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Physiological effect of *Sargassum acinarium* extract to alleviate salt stress of *Vicia faba*, *Triticum aesativum* and *Zea mays* seedlings

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Abstract

Algal extract showed a significant positive influence either alone or combined with different salt concentrations, on the lengths of the shoot and root, as well as the fresh and dry weight of all the seedlings subjected to the study. The promoting effect of algal extract extend to photosynthetic pigments (chl a, b and carotenoids) was also observed for all salt-stressed seedlings. It was observed that the activity of the enzyme superoxide dismutase (SOD) increased in seedlings treated with salt compared to the stressed seedlings that interacted with algal extract (by 2.5% at both salt concentrations in bean, 2.6% at low salt concentration and 1.15 at high concentration in wheat and 5% at low and 8.4% at high salt concentration in corn). This was also confirmed by the rate of free radical activity as well as the amount of proline in the shoots of all crop seedlings subject to the study. Hence, the role of algae extracts in alleviating the harmful effect of sodium chloride salt on crop seedlings is clear.

Keywords: Plant crops, antioxidant, *Sargassum acinarium,* extract, proline, ROS

Introduction

According to (Mittler, 2002) [35], oxidative damage at the cellular level that results in cell death is responsible for a large portion of the damage seen in plants under abiotic stress. During ideal development circumstances, the plant antioxidant defence system strictly controls the balance of reactive oxygen species generation and consumption (Hameed *et al*., 2011) [25]. In the world's arid and semi-arid regions, salinity reduces agricultural production by 18 to 43% (Chanthini *et al*., 2022) [11]. Salinity is a major limiting factor in agricultural output, and salt stress has an impact on plant growth at all stages. An osmotic stress brought on by a decrease in soil water availability and an ionic stress brought on by an imbalance of solutes in the cytosol are the two components of the stress that high salt levels produce in plants (Conde *et al*., 2011) [15]. Salinity affects around 20% of the world's farmed land and almost half of all irrigated land Salinity may impact seedling development by limiting the absorption of water and/or lowering gibberellin levels in germinating seeds. (Fercha *et al*., 2014) [19].

Seaweed is a kind of large marine algae that are tolerant of saltwater, rich in micro- and macronutrients, growth regulators, and other natural products that are often used as organic fertiliser. Seaweed is also an important source of oxygen for plants. (Bulgari *et al*., 2019) [9]. Seaweed extracts promote seed germination and early seedling vigour, encourage additional buds, root growth, and raise the validity of vegetables and fruits. (Arioli *et al*., 2015) [4]. SLF has a high concentration of plant growth regulators like auxin, indole-3-acetic acid (Valencia *et al*., 2018) [48] and gibberellins (Flora and Vishnupriya, 2016) [20]. As a result, marine resources, namely brown algae, play an important role in agriculture. Applying seaweed extracts or manures in various ways display a wide variety of favourable reactions, which include improved germination, root growth system, improved fruit quality, plant vigour, disease resistance, and chlorophyll content and leaf area . Seaweed extracts are considered to have active compounds that have a small molecular mass (polyamines and brassinosteroids) and growth hormones (auxins, cytokinin, gibberellins) and all of them work well at modest levels. This is because these positive effects are accomplished with modest dosages of the extracts. For example, Using various marine algae significantly improved wheat seedling development under salt stress conditions.

Sargassum sp. is one of the biggest and most diverse genera of brown seaweeds, with over 400 taxonomically recognised species, and were used as food, feed, and cures in traditional medicine throughout human history (Catarino *et al*., 2023) [10]. With its rapid growth and strong capacity for regeneration, *Sargassum acinarium* is a brown seaweed (*Phaeophyceae*) that forms thick floating masses that cover sizable portions of the water's surface (Pereira, 2019) [38]. (Ismail *et al*., 2019) [29] and reported that, this species might be utilised for agricultural fertiliser. Therefore, The study's objective is to study the effect of *Sargassum acinarium* extract as a biofertilizer to reduce salt stress on crop plants to improve the physiological and enzymatic processes within the plant. The current study aims to determine the effect of *Sargassum acinarium* extract on growth functions and some physiological changes, as well as superoxide dismutase enzyme activity in beans, wheat, and maize seedlings stressed with different concentrations of sodium chloride salt.

Materials and methods

The algal sampling and Plant material

Sargassum acinarium seaweeds were collected from the Mediterranean coast of Egypt, the western coast (Al-Agami region, at latitudes 31° 5' 55.8132" N and 29° 45' 59.9976" E) Alexandria, Egypt. Assembled *Sargassum acinarium* was packaged in polythene bags and carried to the laboratory in an ice box, where they were first rinsed with sea water to remove sand and other substances before being extensively cleansed with fresh water to remove salt. The strategies described in the literature were used to identify the algae species. (Shabaka, 2018, Chapman and Gellenbeck, 1989) [12, 42] and categorised taxonomically in accordance with (PAPENFUSS, 1960) [37]. The identification was based on morphological and ecological (distribution and habitat) characteristics. Sigma-Aldrich supplied all of the chemicals and reagents (Taufkirchen, Germany), and Merck (Darmstadt, Germany). *Vicia faba* (cv. Giza 716), seeds, *Triticum aesativum* (cv. Suds 14), *and Zea mays* (cv. Cairo 1), grains were supplied by Agriculture Research Centre, Giza, Egypt. The healthy grains were properly cleaned (2.5% sodium hypochlorite for 5 minutes) and submerged in clear distilled water after being checked for probable unity of size and shape.

Preparation of algal extracts

The *Sargassum acinarium* seaweed was dried in the sun for seven days. After that, the dry material was completely ground using an electric grinder into very fine particles and then sieved with an 80-mesh sieve, and 4.0% aqueous algae extract was prepared (because of testing several percentages as a preliminary test) by soaking in distilled water for one day at room temperature using a shaking machine, then centrifuged at 3000 round/minute for 15 min. Through a double-layered filter paper, filtration was conducted (Whatman No.1). In the control treatment, distilled water was used in the experiment for comparison with other treatments. The obtained powder was kept at 4°C until needed by placing it in moisture and air proof containers (Makawita *et al*., 2021, El-Sheikh *et al*., 2020) [17, 33].

Seeds germination and cultivation condition

Germination of *Vicia faba*, *Triticum aesativum and Zea mays* seedlings were brought temperature-neutral environment (23 ± 2 ºC) in 0.02 m^2 sterilized plastic containers, each contains 780 g of sandy soil that has been disinfected by acid washing. Seeds were tested and sorted into two groups.: one group was soaked in liquid algal extract for 24 hours at room temperature, while the other group, representing the control seeds were steeped for 24 hours in distilled water. as well. Both groups were irrigated by different concentrations of salt (0.0, 25, 150 mM NaCl), such that each dish contains 10 seeds, which were irrigated with equal amounts (20 ml) of different treatments and kept in the darkness for two days. The photoperiod was 10:14 hours (light/darkness). At 12th-day, the seedlings were collected to determine growth parameters (radicle and plumule lengths, fresh and dry weights), pigment contents in leaves, superoxide dismutase enzyme activity, total prolins and antioxidant capacity, in addition to the physicochemical and hormonal investigation of *Sargassum acinarium* seaweed extracts.

Phytochemical analysis

Estimation of photosynthetic pigments

Fresh leaves were weighted and mixed with 5ml of 85% cold acetone. After homogenizing, the samples were kept in refrigerator for 24 hours. Following the incubation time, samples underwent a 15-minute centrifugation at 5000 g. Extract stored in the refrigerator until the next day. A suitable volume of the acetone extract was diluted, at 663, 644, and 452.5 nm, its color intensity was then measured (Metzner *et al*., 1965) [34]. The experimental plant's pigment concentration was calculated using the following equations:

$$\text{Chl } a = 10.3A_{663} - 0.918\ A_{644}$$
$$\text{Chl } b = 3.87A_{663} - 19.7\ A_{644}$$

$$\text{Carotenoids} = 4.2\ E\ 452.5 - (0.0264\ \mathit{Chl\ a} + 0.426\ \mathit{Chl\ b})$$

In terms of fresh weight, pigment fractions were represented as µg/g.

Antioxidant assay

A 0.5 g of fresh plant leaves were crushed in liquid nitrogen and 50 mM cold phosphate buffer, 8 ml of which were homogenized of pH 7.0 (Beauchamp and Fridovich, 1971) [7]. Centrifuging the homogenates at 1971 g for 20 minutes separated the mixtures. It was the supernatants were stored at -80 °C which further utilized as a raw extract for an enzymatic analysis.

The extract of the enzyme was introduced into the reaction mixture, which included 9.9 mM L-methionine, 0.057 mM nitro blue tetrazolium, 50 mM potassium phosphate buffer pH 7.8, 0.025% Triton X-100, and 0.0044% riboflavin. The reaction was then illuminated for 15 min with 30 W fluorescent lamp. To evaluate the activity of SOD, the synthesis of formazan resulting from photochemical reduction of nitro blue tetrazolium (NBT) in the presence of SOD [EC 1.15.1.1] was observed (Beyer Jr and Fridovich, 1987) [8]. After turning off the light, the absorbance was measured at 560 nm, and the activity of SOD was estimated using the extinction value of 21.1 $mM^{-1}\ cm^{-1}$.

Antioxidant capacity DPPH Free radical scavenging activity (RSA)

Sargassum acinarium dilute thallus extracts' antioxidant activity was assessed with DPPH RSA. Using the 1,1-diphenyl-2-picryl hydrazyl (DPPH) method, the free RSA of the diluted thallus extract of *Sargassum acinarium* was examined. The stock solution was created by dissolving 24 milligramme of DPPH in 100 mL of methanol. In a test tube, add 3 mL of DPPH working solutions and 100 µL of thallus extract. A standard usually consists of 100 µL of methanol and three millilitres of DPPH solution. After that, for half an hour, the tubes were kept completely dark. Therefore, the absorbance was measured at 517 nm. The following formula was used to compute the percentage of antioxidants or RSA (Valko *et al*., 2007) [49]:

$$\%\ \text{Antioxidant activity} = [(Ac - As) \div Ac] \times 100$$

(Ac) at t = 0 min, is the absorbance of the blank; (As) represents the antioxidant's absorbance at t = 30 minutes.

Estimation of total proline

The total proline was detected in the dry plant samples according to (Bates *et al*

., 1973) [6]. 0.5 g dry leaves were grinded in (5 ml) of 3% sulphosalicylic acid and centrifuged at 10000 g for 20 min. 2 millilitres of acid ninhydrin reagent (1.25% ninhydrin heated in a boiling water bath for one hour with 30 millilitres of glacial acetic acid, 20 millilitres of 6M phosphoric acid, and 2 millilitres of acetic acid) were mixed with 0.1 millilitres of supernatant. An ice bath was used to stop the reaction. Toluene chromophore was measured at 520 nm after drops of toluene were introduced to the reaction mixture. Using a proline-prepared calibration curve, the total proline was determined as mg/g d.m.

Physio-chemical and growth promoting substances of *Sargassum acinarium* extract

The extract from *Sargassum acinarium* was measured for physical attributes including colour and pH. The American Public Health Association's guidelines (1995) were used to estimate the chemical components included in seaweed extract, including magnesium, iron, chloride, sulphate, copper, sodium, calcium, zinc, nitrate, cobalt, phosphate, potassium, and manganese. Additionally, in order to calculate the concentrations of plant growth regulators such cytokinin, auxin, and gibberellin, *Sargassum acinarium* liquid extract was prepared. (Ünyayar *et al*., 1996) [47].

Statistical analysis

A statistically significant level between treatments was assessed for all outcomes, and Fisher's individual error rate was used to plate the one-way analysis of variance (ANOVA) design. The means were compared using the least significant difference method. a 5% LSD, or least significant difference using the Minitab program version 16, three replicas of each experiment were run.

Results

Table 1 displays the physiochemical parameters of the brown alga *Sargassum acinarium*. The extract had a dark color and a pH of 6.7. Similarly, sodium (7.328 mg/L) and magnesium (18.25 mg/L) accounted for a major amount in the micro elemental analysis, whereas other elements were present in significant amounts. Cytokinin (8.98 mg/L) was found in greater concentrations than gibberellin and auxin in growth hormone study.

The brown alga *Sargassum acinarium*'s physiochemical characteristics are shown in Table 1**.** The extract's pH was 6.7 and it had a dark physical appearance. In the same way, the micro elemental analysis magnesium (18.25 mg/L), sodium (7.328 mg/L) and copper (4.031 mg/L) accounted for a significant amount, and additional components were found in significant amounts. Cytokinin (8.98 mg/L) was found to be more abundant than auxin and gibberellin in the growth hormone study.

Table 1: Physico-chemical and hormone analysis of seaweed extracts of Sargassum *acinarium*

Physical parameters	
Color	Brown
pH	6.7
Chemical parameters (mg/L)	
Copper	4.031
Manganese	0.049
Zinc	1.793
Iron	0.449
Potassium	1.121
Magnesium	18.25
Cobalt	0.177
Sodium	7.328
Growth hormones (mg/L)	
Auxin	3.2
Cytokinin	8.98
Gibberellin	4.8

Growth criteria

The impact that algae extracts have on the length of shoot and root exhibited a remarkable effect on seedlings of investigated seedlings. Figure 2 shows the effect of different NaCl salt concentrations (25mM and 150mM) singly or combined with *Sargassum acinarium* extract (4.0%) on the shoot height and root length of *Vicia faba*, *Triticum aesativum* and *Zea mays* seedlings (12 days old). The remarkable rise in the length of both shoot (by 9% in bean, 6.6% in wheat and 2.4% in corn) and root (by 7.9% in bean, 7.6% in wheat and 6.8% in corn) was observed in all seedlings treated with the algal extract compared to the control. It was discovered that both shoots benefited noticeably from the algal extract (by 8% in bean, 12.5% in wheat and 11.3% in corn) and root (16.8% in bean, 19.5% in wheat and 6.5% in corn) length, interacting with the low salt concentration compared to the salt concentration alone for all seedlings. The effect was also clear in the case of the algal extract interacting with the high salt concentration compared to the salt concentration alone in both shoot (by 4.8% in bean, 4.3% in wheat and 10.1% in corn) and root (by 34.2% in bean, 8.8% in wheat and 16.25% in corn) length, it was noted that the shoot of corn seedlings was the most responsive to the algal extract's impact on different salt concentrations. While the roots of beans and corn are the seedlings most responsive regarding the impact of algae extract on different salt concentrations. In comparison to the control, Figure 3 demonstrates that the algal extract significantly impacted each seedling's fresh and dry weight. The algal extract had a noticeable influence on both fresh (by 3.75% in bean, 6.5% in wheat and 9.9% in corn) and dry (4.5% in bean, 8.8% in wheat and 6.8% in corn) weight in all treated seedlings in comparison to the control. Also, The fresh weight of all seedlings was clearly affected by the interaction of the algal extract with varying salt concentrations, while the dry weight in the case of low salt concentration (by 10.5% in bean, 8.06% in wheat and 9% in corn) was more noticeable than the high concentration (by 14.3% in bean, 8.2% in wheat and 16.9% in corn) when compared to salt concentrations alone. The response of different seedlings to the algae extract in the fresh weight state was slightly more evident than the response of seedlings in the dry weight state at different salt concentrations.

Fig 1: The seedlings at 12th day (a) *Vicia faba*, (b) *Triticum aesativum* and (c) *Zea mays* seedlings as affected by NaCl salt (25mM and 150mM) singly or combined with *Sargassum acinarium* extract (4.0%)

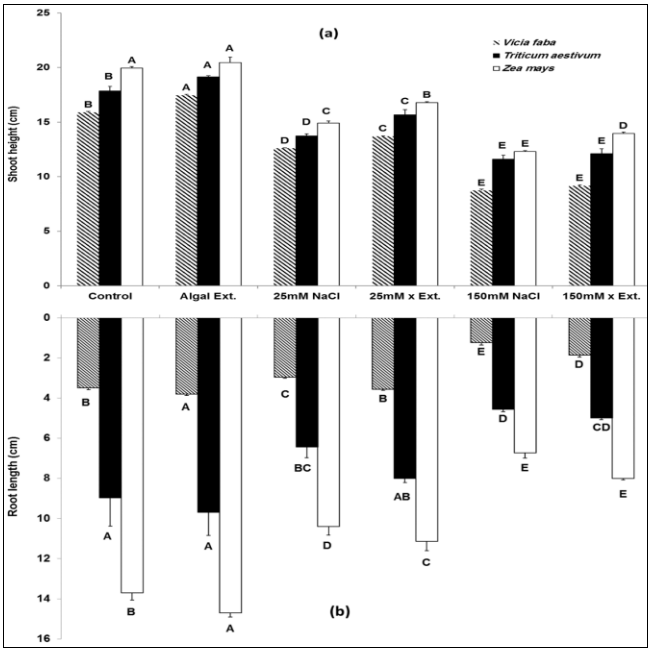

Fig 2: Effect of NaCl salt (25mM and 150mM) singly or combined with *Sargassum acinarium* extract (4.0%) on the shoot height and root length of *Vicia faba*, *Triticum aesativum* and *Zea mays* seedlings at 12th day. Error bars represented a standard error of the mean (SE), while the different letters on top of the error bars show significant differences at P ≤ 0.05

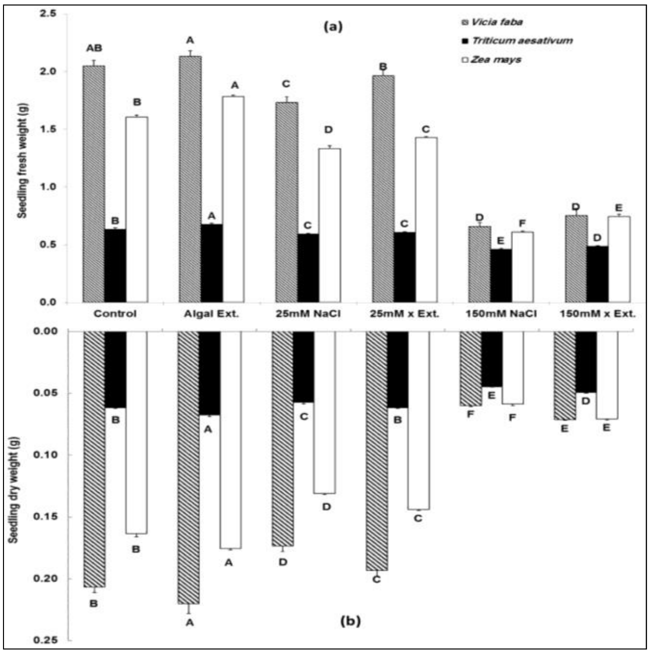

Fig 3: Effect of NaCl salt (25mM and 150mM) singly or combined with *Sargassum acinarium* extract (4.0%) on the fresh and dry weights of *Vicia faba*, *Triticum aesativum* and *Zea mays* seedlings at 12th day. Error bars represented a standard error of the mean (SE), while the different letters on top of the error bars show significant differences at P ≤ 0.05

Pigment contents

Figure 4 shows effect of NaCl salt (25mM and 150mM) singly or combined with *Sargassum acinarium* extract (4.0%) on the pigments of (a) *Vicia faba*, (b) *Triticum aesativum* and *(*c) *Zea mays* seedlings (12 days old). As in Figure 4(a), the algal extract has a noticeable positive effect on chlorophyll a, chlorophyll b, as well as total pigments when compared to the control of *Vicia faba* seedlings. Likewise, the same effect occurs when the algal extract interacts with different salt concentrations when compared to salt concentrations alone. While the effect of algal extract, whether alone or combined with different salt concentrations, on carotenoids was not significant.

In Figure 4(b), the algal extract had no significant effect when applied alone on chlorophyll (b) and the total pigments of wheat seedlings, while the effect was positive in the case of chlorophyll (a). Likewise, when the algal extract was mixed with low and high salt concentrations, the effect was noticeable and very clear in the case of chlorophyll (a) of wheat seedlings. While the effect of the algal extract was positive on increasing the percentage of carotenoids when interacting with the high salt concentration (150 mM) when compared to the salt concentration alone.

In Figure 4(c), the effect of the algae extracts on the photosynthetic pigments of corn seedlings, where the effect of the algal extracts had a considerable influence on chlorophyll (a) when compared to the control of corn seedlings, while the interaction of the algal extract with different salt concentrations was unclear in relation to total pigments, chlorophyll (a), and chlorophyll (b) of corn seedlings. The algal extract's impact in general was not positive on the carotenoid contents of corn seedlings, whether whether or not there is salt present. The response of the bean plant treated with different salt concentrations under the influence of the algal extract on the different pigments was more obvious than corn and finally wheat.

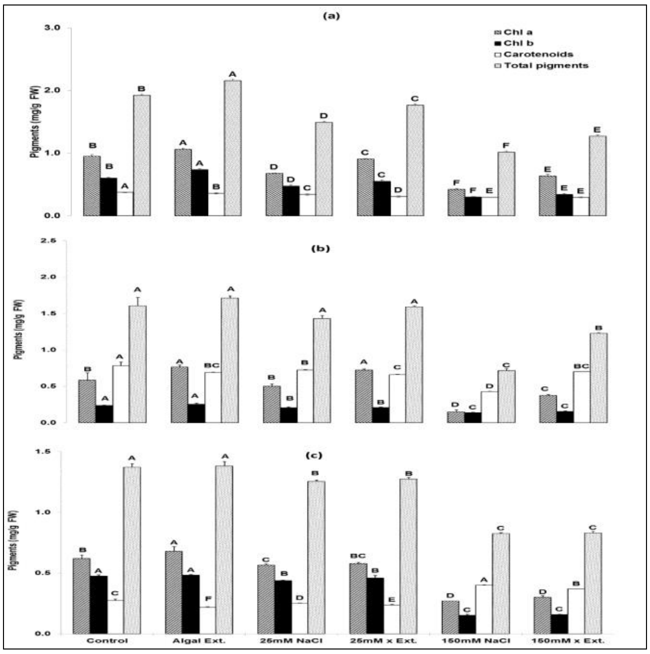

Fig 4: Effect of NaCl salt (25mM and 150mM) singly or combined with *Sargassum acinarium* extract (4.0%) on the pigments of **(a)** *Vicia faba*, **(b)** *Triticum aesativum* and **(c)** *Zea mays* seedlings at 12th day. Error bars represented a standard error of the mean (SE), while the different letters on top of the error bars show significant differences at P ≤ 0.05

Activities of antioxidant enzymes

Figure 5 shows the effect of NaCl salt (25mM and 150mM) singly or combined with *Sargassum acinarium* extract (4.0%) on the superoxide dismutase activities of *Vicia faba*, *Triticum aesativum* and *Zea mays* shoot seedlings**.** As in the case of all seedlings, the algal extract alone had a slightly lower activity of the enzyme superoxide dismutase (SOD) as opposed to the control, as the activity of the enzyme was elevated at different salt concentrations alone in all seedlings as opposed to the control, but the interaction of the algal extract with the different salt concentrations led to a significant inhibition of the enzyme activity compared to individual salt concentrations for all seedlings. The effect of increasing and decreasing enzyme activity was almost similar in the case of the response of all seedlings under study to the algal extract compared to the treatments not affected by the algal extract.

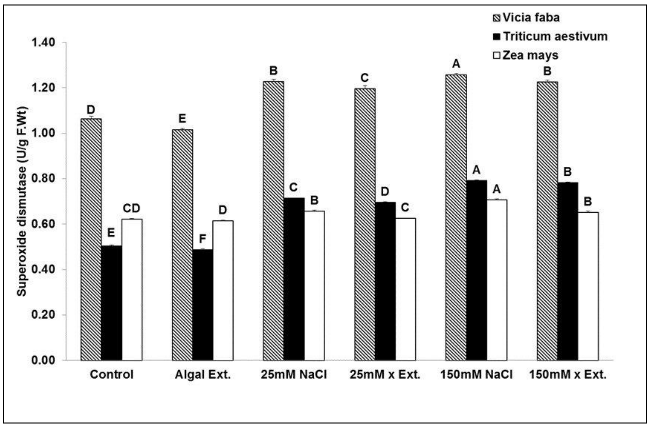

Fig 5: Effect of NaCl salt (25mM and 150mM) singly or combined with *Sargassum acinarium* extract (4.0%) on the superoxide dismutase activities of *Vicia faba*, *Triticum aesativum* and *Zea mays* shoot seedlings at 12th day. Error bars represented a standard error of the mean (SE), while the different letters on top of the error bars show significant differences at $P \leq 0.05$

Total proline contents

It is clear from Figure 6 that the algal extract's impact on the total amount of proline was positive in the case of bean and corn seedlings as opposed to the control, while the interaction of the algae extract with different salt concentrations led to a significant inhibition of the total amount of proline for all seedlings (*Vicia faba*, *Triticum aesativum* and *Zea mays*) when compared to the salt concentrations alone. When compared to the bean plants, the algal extract clearly had a good impact to the other plants studied, in inhibiting the harmful effect of salt, which was evident in the significant decrease in the total amount of proline.

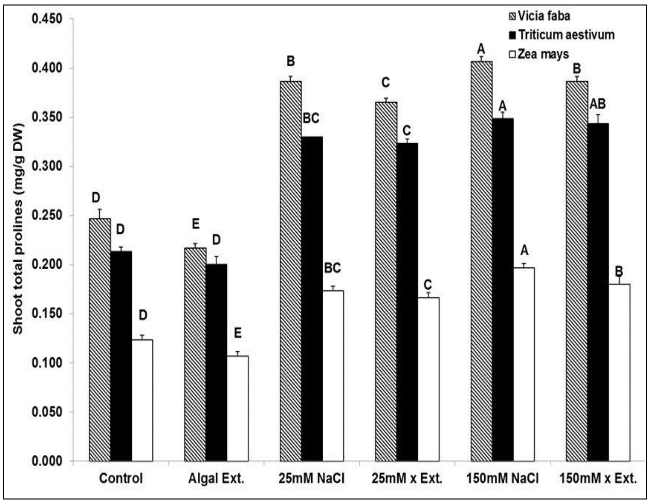

Fig 6: Effect of NaCl salt (25mM and 150mM) singly or combined with *Sargassum acinarium* extract (4.0%) on the total proline contents of *Vicia faba*, *Triticum aesativum* and *Zea mays* shoot at 12th day. Error bars represented a standard error of the mean (SE), while the different letters on top of the error bars show significant differences at $P \leq 0.05$

Total antioxidant activity

Figure 7 shows the effect of NaCl salt (25mM and 150mM) singly or combined with *Sargassum acinarium* extract (4.0%) on the total antioxidant activity of *Vicia faba*, *Triticum aesativum* and *Zea mays* shoot seedlings. As the algal extract had a significant effect on the rate of free radical activity compared to the control, and this became clearer when the effect of the algal extract overlapped with low and high salt concentrations, as it inhibited the activity of free radicals for all seedlings compared to the salt concentrations individually. The effect of the significant increase in antioxidants was clear at high salt concentrations, and in general the effect was more responsive in corn plants, followed by wheat and then beans.

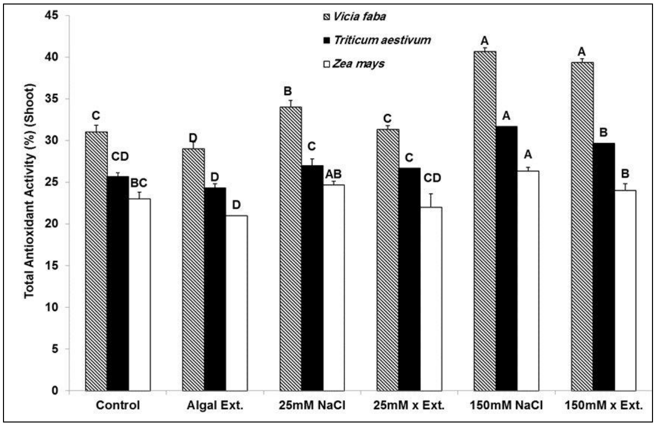

Fig 7: Effect of NaCl salt (25mM and 150mM) singly or combined with *Sargassum acinarium* extract *(4.0%)* on the total antioxidant activity of *Vicia faba*, *Triticum aesativum* and *Zea mays* shoot seedlings at 12th day. Error bars represented a standard error of the mean (SE), while the different letters on top of the error bars show significant differences at $P \leq 0.05$

Discussion

Properties of Physio-Chemicals Examination of the Seaweed Extract from *Sargassum acinarium*:

Macroalgal liquid extracts are rich sources of macro-, micronutrients, vitamins, and different plants growth regulators (auxins, gibberellins, and cytokinins) . *Sargassum acinarium* liquid extract's (SLE) physio-chemical characteristics have been examined. The SLE have high growth stimulation potential with the different plants . The observed higher cytokinin content (than auxin and gibberellins) in the SLE have been reported in earlier findings as in the Table 1. For example, (Vijayanand *et al.*, 2014) [50] found that the brown algae *Sargassum wightii* (*S. wightii*) and *Stoechospermum marginatum* were reported to have greater cytokinin concentrations (Ramya *et al.*, 2015) [39], than auxin and gibberellins. The SLE showed to contain different ratio of micro-, macro elements, and vitamins, they are necessary for plant development and other functions. The experiment's findings concurred with those of the studies by (Sutharsan, 2017) [46].

Effect SLE on seedling germination (shoot and root length as well as fresh and dry weight)

Figures 2 and 3 show that the green *Sargassum acinarium* crude extract improved *Vicia faba* seed germination, root and shoot length, and lateral root number. These findings are in line with those of (El-Sheekh, 2000) [16]. The germination %, radicle and hypocotyl length, total seedling length, dry weights, and seedling biomass of pepper (*Capsicum annuum*) were all reportedly enhanced by *Fucus spiralis* extract (Baroud *et al*., 2019) [5]. Furthermore, soaking the *Vigna radiata* seeds with the *Sargassum* sp. compared to the control, growth was improved by the 40% liquid extract (this discovery is because to the concentrated quantity of growth regulators in SLE, which would have enhanced photosynthesis, boosting vegetative development. (Makawita *et al*., 2021) [33].

Plants under salt stress experience osmotic stress, consumes more energy to absorb water and nutrients, and led to lower their rate of development and biomass accumulation (Sariñana-Aldaco *et al*., 2022) [40]. When a plant raises its respiration rate in order to generate more energy for water absorption, this impact is comparable to that of water stress (Che-Othman *et al*., 2020) [13, 14]. According to our findings, the usage of SLE enhanced these indicators. Various studies suggest that SLE has a favourable effect on plant growth and biomass. (Abdel Latef *et al*., 2017) [2] found that *Sargassum muticum* extracts (1%) had a good effect on chickpea crop development characteristics and biomass in the presence of NaCl stress (50 and 150 mM). Conversely, (Gharib *et al*., 2014) [21] stated that rosemary plants experience salt stress (100 mM NaCl) showed higher biomass (the weights of the shoot, both fresh and dry) and growth characteristics (height) when extracts from Sargassum latifolium were applied topically (0.4%). The development of the plants in stress-free conditions was likewise enhanced by the extracts, according to their statement, at all concentrations employed.

Pigment contents

Osmotic stress brought on by the solute absorption system being saturated or the high demand for the energy requirements of such systems might be the reason of the growth barrier (Shaddad *et al*., 2014) [43]. The varying salt levels had a significant impact on pigment biosynthesis (Chl.a, Chl.b, and Car.), as did the responses of *Vicia faba*, *Triticum aesativum*, and *Zea mays* seedlings. This study's findings are in line with those of (Hameed and Ashraf, 2008) [26].. It has also been reported that Cl^- may be more injurious than Na^+, causing leaf chlorosis in several plant species (Shaddad *et al*., 2014) [43].

Additionally, the effectiveness of SLE to promote growth may be influenced by the availability of macro and micronutrients (Shaddad *et al*., 2014, Sathya *et al*., 2010) [41, 43]. In most cases, applying marine algae extract to the seedlings decreased salt stress's inhibitory influence on photosynthetic pigment formation while also dramatically increasing the stimulatory effect when compared to control plants. The presence of magnesium, the primary component for chlorophyll synthesis, may be the cause of the rise in photosynthetic pigments in faba bean, wheat, and zea plants (Kalaivanan *et al*., 2012) [30]. Concerning the same issue, (El-Sheekh, 2000) [16] discovered that crude extracts of several seaweeds increased *V. faba's* total soluble sugars and chlorophyll content. The used algal extract enhanced seedling growth to varying degrees. Likewise, (Baroud *et al*., 2019) [5] said that the usage of aqueous extracts of brown algae resulted in a significant increase in the total sugar content of pepper plants. (Mohammed *et al*., 2023) [36].

Activities of antioxidant enzymes

The current study's results (Fig. 5) demonstrated that considerable rises in superoxide dismutase (SOD) activity in the shoot seedlings of the three tested plants were detected during their growth under the two administered NaCl levels. According to (Hegazi *et al*., 2015) [27], salt stress dramatically boosted superoxide dismutase (SOD) enzymatic activity in eggplants. The increase in enzyme activity might be attributed to salt-stressed plants developing a sophisticated antioxidant system to repair salt-induced damage. Also, Several studies have documented changes in the antioxidant enzymes' activity in salty environments, (Ibrahim, 2016) [28] discovered that salt challenged wheat plants revealed that antioxidant enzymes including superoxide dismutase (SOD) were much more active. Additionally, increases in antioxidant enzyme activity have been noted by (Fayez and Bazaid, 2014) [18] on barley, (Sharaf and El-Monem, 2010) [44] on wheat shoot.

Salt stress's effects on antioxidant enzymes are complex in this regard and vary depending on the genotypes, plant species, and treatment duration (Zhu *et al*., 2021) [51]. In the current investigation, the administration of *Sargassum* liquid extract (SLE) resulted in significant changes to the antioxidant enzymes' assessed activity. Under saline circumstances, using *Sargassum* extract for treatment significantly suppressed increases in SOD activity (Fig. 5). When *Sargassum* was administered at 150 mM NaCl, the most significant effects were seen. Antioxidant enzyme activity, including superoxide dismutase, was significantly elevated in plants under salinity stress. Seaweed treatment may have an enhancing impact because it stimulates K absorption and can reduce the inhibitory effect of Na toxicity, restoring growth. To further protect against adverse environmental conditions, the usage of seaweed increased the levels of antioxidant enzymes (SOD) (Zhu *et al*., 2021) [51].

Algal extracts include bioactive compounds, such as flavonoids, proline, and plant hormones, may help to alleviate salinity stress. (Kasim *et al*., 2015) [31] discovered that antioxidative system components like catalase and peroxidase are activated by *Sargassum* or *Ulva* seaweed extracts, which mitigate the oxidatively damaging effects of abiotic stress, in addition to directly. Furthermore, presoaking wheat grains in different amounts of seaweed extract increased SOD and CAT activity as the amount of algal extract increased.

The use of algal extracts appeared to alleviate seawater salt stress in wheat plants by lowering Na^+ levels while increasing photo-synthetic pigment content. Additionally, an increase in antioxidant defence capabilities, such is antioxidant systems that are enzymatic and non-enzymatic, was linked to the improvement of algal extracts on salinised wheat plants. In addition to maintaining several physiological functions of wheat plants, such as photosynthetic activity and production, this helps to minimise oxidative damage to functional molecules. By strengthening the antioxidant defence system, which is one of the elements for wheat plants to tolerate salt, the application of algae extracts may provide protection against this oxidative stress, according to (Abd El-Baky *et al*., 2020) [1], it shown how irrigating wheat plants with 10 and 20% (v/v) sea water causes oxidative damage. As a result, when treated with algal extracts, wheat plants may be irrigated with brackish water at a concentration of 20%.

Total proline contents

Figure (6) shows that proline levels in the shoots of all three seedlings rose considerably in reaction to salt stress. Proline, as an osmolyte, plays an important function in osmoregulation (Annunziata *et al*., 2019) [3], moreover, they can switch out molecules to alter structures, preserve membrane integrity under stress, preserve macromolecule activity, scavenge ROS, stabilise photosynthetic activity, and initiate the activation of genes in *G. hirsutum* L. that control oxidative stress responses (Hamani *et al*., 2020) [24], *Zea mays* L. (Chen *et al*., 2020) [13, 14], and rice (Hafez *et al*., 2021) [23]. Seaweed extract's bioactive compounds enhance the synthesis of metabolites (stress-relieving amino acids), membrane permeability, and osmolyte/ion transport to enhance resistance to abiotic stress by altering turgor pressure and water capacity of plants. These bioactive compounds also have a positive impact on plants' overall growth. In our investigation, proline concentration rose in salt stress and with the administration of SLE compared to the control.

According to the findings (Fig. 6), using *Sargassum* extract produced significant differences in proline concentration in the shoots of the three seedlings. *Sargassum* extract treatment considerably raised proline levels in *Zea* (corn) shoots compared to other treatments. Similar result with (Khan *et al*., 2022) [32], found that okra (Abelmoschus esculentus) had higher levels of proline during salt stress as compared to the control. The evidence for this is that SLE supplementation under salt stress causes an increase in endogenous proline level as well, which accounts for the enhanced resistance to salt stress

Total antioxidant activity

According to the results of this investigation, salt stress had a substantial effect on total antioxidant activity (TAA) **(Fig. 7)**. (Fayez and Bazaid, 2014) [18] discovered that salt and water strains dramatically elevated barley TAA, which rose as stress increased. , the mechanisms involved in the diverse effects of seaweed extracts on plant metabolism have been clarified by recent gene expression studies. Significant enzymes involved in nitrogen metabolism (cytosolic glutamine synthetase), glycine betaine synthesis (betaine aldehyde dehydrogenase and choline monooxygenase), and antioxidant capacity (glutathione reductase) were linked to these effects through increases in transcript abundance. Following treatment with seaweed extract, chalcone isomerase activity increased, the production of phenylpropanoid plant defence compounds and flavanone precursors depends on this crucial enzyme. The current study's findings (Fig. 7) demonstrated that significant variations in TAA were seen in the studied plants' shoots following treatment with *sargassum* extract. Analogous findings have been documented by (Sofy *et al*., 2017) [45], plants grown in an abiotic stress condition at 75 mM NaCl and treated with 30% Sargassum extract exhibited significant levels of antioxidant activity and polyphenol content. Furthermore, (Guinan *et al*., 2012) [22] reported that the high antioxidant content of *Ascophyllum nodosum* seaweed extracts may minimise oxidative damage and encourage plants' ability to withstand abiotic stress, which explains why seaweed extracts are excellent at improving growth under abiotic stress circumstances.

Conclusions

The chemical composition of the SLE employed in the current investigation demonstrated a strong overall antioxidant capacity with higher DPPH and proline levels. Due to the high concentration of antioxidants that scavenge the ROS produced when salt stress occurs, SLE supplementation may have promoted resistance to salt stress. Superoxide dismutase (SOD), glutathione reductase (GR), and APX are all included in the intricate defensive system of antioxidants. that plants activate in response to salt stress. This system scavenges excessive ROS, depletes free radicals, and generally protects plants from cellular stress. SLE's growth-enhancing benefits might possibly be linked to the macro- and microelements discovered in this study, which are consistent with earlier research. The minerals found in seaweed extracts, such as zinc, copper, boron, and cytokinesis, are also known to be easily absorbed by plants and to promote growth and productivity by enhancing root development, cell division, and cell elongation. Additionally, consistent with earlier findings, SLE supplementation significantly decreased salt-induced toxicity by modifying the ionic status (K+/Na+, Mg2+/Na+, and Ca2+/Na+ ratios) in *Vicia faba* (faba bean), Triticum aesativum (wheat), and Zea mays (corn) cultivated under various salt stressors.

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Declaration

Conflict of Interest: I declare that I have no direct or indirect personal interest in any topic that might interfere with my obligations as a researcher and instructor at Damanhur University's Faculty of Science, Botany, and Microbiology Department.

Ethical approval: This study was carried out in accordance with the objectives and standards established by Damanhur University Faculty of Science.

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