EFFECT OF SEAWEED EXTRACT OF *CODIUM FRAGILE* ON GERMINATION BEHAVIORS OF SOYBEAN *(Glycine max* (L.) Merr.) UNDER SALT STRESS

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ABSTRACT

Abiotic stresses are major constraints on worldwide crop production, and salinity is one in all the most important issues touching regarding simple fraction of the irrigated land on earth. Soybean (*Glycine max (L.) Merr*) is avital crop occupying an oversized space, wherever salt stress is affecting almost every aspect of the physiology and biochemistry of plants and considerably reduces yield and limit agricultural production worldwide. In the present investigation, a trial has been made to study the effect of seaweed liquid extract (SWE) from *Codium fragile* at completely different concentrations on seed germination and seedling growth of Soybean (*Glycine max (L.) Merr*) under salt stress.

Keywords: *Codium fragile, liquid seaweed extract, Salt stress, Seedling growth, Soybean.*

1. INTRODUCTION

Seaweeds are one in all the foremost vital marine resources of the world. Seaweed extracts are marketed for several years as fertilizer additives and useful results from their uses are illustrated by Booth [1]. Different varieties of seaweed preparation like LSF (Liquid Seaweed Fertilizer), SLF (Seaweed Liquid Fertilizer) LF (Liquid Fertilizer), and either whole or finally sliced and powered as algal manure are used and every one of them are reportable to provide useful effects on cereals, pulses, and flowering plant. Seaweed contains all the trace **components** and growth hormones **needed** by plants.

Recently there's a growing concern over the utilization of seaweed liquid fertilizer (SLF). Today, there's a high demand for environment friendly agriculture for production of quality and healthy food to nourish the increasing population [2].

Salinity is bothered as a result of it's one in every of the foremost necessary abiotic stress factors limiting plant growth [3], affecting virtually every aspect of the physiology and biochemistry of plants and significantly reduces yield [4]. A biotic stresses can reduce the yield of major crops [5] and limit agricultural production worldwide. Soybean (Glycine max) is one the most inexperienced herbaceous plant crops, it's thought of mutually of the essential sources of proteins, carbohydrates, vitamins and mineral salts which are essential for human nutrition [6].Many crop plants including leguminous crops are liable to cell harm from high salinity and might survive solely with diminished yields. That's why; this study was undertaken to check the impact of seaweed liquid fertilizer extract of *Codium fragile* on germination behavior of Soybean grains (*Glycine max (L.) Merr*) cultivars under salt stress.

2. MATERIALS AND METHODS:

2.1. Experimental Material

Seeds of Soybean (*Glycine max (L.) Merr*) were obtained from the breeding program of Agriculture Research, Dokky, Cairo, Egypt

2.2. Seed Material

Germination experiment was conducted among the laboratory to analyze the impact of sodium chloride salinity additional with seaweed liquid extract or not, on germination behavior and seedling growth of Soybeans. Seeds employed during this study were *Glycine max (L.) Merr*. This plant belongs to the family of Fabaceae. It's sensitive to salt stress

2.3. Collection of Seaweeds

Seaweeds *Codium Fragile* (Chlorophyceae) utilized during this study were collected from the coastal area of Hurghada, Egypt during June, 2017. Morphologically distinct thallus of algae were placed in polythene bags and transported to the laboratory. Samples were washed completely using tap water to remove the salt.

2.4. Seaweeds Treatment

Seaweeds were shade dried for four days, followed by oven dry for 12h at 60°C. Then the materials were hand crushed and created as coarse powder, was additional with distilled in a ratio of 1:20 (w/v) and boiled at 121^0 C for 30 minutes. The hot extracts were filtered through a double-layered cheese cloth and allowed to cool at room temperature [7]. The ensuring supernatant was taken as 100% seaweed liquid extracts. Seaweed liquid extracts were prepared with different doses: control (0%), 0.5% and 1.0%

2.5. Seed Treatment

Ten Seeds of Soybean were placed in 9 cm Petri dishes on a two layers of filter paper (Whatman 41). Salt stress was induced by sodium chloride (NaCl). Three sets were treated with (0, 50, 100 and 150 mM) of NaCl and were considered as control as they don't receive extract of *Codium fragile*. Seeds under study were treated with salt at varied concentrations ((0, 50, 100 and 150 mM of NaCl) and each concentration was supplemented with seaweed extracts (SWE) of *Codium fragile* separately at two different doses (C1: 0.5% and C2: 1.0%). All sets were labeled as control (non-treated seeds with seaweeds extract of *Codium fragile*); C1 (Seeds supplemented with 0.5% of *Codium fragile* extract); C2 (seeds supplemented with 1.0% of *Codium fragile* extract).

Seeds were placed on top of the filter paper wetted with 5 ml of each different concentrations of seaweed extracts within the Petri dishes and were unbroken under photoperiod for 14 days. The culture room temperature was maintained at 25°C. Seed germination was recorded daily up to day 14 after the start of the experiment. After fourteen days, seedlings were taken for the observations. Parameters measured in this experiment were:

Total seed germination rate (TG) measured within the sixth day using the formula TG (%) = (total number of germinated seeds/ total seed) x 100.

Mean germination time (MGT) calculated according the formula of Ellis and Roberts [8]. MGT= $\Sigma(ni/di)$. With ni: number of germinated seeds and di: day of counting.

2.6. Greenhouse Growth Bioassay

Soybean plants were grown under greenhouse under 16-h light regime at 25 °C and 8-h dark regime at 18 °C. Seeds were planted in pots at a depth of 0.5 cm below the soil level and were allowed to germinate. After germination in each pot, five healthy plants were retained and other plants were removed. The experiment was made in triplicates. Plants were grown into plastic pots containing sand and clay (50% - 50%). They were also irrigated separately with saline water which was added with sodium chloride (NaCl) at different concentrations (0, 50 100 and 150mM) every third day. Potted plants were grown for 7 weeks in a greenhouse at 25±2 °C, in 85 % relative humidity.

The experiment comprised of five treatments, (control, water spray), 0.5 and 1.0%; (volume/volume; v/v) of seaweed extract in water. Sprays of *Codium fragile* -derived extract were applied, twofold per week, at the seed plant (30 days after sowing). Morphological characteristics such as Radicle length, dry weight of shoot &root were measured.

2.7. Gas Chromatography and Mass Spectrometry Analysis of SLF

The GC-MS analysis of algal extracts was analyzed by GC-MS in the National Research Center of Chromatography Unit in Dokky Street, Cairo, Egypt. The GC-Mass method is a direct and fast analytical approach for identification of algal extracts components the analysis was performed by using a GC, Agilent 5977

A MSD with a direct capillary interface and fused silica capillary column Hp-5 ms. Extracts were injected under the following condition. Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:10 using the following temperature program: 40 C for 1 min; rising at 4.0 C/min to 160 C and held for 6 min; rising at 6 C/min to 210 C and held for 1min. The injector and detector were held at 210 o C. Diluted samples (1:10 hexane, v/v) of 0.2 µL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library). and those in the literature data [9]

Statistical Analysis

The mean values from control and treated samples were analysis using SPSS (version 21.0). Data presented are means ±standard errors (SEs) of three replicates

RESULTS AND DISCUSION

3.1. <u>Laboratory Conditions</u>
3.1.1. Total Germination

The mean germination was reduced in Soybean seeds (*Glycine max (L.) Merr*) genotypes as response to salinity stress. The decrease with in the germination rate was found to be additional with the increasing concentration of NaCl. Once the treated seeds were supplemented with SWE (0.5 and 1.0%) on NaCl salt, the germination rate was inflated (Fig. 1 and 2). Total germination from each treated and non-treated seeds with SWE of *Codium fragile* decreased significantly with increasing NaCl salinity. However, this reduction in total germination was significantly higher for non-treated seeds, compared to treat ones. Data suggested a reduction of about 50% on total germination rate due to an increase in salinity from 0 to 100 mM. Results indicated that application of seaweeds extract increase germination rate in bean cultivars using SWE S1 (0.5%) and S2 (1.0%) when compared to control seeds (table. 1).

In general, increasing salinity causes a decrease in Soybean germination; this might ensue to the ototoxic effects of Na^+ and Cl^- within the method of germination [10]. It seems jointly that salinity stress affects seed germination via the limitation of seed water absorption [11], excessive use of nutrient pool [12] and creation of disorders in protein synthesis.

The effects of seaweed extracts on germination of various crops are shown by several authors [13], [14]. It had been indicated that seaweeds extracts induce outflow of inhibitors probably absisic acid from the seeds that improve germination percentage [15]. The involvement of growth regulating substances in seaweed extracts like ethylene, kinetin and gibberellic acid were effective on reversal of induced dormancy in seeds [16].

The ameliorator results of SWE could also be due to the expansion hormones obtainable which might have triggered First State novo the synthesis of hydrolytic enzymes. This present study is in accordance with the earlier results of [17], [18] and [19]. It should due to ensue from the presence of growth-promoting substance in SWE [20].

3.1.2. Mean Germination Time (MGT)

Results showed that salinity significantly increase mean germination time (MGT) for every treated and non-treated bean seeds with seaweed extract. However, treated seeds with *Codium fragile* extract have lower MGT compared to manage seeds. Data in Table 1 show also that rising concentration of NaCl delayed significantly MGT in soybean

(L.) Merr genotypes; this delay was less pronounced in treated seeds with SWE. In keeping with [21], most plants are sensitive to salinity throughout germination and seedling growth. This can be in agreement with our study whereby a lower mean germination time was found with treatments of *Sargassum dentifolium*.The probable reason for early emergence of the seaweed extract treated seeds perhaps because of the completion of pre-germination metabolic activities creating the seed prepared for radicle protrusion and also the SWE treated seeds germinated before long when planting compared with untreated ones [22].

Furthermore, seaweed extracts contain varied betaines and betaine-like compounds [23], [24]. In plants, betaines function a compatible substance that alleviates diffusion stress elicited by salinity stress. Those compatibles solutes are shown to play a district in successful formation of somatic embryos from cotyledonary tissues and mature seeds of tea [25]. All that information supports our results regarding seed germination and improved mean germination time with SWE.

Table 1. Effect of *Codium fragile* extract on germination of *Glycine max (L.) Merr* Soybean cultivar under salt stress.

	0 mM (NaCl)			50 mM (NaCl)			100 mM (NaCl)			150 mM (NaCl)		
	CONTR0L (0 %)	C1 (0.5%)	C2 (1%)	CONTROL (0 %)	C1 (0.5%)	C2 (1%)	CONTROL (0 %)	C1 (0.5%)	C2 (1%)	CONTROL (0 %)	C1 (0.5%)	C2 (1%)
TG	98.52 ±0.65	99.15 ±0.75	100 ±0.74	92.59 ±0.70	94.72 ±0.69	97.72 ±0.43	50.52 ±0.55	62.52 ±0.68	68.52 ±0.70	IE	10.10 ±0.61	19.21 ±0.55
MGT	2.01 ± 0.35	1.95 ± 0.55	1.37 ±0.44	2.91 ± 0.15	2.21 ±0.15	2.01 ±0.15	4.521 ±0.15	3.91 ±0.15	3.11 ±0.15	IE	5.52 ±0.77	4.91 ±0.80

IE injurious effects and the plant failed to survive

3.1.3. Radicle Length

Salinity had a restrictive result on radicle length for every treated and un-treated seeds (Fig. 1). However, this result was considerably less pronounced in seedlings from treated seed with *Codium fragile* extract compared with control seeds of *Glycine max (L.) Merr* cultivar. The applying of SWE improved the growth of the radicle considerably. The improved in radicle length is additionally as a result of presence of some growth promoting substances like IAA and IBA, Gibberellins, Cytokinins, micronutrients and amino acids [26].

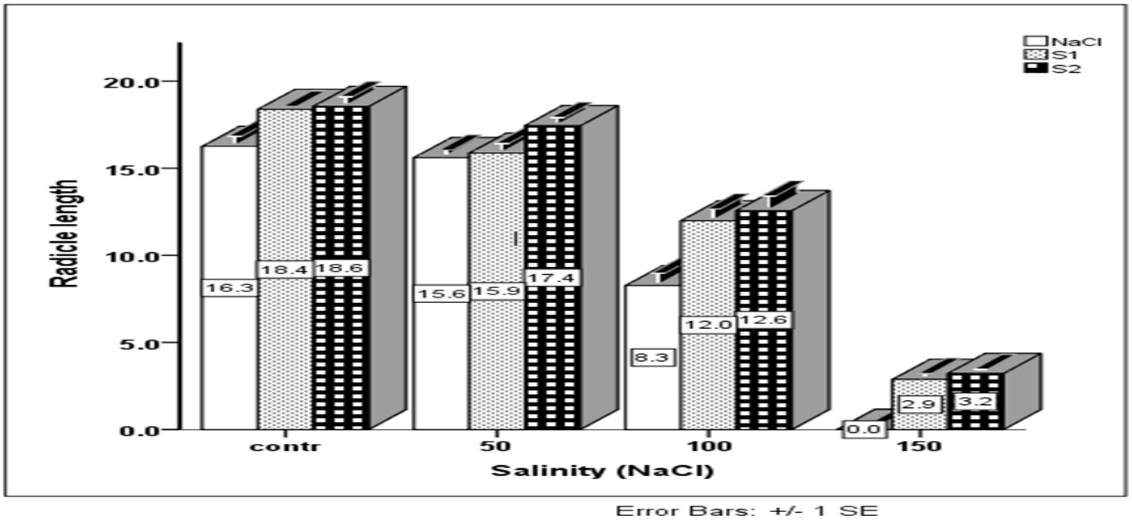

Fig.1. Effect of *Codium Fragile* extract on radicle length (mm) of Soybean *Glycine max (L.) Merr* under different concentration of NaCl.

3.2. <u>Greenhouse Conditions</u>

3.2.1. Shoot Dry Weight

The increase in shoots characteristics could be because of the Auxins content within the seaweed extracts which have an effective role in cell division and enlargement. This leads to an increase in shoot growth and plant fresh and dry weights [27]. This positive impact could be because of the minerals Zn, Cu and B content in the seaweed extracts, which have a good role in biological process and enlargement [28], or may also because of the macronutrient content in seaweed extracts, that have a good role in plant nutrition like nitrogen, potassium and phosphorous, very essential for the growth and development of the plant [29].

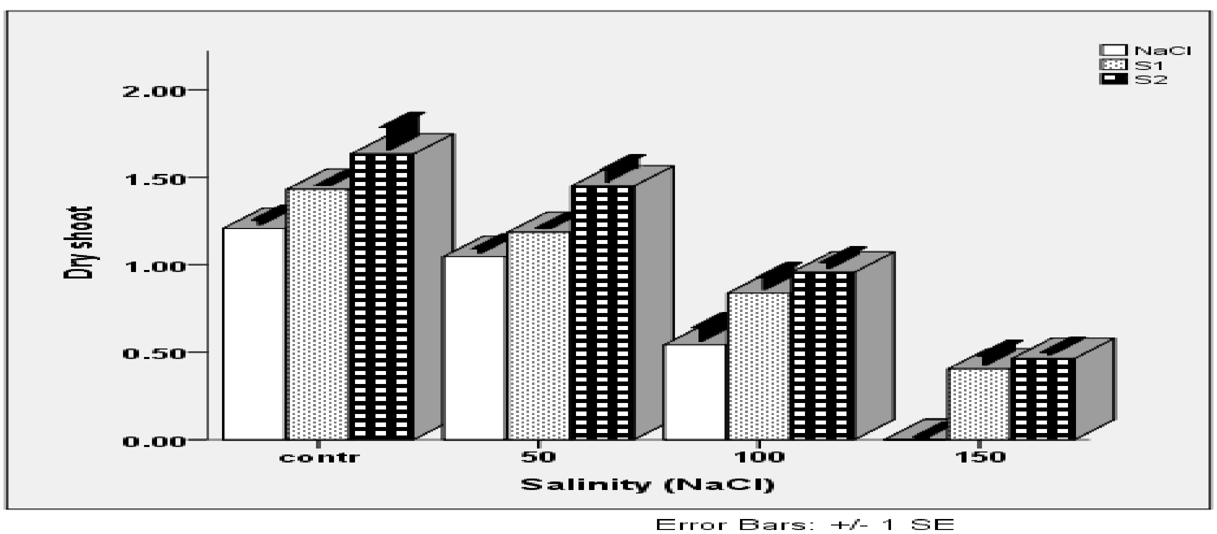

Fig.2. Effect of *Codium Fragile* extract on shoot growth of Soybean *Glycine max (L.) Merr* under different concentration of NaCl.

3.2.2. Root Dry Weight

Increasing salinity reduced bean roots dry weight for every treated and un-treated seeds. In fact, the increase in salt concentration in culture medium brought down root dry weight from 0.47 g at 0 mM to reach 0.22 g at 100mM (Fig. 3). However, seeds supplemented with SWE showed better performance than non-supplemented seeds. Data in Fig. 3 shows that seeds supplemented with 0.5 % and 1.0 % of SWE enhanced beans root dry weight (RDW) as compared to control.

Reduction in rooting growth (dry weight) was visible in salt stressed seeds with increasing concentration of NaCl. However, the application of SWE improved growth that was visible in seeds supplemented with SWE of *Codium fragile*.

Reduction in growth rate as a results of salt stress rumored in many others species [30], [31]. Salinity has diffusion and specific ionic effects on growth [32]. Similarly, toxic ion accumulation (Na+ and Cl-) negatively have a sway on plant metabolism [33]. It's additionally been rumored that salinity suppresses the uptake of essential nutrients like P and K [34] that might adversely have an effect on growth. [35] have reported that salinity reduced fresh and dry weight of maize.`

Seaweed include macro and microelement nutrients, amino acids, vitamins, cytokinins, auxins, and abscisic acid that have a sway on cellular metabolism in treated seeds, leading to raised growth [36], [37]. Additionally, seaweeds contain precursors of elicitor compounds that promote germination [38].

Another likelihood is that the presence of polysaccharides in SWE, as sugars that are well-known to reinforce growth in a similar manner to hormones [39]. Zeatin is another candidate for induction of rooting in plants by seaweed [40].

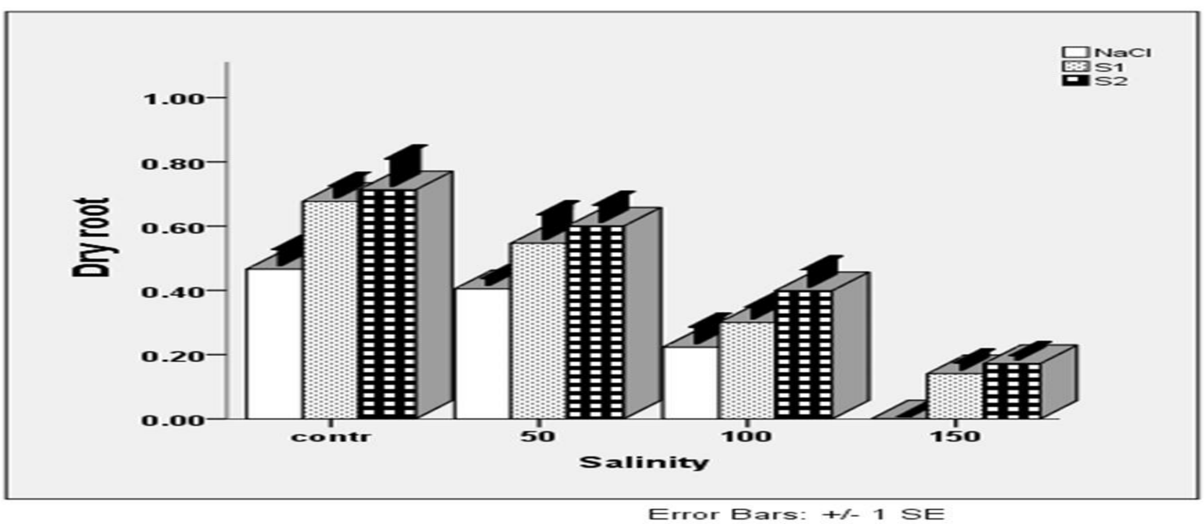

Fig.3. Effect of *Codium fragile* extract on root growth of Soybean *Glycine max (L.) Merr* under different concentration of NaCl.

3.3. Gas Chromatography and Mass Spectometry Analysis

The GC-MS analysis revealed the presence of varied compounds present in the SLF sample.The gas chromatogram of the extract showed 38 peaks. However, only 7 major compounds were identified to have plant growth regulation properties **(Table 2).** The major compound present in the SLF extract of *C. fragile* was benzene, 1,1'-(1-chloro-1,2-ethenediyl) bis; - diclofenac, methyl ester; benzene, 1-chloro-4-(2- phenylethenyl)-; benzene, 1,1-'(chloroethenylidene) bis- ; benzene, 1-chloro-3-(2- phenylethenyl)-, (E)- ; benzene acetic acid; oleic acid; Diclofenac, Methyl ester & penta deconic acid. All these compounds were found to own one or a lot of plant growth promoting activities (Table 2). The presence of those compounds gave associated degree insight into the plant growth regulating properties for increasing the crop yield in addition on improve the soil fertility [41].

Table 2 - GC-MS Profile of SLF prepared from Codium fragile. Compounds with reported plant growth promotion activity only are shown.

S No. Compound Name	M.W.	Formula	R.T.	Biological activity on plant growthPromotion*
1. Benzene, 1, 1'-(1- Chloro -1, 2- Ethenediyl)bis	214	C14H11Cl	26.49 -	Pesticide, fungicide and growth regulator
2. Benzene acetic acid, 2- [(2, 6- Dichlorophenyl) amino]	295	C14H11O2 NCl2	28.87	Plant growth regulator, algaecide and herbicide
3. Benzene, 1-Chloro-4-(2- . Phenylethenyl)	214	C14H11Cl	20.77	Pesticide
4. Benzene, 1-Chloro-3 - (2- Phenylethenyl) -, (E)	214	C14H11Cl	24.80 -	Pesticide; for growth regulating activities
5. (Oliec acid) cis-9-Octa deconic acid	282	C18H34O2	30.45	reductase inhibitor, insectifuge, flavor
6. Diclofenac, Methyl ester	309	C15H13O2 NCl2	20.30	Herbicide
7. penta deconic acid	242	C15H30O2	18.45	antioxidant

- **[42] Source: Dr. Duke's phytochemical and ethnobotanical databases [online database]**

4- CONCLUSION

It is clear from the present study that seaweeds extract of *Codium Fragile* have associate degree ameliorated impact on Soybean seeds under salt stress owing to the presence of growth hormones, nutrients and different necessary physiochemical compounds such as Benzene, 1, 1'-(1-Chloro-1,2-Ethenediyl)bis. Therefore the supplementation of SWE may be used as a biological modification in soil reclamation technique which might boost food production not solely in cultivated lands however conjointly in barren soils accumulated with salt. More study will be to test the influence of SWE on later growth and yield of Soybean cultivated in salt stress.These results urged that the seaweed liquid fertilizers are recommended to the farmers for influencing early seeds germination and growth parameters has been increased as and growth parameters ecofriendly.

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