were reported by Kalaivanan and Venkatesalu (2012) that 100% seed germination of *Vigna mungo* was recorded with the lowest concentration of *Sargassum myriocystum* extract (20%). However, 100% seed germination of *V. mungo* was achieved with an even lower concentration (2.5%) of *Ulva reticulata* extract (Ganapathy Selvam et al. 2013).

Besides increasing the seed germination rate, the growth parameters such as radicle and epicotyls were also enhanced under low SLE concentration (1%). Similar effect of SLE prepared from *U. reticulata* on *V. mungo* was reported by Ganapathy Selvam et al. (2013). This can be attributed to the existence of high N, P and K levels in the SLE of *C. linum*. These macronutrients serve as soil conditioners by enhancing nutrient and water absorption by plants and improving proliferation of biota that support plant growth in the rhizosphere (Fan et al. 2011). These microbiotas assimilate the inorganic NPK into the organic form, increasing the bioavailability of nutrients for plant uptake (Kramer et al. 2006, Yakhin et al. 2017, Raghunandan et al. 2019).

Layek et al. (2019) showed that the increased use of doses of *Gracilaria edulis* and *Kappaphycus alvarezii* extracts on maize could reduce the soil nutrient content. The biostimulant mechanism of SLE is mostly attributed to their richness in essential nutrients for the plant and hormones found in seaweed (Spinelli et al. 2010). Indeed, these elements play an important role in improving cell size and amitosis. As well, the cumulative impacts of cytokinins and auxins enhance root-shoot development.

In this study, the physicochemical characterization of SLE from *C. linum* showed an extract rich in mineral. The obtained values are comparable to those reported for SLEs prepared from different algal species such as *Ulva reticulata* and *Codium tomentosum* (Ganapathy Selvam and Sivakumar 2013; Mohy El-Din, 2015) which also have a neutral pH.

On a qualitative point of view, several studies have focused on the chemical composition of algal extracts in order to be used as fertilizer for different plants. Sivasankari et al. (2006) demonstrated that the SLEs of *Sargassum wightii* and *Caulerpa chemnitzia* from Indian coasts have similar mineral composition in slightly different proportions. Divya et al. (2015)also confirmed the presence of the same chemical elements in the composition of *Ulva lactuca* extract with lower contents than the extract prepared in the present study. The observed difference in the composition of SLE could be justified by the extraction process or by the variation in the biochemical composition of the original algal species which in turn varies according to several biotic and abiotic factors.

The application of *C. linum* SLE has also enhanced the total chlorophyll, chlorophyll (a) and chlorophyll (b) contents and the highest values were recorded in 1% treatment. Beyond this concentration, these variables decreased substantially and specially as compared to control.

Chebil Ajjabi et al. (2019) demonstratedthat same concentration (1%) has also stimulated the chlorophyll content of *Triticum turgidum* as compared to control and to the other SLE concentrations. Higher concentrations of SLE have decreased the chlorophyll contents. Our findings are also in accordance with those of the Blunden 1977, when the *Ascophyllum nodosum* extract was implemented at low concentrations to the soil or foliage, the tomatoes produced leaves that had higher chlorophyll content than those of the untreated tomatoes.

According to our results, the increased chlorophyll content at the lowest concentration of SLE could be owing to the existence of substantial amount of magnesium in the liquid extract of *C. linum*. Michalak and Chojnacka (2013) indicated that high contents of elements like Cu, Fe and Mg in Baltic seaweed, and consequently in their extracts, are linked to their stimulating effect on chlorophyll synthesis. The present study showed that *C. linum* SLE, rich in mineral composition, stimulated the productivity of the plant while increasing the chlorophyll content.
Nevertheless, it is suggested that the increased chlorophyll content in plant leaves treated with seaweed extract is dependent on the presence of betaines and the presence in number and size of the chloroplast (Meno and Srivastava 1984, Whapham et al. 1993). Ganapathy selvam and Sivakumar (2013) observed a substantial increase in the amount of stomata and epidermal cells in *Vignamungo* treated with *Ulva reticulata* liquid.

5. Conclusion

Considering the above findings, the present study is an important step towards the utilization of *C. linum* extract to improve the growth of Chickpea *C. arietinum*. The lowest concentration of SLE (1%) yielded the best germination rates and growth of the different Chickpea organs. These findings support the bio-stimulating potential of *C. linum* SLE, which can be used as economic, renewable, effectiveand environmentally friendly biofertilizers. The green macroalgae *C. linum* represents a renewable marine source, economically sustainable for agricultural application and does not require specialized methods forthe SLE preparation or formulation for application.

However, further researches are necessary, though, to confirm the impact of *C. linum* SLE in various applications and on different crop species.

References

1. Abdel-Mawgoud, A.M.R., Tantaway, A.S., Hafez, M.M. & Habib, H.A.M. (2010). Seaweed extracts improves growth, yield and quality of different watermelon hybrids. *Research journal of agriculture and biological sciences*, 6(2), 161-168. https://www.aensiweb.net/AENSIWEB/rjabs/rjabs/2010/161-168.pdf
2. Ali, O., Ramsubhag, A. & Jayaraman, J. (2021). Biostimulant Properties of Seaweed Extracts in Plants: Implications towards Sustainable Crop Production. *Plants*, 10(3), 531. https://doi.org/10.3390/plants10030531
3. Arnon, D.I. (1949). Copper enzymes in isolated chloroplast, polyphenol oxidase in *Beta vulgaris*. *Plant Physiology*, 24(1), 1-15. https://doi.org/10.1104/pp.24.1.1
4. Blunden, G. (1977). Cytokinin Activity of Seaweed Extracts. In: Faulkner, D.J., Fenical, W.H. (eds) *Marine Natural Products Chemistry*. Nato Conference Series, vol 1. Springer, Boston,MA. https://doi.org/10.1007/978-1-4684-0802-7_27
5. Campobenedetto, C., Agliassa, C., Mannino, G., Vigliante, I., Contartese, V., Secchi, F. & Bertea, C.M.A. (2021). Biostimulant based on beaweed (*Ascophyllum nodosum* and *Laminaria digitata*) and yeast extracts mitigates water stress effects on tomato (*Solanum lycopersicum* L.).*Agriculture,* 11(6), 557.

https://doi.org/10.3390/agriculture11060557

6. Chanthini, K.M.P., Senthil-Nathan, S., Stanley-Raja, V., Thanigaivel, A., Karthi, S., Sivanesh, H., Narayanan, S.S., Palanikani, R. & Soranam, R. (2019). *Chaetomorpha antennina* (Bory) Kützing derived seaweed liquid fertilizers as prospective bio-stimulant for *Lycopersicon esculentum* (Mill). *Biocatalysis and Agricultural Biotechnology*, 20, 101190, 1-9. https://doi.org/10.1016/j.bcab.2019.101190
7. Chebil Ajjabi, L., Ben Ammar, I., Ktari, L., Cherif, D., Cherifi, H. & Sadok, S. (2019). Effet de l'extrait liquide de l'algue verte *Chaetomorpha linum* (Müller) Kütz., 1849 sur la germination et la croissance du blé dur *Triticum turgidum* l. subsp. Durum. *INSTM Bulletin,* 46, 133-145. https://n2t.net/ark:/68747/INSTM.Bulletin.v46.212
8. Chouliaras, V., Tasioula, M., Chatzissavvidis, C., Therios, I. & Tsabolatidou, E. (2009). The effects of a seaweed extract in addition to nitrogen and boron fertilization on productivity, fruit maturation, leaf nutritional status and oil quality of the olive (*Olea europaea* L.) cultivar Koroneiki. *Journal of the Science of Food and Agriculture*, 89(6), 984-988. https://doi.org/10.1002/jsfa.3543
9. Coolbear, P., Francis, A. & Grierson, D. (1984). The effect of low temperature pre-sowing treatment on the germination performance and membrane integrity of artificially aged tomato seeds. *Journal of Experimental Botany*, 35 (11), 1609-1617. https://doi.org/10.1093/jxb/35.11.1609
10. Cozzolino, E., Di Mola, I., Ottaiano, L., El-Nakhel, C., Rouphael, Y. & Mori, M. (2021). Foliar application of plant-based biostimulants improve yield and upgrade qualitative characteristics of processing tomato. *Italian Journal of Agronomy*, 16 (2), 1825. https://doi.org/10.4081/ija.2021.1825
11. Crouch, I.J. & Van Staden, J. (1993). Commercial seaweed products as biostimulants in horticulture. *Journal of Home & Consumer Horticulture*, 1(1), 19-76. https://doi.org/10.1300/J280v01n01_03
12. Divya, D., Gopinath, L. R., Merlin Christy, P. (2015). A review on current aspects and divers prospects for enhancing biogas production in sustainable means. *Renewable and Sustainable Energy Reviews*, 42, 690-699. https://doi.org/10.1016/j.rser.2014.10.055
13. El-Sheekh, M. & El-Saied A. (2000). Effect of crude seaweed extracts on seed germination, seedling growth and some metabolic processes of *Vicia faba* L. *Cytobios*, 101(396), 23-35.
14. Farooq, M., Basra, S. M. A., Hafeez, K. & Ahmad, N. (2005). Thermal hardening: a new seed vigor enhancement tool in rice. *Journal of Integrative Plant Biology,* 47(2),187-193. https://doi.org/10.1111/j.1744-7909.2005.00031.x
15. Frioni, T., Sabbatini, P., Tombesi, S., Norrie, J., Poni, S., Gatti, M. & Palliotti, A. (2018). Effects of a biostimulant derived from the brown seaweed *Ascophyllum nodosum* on ripening dynamics and fruit quality of grapevines. *Scientia Horticulturae*, 232, 97-106. https://doi.org/10.1016/j.scienta.2017.12.054
16. Ganapathy Selvam, G. & Sivakumar, K. (2013). Effect of foliar spray from seaweed liquid fertilizer of *Ulva reticulata* (Forsk.) on *Vigna mungo* L. and their elemental composition using SEM- energy dispersive spectroscopic analysis. *Asian Pacific Journal of Reproduction,* 2(2), 119-125.

https://doi.org/10.1016/S2305-0500(13)60131-1

17. Ganapathy Selvam, G., Balamurugan, M., Thinakaran, T. & Sivakumar, K. (2013). Developmental changes in the germination, growth and chlorophyllase activity of *Vigna mungo* L. using seaweed extract of *Ulva reticulata* Forsskål. *International Research Journal of Pharmacy,* 4, 252-254.
18. Gibilisco, P. E., Lancelotti, J.L., Negrin, V.L. & Idaszkin, Y.L. (2020). Composting of seaweed waste: Evaluation on the growth of *Sarcocornia perennis*. *Journal of Environmental Management*, 274, 111193. https://doi.org/10.1016/j.jenvman.2020.111193
19. Godlewska, K., Michalak, I., Tuhy, Ł. &Chojnacka, K. (2016). Plant Growth Biostimulants Based on Different Methods of Seaweed Extraction with Water. *BioMed Research International,* 4,5973760,1-11. https://doi.org/10.1155/2016/5973760
20. Hassan, S. M., Ashour, M., Sakai, N., Zhang, L., Hassanien, H. A., Gaber, A., & Ammar, G. (2021). Impact of seaweed liquid extract biostimulant on growth, yield, and chemical composition of cucumber (*Cucumis sativus*). *Agriculture,* 11(4), 320. https://doi.org/10.3390/agriculture11040320
21. Hernández-Herrera, R. M., Santacruz-Ruvalcaba, F., Ruiz-López, M.A., Norrie J. & Hernández-Carmona, G. (2014). Effect of liquid seaweed extracts on growth of tomato seedlings (*Solanum lycopersicum* L.). *Journal of Applied Phycology,* 26, 619-628. https://doi.org/10.1007/s10811-013-0078-4
22. Hong, D. D., Hien, H. M., Son, P. N. (2007). Seaweeds from Vietnam used for functional food, medicine and biofertilizer. *Journal of Applied Phycology*,19, 817-826. http://dx.doi.org/10.1007/s10811-007-9228-x
23. Kalaivanan, C. & Venkatesalu, V. (2012). Utilization of seaweed *Sargassum myriocystum* extracts as a stimulant of seedlings of *Vigna mungo*. *Spanish Journal of Agriculture Research,* 10(2), 466-470. https://doi.org/10.5424/sjar/201210 2-507-10
24. Kapur, B., Çeliktopuz, E., Saridas, M. A., Kargi, S. P. (2018). Irrigation regimes and bio-stimulant applicationeffects on yield and morpho- physiological responses of strawberry. *Horticultural Science and Technology,* 36(3), 313-325. http://dx.doi.org/10.12972/kjhst.20180031
25. Karthik T. & Jayasri M.A. (2023). Systematic study on the effect of seaweed fertilizer on the growth and yield of *Vigna radiata* (L.) R. Wilczek (Mung bean). *Journal of Agriculture and Food Research*, 14, 100748. https://doi.org/10.1016/j.jafr.2023.100748
26. Karthikeyan, K. & Shanmugam, M. (2016). Development of a protocol for the application of commercial bio-stimulant manufactured from *Kappaphycus alvarezii* in selected vegetable crops. *Journal of Experimental Biology and Agricultural Sciences*, 4, 92-102.
27. Kotowski, F. (1962). Temperature relations to germination of vegetable seed. *Proceedings of American Society of Horticultural Science*, 23, 176-184.
28. Ksouri, J., Ben said, R. & Beji, O. (1997). Evaluation des potentialités qualitatives naturelles des gracilaires (Algues rouges) du lac Nord de Tunis. *Bulletin de l'INSTM*, 24, 15-25.

https://n2t.net/ark:/68747/INSTM.Bulletin.v24.856

29. Kramer, S. B., Reganold, J. P., Glover, J. D., Bohannan, B. J. M. & Mooney, H. A. (2006). Reduced nitrate leaching and enhanced denitrifier activity and efficiency in organically fertilized soils. *Proceedings of the National Academy of Sciences*, 103 (12), 4522-4527. http://dx.doi.org/10.1073/pnas.0600359103
30. Latique, S., Elouaer, M.A., Chernane, H., Hannachi, C. & El Kaoua, M. (2017). Alleviation of Salt Stress in Durum Wheat (*Triticum durum* L.) seedlings through the application of liquid seaweed extracts of *Fucus spiralis*. *Communications in Soil Science and Plant Analysis,* 48(21), 2582–2593. http://dx.doi.org/10.1080/00103624.2017.1416136
31. Latique, S., Mrid, R. B., Kabach, I., Kchikich, A., Sammama.H., Yasri, A., Nhiri, M., El Kaoua, M., Douira.A. & Selmaoui, K. (2021). Foliar Application of *Ulva rigida* Water Extracts Improves Salinity Tolerance in Wheat (*Triticum durum* L.). *Agronomy,* 11(2), 265. https://doi.org/10.3390/agronomy11020265
32. Layek, J., Das, A., Ghosh, A., Sarkar, D., Idapuganti, R. G., Boragohain, J., Yadav, G. S.& Lal, R. (2019). Foliar application of seaweed sap enhances growth, yield and quality of maize in Eastern Himalayas. *Proceedings of the National Academy of Sciences, India,* 89, 221-229. https://doi.org/10.1007/s40011-017-0929-x
33. Lichtenthaler, H. & Wellburn, A.R. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions,* 11(5), 591-592. http://dx.doi.org/10.1042/bst0110591
34. Lopez-Mosquera, M.E., & Pazos, P. (1997). Effects of seaweed on potato yields and soil chemistry. *Biological Agriculture & Horticulture,* 14(3), 199-206.https://doi.org/10.1080/01448765.1997.9754810
35. Menon, K. K. G. & Srivastava, H. C. (1984). Increasing plant productivity through improved photosynthesis. *Proceedings/ Indian Academy of Sciences*, 93(3), 359-378. https://doi.org/10.1007/BF03053089
36. Michalak, I., & Chojnacka, K. (2013). Use of extract from Baltic seaweeds produced by chemical hydrolysis in plant cultivation. *Przemysl Chemiczny,* 92, 6, 1046-1049.
37. Mohanty, D. & Adhikary, S. P. (2018). Standardization of protocol for preparation of liquid extracts from seaweeds, quantification of their phytohormones and application for crop improvement. *Indian Journal of Geo-Marine Sciences*, 47(7), 1364-1372.
38. Mohy El-Din, S. M. (2015). Utilization of seaweed extract as bio-fertilizers to stimulate the growth of wheatseedlings. *Egyptian Journal of Experimental Biology*, 11(1), 31-39.
39. Nabti, E., Jha, B. & Hartmann, A. (2017). Impact of seaweeds on agricultural crop production as biofertilizer. *International Journal of Environmental Science and Technology*, 14, 1119-1134. https://doi.org/10.1007/s13762-016-1202-1
40. Nichols, M.A. & Heydecker, W. (1968). Two approaches to the study of germination data. *Proceedings of the International Seed Testing Association*, 33, 531-540.
41. Raghunandan, B.L., Patel, N.M., Dave, H.J. & Mehta, D.M. (2019). Natural occurrence of nucleopolyhedrovirus infecting fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera:

Noctuidae) in Gujarat, India. *Journal of Entomology and Zoology Studies,* 7(2), 1040-1043.

42. Ramarajan, S., Henry Josep, L. & Saravana Ganthi, A. (2012). Effect of seaweed liquid fertilizer on the germination and pigment concentration of soybean. *Research and Reviews: A Journal of Crop Science and Technology*, 1(2), 1-5
43. Rathore, S. S., Chaudhary, D. R., Boricha, G. N., Ghosh, A., Bhatt, B. P.,Zodape, S. T. & Patolia, J. S. (2009).Effect of seaweed extract on the growth,yield and nutrient uptake of soybean(Glycine max) under rainfed conditions.*South African Journal of Botany,* 75(2),351-355. https://doi.org/10.1016/j.sajb.2008.10.009
44. Rouphael, Y., Giordano, M., Cardarelli, M., Cozzolino, E., Mori, M., Kyriacou, M.C., Bonini, P. & Colla, G. (2018). Plant-and Seaweed-Based Extracts increase yield but differentially modulate nutritional quality of greenhouse Spinach through biostimulant action. *Agronomy*, 8(7), 126. https://doi.org/10.3390/agronomy8070126
45. Sekar, R., Thangaraju, N., & Rengasamy, R. (1995). Effect of seaweed liquid fertilizer from *Ulva lactuca* on *Vigna unguiculata* L. (WALP). *Phykos,* 34 (1-2), 49-53.
46. Sivasankari, S., Venkatesalu, V., Ananthara, M. & Chandrasekaran, M. (2006). Effect of seaweed extracts on the growth and biochemical constituents of *Vigna sinensis*. *Bioresource Technology,* 97(14), 1745-1751. https://doi.org/10.1016/j.biortech.2005.06.016
47. Sleïmi, N., Lachaâl, M. & Andelly, C. (1999). Responses of Some Tunisian chickpea (Cicer arietinum) varieties to salinity in nutrient solution. *International chickpea and pigeonpea newsletter*, 6, 23-25.
48. Sleïmi, N., Lachaâl, M., Andelly, C., Soltani, A. & Hajji, M. (2001). Physiological behaviour of two chickpea Tunisian varieties irrigated with saline nutrient solution. In: Horst, W.J., et al. *Plant nutrition: food security and sustainability of agro-ecosystems through basic and applied research*. *Developments in Plant and Soil Sciences*, vol 92. Springer, Dordrecht. https://doi.org/10.1007/0-306-47624-X_197
49. Spain, O., Hardouin, K., Bourgougnon, N. & Michalak, I. (2022). Enzyme-assisted extraction of red seaweed *Solieria chordalis* (C. Agardh) J.Agardh 1842 the starting point for the production of biostimulants of plant growth and biosorbents of metal ions. *Biomass Conversion and Biorefinery,* 14, 1621-1635. https://doi.org/10.1007/s13399-022-02456-7
50. Spagnuolo, D. & Prisa, D. (2021). Evaluation of growth parameters on *Carpobrotus edulis*, *Kalanchoe daigremontiana* and *kalanchoe tubiflora* in relation to different seaweed liquid fertilizer (SLF) as a biostimulant. *International journal of current microbiology and applied sciences* 10(10), 67-76. https://doi.org/10.20546/ijcmas.2021.1010.010
51. Spinelli, F., Fiori, G., Noferini, M., Sprocatti, M. & Costa, G. (2009). Perspectives on the use of a seaweed extract to moderate the negative effects of alternate bearing in apple trees. *Journal of Horticultural Science and Biotechnology,* 84(6), 131-137. http://dx.doi.org/10.1080/14620316.2009.11512610
52. Taylor, I.E.P. & Wilkinson, A.J. (1977). The occurrence of gibberellins and gibberellins-like substances in algae.

Phycologia, 16, 37-42. https://doi.org/10.2216/i0031-8884-16-1-37.1

53. Thirumal Thangam, R., Rani, M.V. & Marian, S.P. (2003). Effect of seaweed liquid fertilizer on the growth and biochemical constituents of *Cyamopsis tetragonoloba* (L.) Taub. *Seaweed Research and Utilization,* 25, 99-103.
54. Venkataraman, K.V., Mohan, V.R., Murugeswari, R. & Muthusamy, M. (1993). Effect of crude and commercial seaweed extracts on seed germination and seedling growth in green gram and black gram. *Seaweed Research and Utilization,*16 (1-2), 23-27.
55. Verkleij, F.N. (1992). Seaweed extracts in agriculture and horticulture: *A review. Biological Agriculture and Horticulture,* 8(4), 309-324. https://doi.org/10.1080/01448765.1992.9754608
56. Whapham, C. A., Blunden, G., Jenkins, T. & Hankins, S. D. (1993). Significance of betaines in the increased chlorophyll content of plants treated with seaweed extract. *Journal of Applied Phycology*, 5, 231–234. https://doi.org/10.1007/BF00004023
57. Yakhin, O.I., Lubyanov, A.A., Yakhin, I.A., & Brown, P. H. (2017). Biostimulants in plant science: a global perspective. *Frontiers in Plant Sciences*, 7, 2049, 1-32. https://doi.org/10.3389/fpls.2016.02049