

Microalgae as a Biofertilizer on Oat Seed Germination

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Abstract: Algae are a large and very diverse group of prokaryotic and eukaryotic organisms, autotrophic organisms. These organisms have the ability and potential to produce many secondary metabolites such as proteins, vitamins, amino acids and plant growth regulators and are widely distributed. The effect of different concentrations of *Spirulina platensis* and *Cladophora* extract on oat seeds (*Avena sativa* L.), germination percentage, germination speed, plumule length, radicle length, seedling length, seed vigor, fresh weight and dry weight were studied. Treatment 3 showed the highest germination rates for *S. platensis* extract 88.220%, 2.51333seed day⁻¹, 5.58333 cm, 5.88333 cm, 11.47333 cm, 10110.2, 0.975 g and 0.952 g, while the lowest percentage of treatment 4 was 59.217%, 1.40667, 3.74000 cm, 3.38667 cm, 7.65000 cm, 5084.9, 0.78533 g and 0.37800 g, respectively. While the seed treatment with *Cladophora* algae extract in treatment 4 showed a germination rate of 84.040%, 2.44667seed day⁻¹, 5.50667 cm, 5.32667 cm, 8333310 cm, 2 9104, 0.91533 g and 0.552167, while the lowest germination rate in treatment 4 was also 45.943%, 1.316637, 0003.39 cm, 3.31000 cm, 7.22667 cm, 2933.9, 0.71733 g and 0.356333 g compared to treatment zero. This study has shown that algae extracts have a positive effect on the germination of oat seeds and that it is possible to produce a commercial and environmentally friendly biofertilizer by using different concentrations of algae extracts.

1 INTRODUCTION

Modern agriculture plays an important role in overcoming the food requirements of the growing world population, which leads to an increased reliance on traditional agriculture that uses chemical fertilizers to increase yields, the main problem that is increasing rapidly now is the excessive use of chemical fertilizers in abundance, including increasing the acidity of the soil due to the decrease in organic matter in it. As well as it kills the beneficial microbes in the soil and responsible for fixing nitrogen in the end these practices lead to a decrease in soil fertility [1], [2].

The rising and increasing global demand for food and increasing environmental impacts due to this intensification of agriculture highlight the critical need for sustainable agricultural methods to improve crop productivity and quality [3]. The global shift towards organic products is driven by the growing demand for sustainable farming practices that promote environmental health,

improve soil fertility, and produce food free of harmful chemicals. Agricultural development and management depend heavily on the impact of growth stimulants in boosting crop production, yet chemical growth stimulants pose a major threat to the environment and human health [4].

Algae are a good source of many nutrients such as fiber, minerals, proteins, vitamins and fatty acids that occupy an important place in nutrition, compared to other plants [5].

The presence of an important compound in algae can promote the growth of plants such as auxins, cytokinins and betaines [6]. The latter can affect the development of the root system of the plant [7].

Furthermore, macro and micronutrients can help improve the growth of various vegetables [8], [9]. Oats (*Avena sativa* L.), which is one of the important crops of the gramineae family, has several uses, including as food crops for humans and animals, and the importance of growing oats in recent pots has increased because its grains contain a high percentage of proteins, vitamins, minerals, antioxidants and a high percentage of fiber that is

not soluble [10]. The most productive countries are Russia, the United States of America, Canada and Australia, and 74% of world production is used in animal nutrition in various forms, especially horses and poultry and then ruminants [11]. It has been investigated whether these extracts have a stimulatory effect on seed germination and it is aimed to produce an biostimulant from these extracts.

2 MATERIALS AND METHODS

This experiment was carried out in the winter season of 2024, at the University of Diyala, Laboratories of the College of Education for Pure Sciences (33°40'44.0"N 44°36'00.6"E), which is located about 9.9 km south from the city center, where in 2024 the algae samples were thoroughly and carefully examined and laboratory cleaned of parasites and dust, dried and ground into a fine powder.

2.1 Cultivation and Harvesting

S. platensis isolates were obtained from (Egypt, Cairo, National Research Center, Algae Biotechnology Unit), flask using standard zarrouk culture medium [12], [13], with air presence. The biomass was harvested by centrifugation on day 20 of planting. The biomass was dried in an oven at a temperature of 45 °C for 24 hours and then ground with a grinder and stored at +4 °C. Samples of *Cladophora* algae were obtained locally from Iraq / Wasit Governorate /Al-Ssaouira, Al-Shehaimiya area (30.035840N, 31.205119E).

They were cleaned and washed thoroughly and carefully to get rid of dust and plankton residues, dried at room temperature, ground by grinding and storing at [+4°C.

2.2 Cell Extract

The dried biomass was placed in distilled water at a concentration of 150 kg.L⁻¹. To obtain the cell extract with a concentration of 15% W/V, the extract was filtered by Washman filter papers thick 0.34 mm and then a centrifuge was used to remove of the biomass residue at 22° C, and 6000*g for 6 minutes. The resulting extracted liquid was then collected in a vial covered with aluminum foil and stored in a cool room at 4°C. Macro and micro elements of both extracts were measured by (AAS - Atomic Absorption Spectroscopy) In Iraq, the Ministry of

Science and Technology. Four solutions of different concentrations were prepared using cell extract [14]:

- T1 , Control 0% W/V (100 ml DIW)
- T2, 10 % W/V (100 ml DIW)
- T3, 20 % W/V (100 ml DIW)
- T4, 30 % W/V (100 ml DIW).

2.3 Seed Collection Center

The seeds of the oat plant were supplied from Iraq and the Ministry of Agriculture Department of Seed Inspection and Certification Baghdad Abu Ghraib district.

2.4 Seed Germination

The germination study of the seeds of the oat plant was conducted using 15 seeds per treatment by 12 experimental units by 4 treatments and 3 replicates. The seeds were placed in Petri dishes containing Whatmann filter leaves soaked in the mentioned treatments and germination was continued to be monitored.

2.5 Measuring Growth Factors

2.5.1 Percentage Measurement Methods

Percentage of germination (%) according to the number of seeds as follows:

$$\text{Percentage germination} = \frac{\text{number of germinating seeds}}{\text{total number of seeds}} \quad (1)$$

2.5.2 Germination Speed (Seeds / Day)

The germination speed was estimated as follows [15]:

$$\text{Germination speed} = \frac{\text{number of germinating seeds}}{\text{days number since the beginning of germination}} \quad (2)$$

2.5.3 Measurement of the Length of the Plumule, Radicle and Seedling (cm)

The length of the plumule, radicle and seedling was measured 10 days after cultivating using a graduated tape measure [16].

2.5.4 Measurement of fresh and Dry Weight (g)

The fresh weight of the seedling was estimated using a sensitive balance model Methier PC 400, while the dry weight was calculated after drying the above parts in a 65°C oven until the weight is stable [15].

3 RESULTS AND DISCUSSION

3.1 Chemical Analysis of Algae Extract

Table 1 shows the macro and micro elements found in algae extracts.

Table 1: Chemical analysis of *S. platensis* and *Cladophora* algae extract.

Micro elements (ppm) <i>S. platensis</i>					
Mn	Fe	B	Cu	Zn	
380	1110	395	99	1200	
Micro elements (ppm) <i>Cladophora</i>					
Mn	Fe	B	Cu	Zn	
210	851	265	76	1110	
Macro elements (%) <i>S. platensis</i>					
K ₂ O	S	Ca	Mg	P ₂ O ₅	N
3.8	1.9	0.9	0.8	7.8	3.9
Macro elements (%) <i>Cladophora</i>					
K ₂ O	S	Ca	Mg	P ₂ O ₅	N
2.8	1.4	0.8	0.9	5.3	2.1

3.2 Germination Percentage

The results of Table 2 show that there are significant differences between the seed activation treatments in the average germination rate, where they show the superiority of the seed stimulation treatment with *S. platensis* extract at a concentration of 20% significantly by giving it the highest average germination rate, which was 88.220% compared to the control treatment (soaking with distilled water), which gave 53.990%, also shows the superiority of the seed stimulation treatment with *Cladophora* extract at a concentration of 20% significantly by giving it the highest germination percentage, which was 84.040% compared to the control treatment, which gave 51.437%, this increase in this treatment was due to its superiority in the average germination speed due to a strong significant relationship between them. Especially since there was a positive significance correlation between the germination percentage and the germination speed, and this was consistent with the study of [17], that the activation of seeds by these extracts leads to a significant increase in the percentage of germination and that the reason for the superiority of this treatment is the role of gibberellic acid in breaking the dormancy of seeds and stimulating germination due to its role in activating the enzymes responsible for germination such as Nuclease, Protease, β -amylase and α -amylase. These enzymes act to digest starch, proteins and nucleic acid and convert them into simple compounds that contribute to the activation of germination. This was confirmed by the results of

the study of [18] these results were consistent with [19], [20] and [21].

As for the germination percentage, the results of Table 3 showed the superiority of the treatment 3 and gave the highest germination rate, which was 2.513 seeds/day, compared to the control treatment, which was 1.306 seeds/day when compared to the control treatment, which was 1.306 seeds/day when treated with *S. platensis* extract, while the highest percentage was 2.446 seeds/day when treated with *Cladophora* algae extract, and the lowest percentage was given at treatment 4, which was 1.316 seeds / day.

3.3 Germination Speed

The results of Table 3 showed that the treatment with *S. platensis* extract had a higher percentage of 2.513 seeds/day compared to the control treatment of 1.306 seeds/day than the treatment with *Cladophora* extract, which gave a lower percentage of 2.446 seeds/day compared to the control treatment of 1.343 seeds/day. The reason for this is that the increase in the concentration of *S. platensis* extract led to a decrease in the concentration of toxic ions, which led to an increase in the rates of seed absorption of water and a rise in cellular metabolic reactions related to the performance of the embryo during the germination stage, which reflected positively on the average speed of seed germination, while the increase in the concentration of *Cladophora* algae extract caused an imbalance in the water potential inside and outside the seed, which reduced the rates of seed absorption of water and decreased cellular metabolic reactions that related to the performance of the embryo during the germination stage and reflected negatively on the average speed of seed germination.

3.4 Length of the Plumule (cm)

The results of Table 4 indicate that there are significant differences between the seed activation treatments in the average length of the plumule, the radicle and the length of the seedling, where the results of the study in both extracts showed more than 20% treatment by giving the highest averages for the above-mentioned trait, which was 5.583, 5.506 cm and 11.473 respectively compared to the control treatment, which were 3.556, 2.680 ,6.383 cm, respectively. The results of Table 3 indicate that there are no significant differences between the extracts of both algae. The highest percentage of *Cladophora* extract was recorded, which were 5.506, 5.366 and 10.833 cm respectively when compared to

the control treatment of 3.340, 2.366 and 5.706 cm respectively.

The superiority of the treatment of 10% by giving the highest average length of the plumule, the radicle and seedling to the role of cytokinins are due to the effect of the hormone cytokinin in increasing the absorption of nutrients, including phosphorus, which affected the growth of oat seedlings. The reason for this is due to the role of ascorbic acid in its oxidative enzyme activity, increasing its vegetative growth and encouraging it in the growth of seedling [22].

3.5 Radicle Length (cm)

The results of Table 5 indicate that there are significant differences between the concentrations of seed activation treatments in the average radicle length of the extract of both algae. The highest percentage of the average radicle length characteristic was recorded for *Cladophora* and *S. platensis* at a concentration of 20%, which were 5.883 and 5.326 cm respectively.

The 20% higher treatment is due to the presence of a percentage of hormones and plant growth regulators that increase the processes of cell division and cellular expansion, which they increase the size of the meristematic region as well as increase the number of cells that carry out division [21].

The reason for its superiority is due to the effect of the hormone cytokinin in increasing the absorption of nutrients, including phosphorus, which affected the increase of root growth of the oat plant [23]. The results of Table 4 also showed that there are significant differences in the treatment between the concentrations of the extract of both algae, where it gave the lowest average for the radicle length trait, which were 3.386 and 3.130 cm respectively, due to the increase in the concentration of salts in the extracts, which increases as the concentration increases, and this in turn works to hinder the absorption of nutrients by seeds, which causes nitrogen deficiency, which hinders the proper growth of the plant [24].

3.6 Length of Seedling (cm)

The results of Table 6 indicate that there are significant differences between the seed activation treatments in the average seedling length. The results show that the seed activation treatments were superior in the extract of both algae at a concentration of 20%, which were 11.473 and 10.833 cm respectively, when compared to the control treatment of 6.383 5.706 cm respectively.

The reason for this is due to the presence of a proportion of plant hormones and plant growth regulators such as gibberellic acid and cytokinin, which in turn work on cellular expansion and by increasing cell division, which leads to an increase in the size of the meristematic region in addition to increasing the number of cells that carry out the division process. This is consistent with the results of [25].

3.7 Seed Vigor (cm)

The results of Table 7 indicate that there are significant differences between the seed activation treatments in the average seed strength, the highest averages of this trait were recorded in both extracts at a concentration of 20%, reaching 10110.2 and 9103.2 respectively. Perhaps the reason for the superiority of both algae *Cladophora* and *S. platensis* with a concentration of 20% in the seed vigor characteristic was due to their superiority in the percentage of germination and the length of the seed. The strength of the seeds represents the product of multiplying the percentage of germination by the length of the seedling and that any increase in the components of these two traits will lead in the result to a positive increase in the average seeds vigor, while the lowest average of the seed strength trait was for the seed soaking factor to extract both algae at a concentration of 30% due to the low germination ratio as in Table 1 and the average seedling length characteristic in Table 5, which reflected negatively on the average seed vigor trait, and the reason for this is that the product of the multiplying of the germination percentage by the length of the seedling represents the seeds vigor.

3.8 Fresh weight (g)

The results of Table 8 indicate that there are significant differences between the seed activation treatments in the average fresh weight trait of the seedling for the extract of both algae at a concentration of 20%.

The results of the study showed that the seed activation treatment in the concentration of 20% in both extracts significantly exceeded the rest of the other treatments by giving it the highest average fresh weight trait of the seed, which were 0.976 and 0.952 g, respectively, when compared with the control treatment, which were 0.383 and 0.373 respectively.

Table 2: Effect of stimulating oat plant seeds with *S. platensis* and *Cladophora* algae extract on average laboratory germination percentage (%).

Type of algae	Algae concentration				Average effect of algae
	0	%10	%20	%30	
<i>S. platensis</i>	53.990 d	77.473 b	88.220 a	59.217 c	69.725a
<i>C. glomerata</i>	51.437 e	65.653 c	84.040 a	45.943 f	61.768b
Average concentration effect*	52.714 d	71.563 c	86.130 a	52.580 e	

Table 3: Effect of stimulating oat plant seeds with algae extract on average laboratory germination speed (seed/day).

Type of algae	Algae concentration				Average effect of algae
	0	%10	%20	%30	
<i>S. platensis</i>	1.306 e	2.363 a	2.513 a	1.406 b	1.897 a
<i>C. glomerata</i>	1.343 d	2.193 b	2.446 a	1.316 c	1.825 b
Average concentration effect*	1.325 d	2.278 b	2.480 a	1.361 c	

Table 4: Effect of stimulating oat plant seeds with *Cladophora* and *S. platensis* algae extract on average plumule length (cm).

Type of algae	Algae concentration				Average effect of algae
	0	%10	%20	%30	
<i>S. platensis</i>	3.5566 d	4.273 b	5.583 a	3.740 c	4.288 a
<i>C. glomerata</i>	3.3400 e	4.063 c	5.506 a	3.390 d	4.075 b
Average concentration effect*	3.448 c	4.168 b	5.545 a	3.565 c	

Table 5: Effect of stimulating the seeds of the oat plant with *Cladophora* and *S. platensis* algae extract on radicle length cm.

Type of algae	Algae concentration				Average effect of algae
	0	%10	%20	%30	
<i>S. platensis</i>	2.580 g	4.843 b	5.883 a	3.386 e	4.173 a
<i>C. glomerata</i>	2.366 h	4.560 c	5.326 b	3.130 f	3.832 b
Average concentration effect*	2.473 e	4.701 c	5.605 a	3.358 f	

Table 6: Effect of stimulating oat plant seeds with *Cladophora* and *S. platensis* algae extracts on seedling length (cm).

Type of algae	Algae concentration				Average effect of algae
	0	%10	%20	%30	
<i>S. platensis</i>	6.383 g	8.583 c	11.473 a	7.650 c	8.522 a
<i>C. glomerata</i>	5.706 h	7.896 d	10.833 b	7.226 e	7.915 b
Average concentration effect*	6.045 e	8.240 c	11.153 a	8.240 d	

Table 7: Effect of stimulating oat plant seeds with *Cladophora* and *S. platensis* algae extract on seed vigor.

Type of algae	Algae concentration				Average effect of algae
	0	%10	%20	%30	
<i>S. platensis</i>	4373.8 d	5927.6 c	10110.2 a	5083.9 c	6148.9 a
<i>C. glomerata</i>	2933.9 f	3626.4 e	9103.2 b	2933.9 f	5101.0 b
Average concentration effect*	3653.9 d	4777.0 c	9616.7 a	4008.9 b	

Table 8: Effect of stimulating oat plant seeds with *Cladophora* and *S. platensis* algae extract on fresh weight.

Type of algae	Algae concentration				Average effect of algae
	0	%10	%20	%30	
<i>S. platensis</i>	0.383f	0.892 b	0.975 a	0.785 d	0.832 a
<i>C. glomerata</i>	0.616 g	0.821 c	0.915 b	0.717 e	0.767 b
Average concentration effect*	0.500 e	0.857 b	0.945 a	0.751 c	

* Averages with similar letters do not differ significantly at the probability level $p \leq 0.05$.

Table 9: Effect of stimulating oat plant seeds with *Cladophora* and *S. platensis* algae extract on dry weight (g).

Type of algae	Algae concentration				Average effect of algae
	0	%10	%20	%30	
<i>S. platensis</i>	0.373 e	0.473 d	0.952 a	0.378 e	0.544 a
<i>C. glomerata</i>	0.774 b	0.450 e	0.512 c	0.334 f	0.518 b
Average concentration effect*	0.574 b	0.462 d	0.732 a	0.356 e	

* Averages with similar letters do not differ significantly at the probability level $p \leq 0.05$.

The reason for this is due to the high rates of cellular metabolism of seeds soaked with extracts due to their content of plant hormones and growth regulators [26]. Which are responsible for activating hydrolysis enzymes of the nutrients stored in the seeds; that led to increasing the strength of the seeds and stimulating the embryo and then giving seedlings with a high fresh weight [27].

Also, the superiority of this treatment in the fresh weight attribute was due to its superiority in the length of the plumule, radicle and seedling (Tables 3, 4 and 5), which reflects positively on the fresh weight. While the seeds soaked with *Cladophora* and *S. platensis* algae extract recorded the lowest average fresh weight trait in the treatment of 30%, which were 0.717 and 0.758 g respectively. The reason for this is that the use of high concentrations leads to an increase in the concentration of salts and this leads to a failure in germination or the emergence of weak seedlings, which reflects negatively on the fresh weight of the seedling [28].

3.9 Dry Weight (g)

As for the dry weight trait of the seedling, the results of Table 9 show that there are significant differences between the averages of the extract of both algae, which recorded the highest percentage of 0.592 and 0.512 g, respectively, due to the thickness of the root and the number of root hairs when compared with the control treatment, which were 0.373 and 0.374 g, respectively, and the lowest percentage of dry weight in the treatment was recorded 30%, which were 0.378 and 0.334 g, respectively.

4 CONCLUSIONS

Algae are a large and very diverse group of prokaryotic and eukaryotic organisms, autotrophic organisms. Algae extracts can be used to produce organic fertiliser. Stimulating the seed by soaking with algae extract resulted in a significant increase in the average traits analysed, indicating its positive

role in breaking the dormancy of the seed and the regular growth of the plant. Also, the use of high concentrations of seaweed extracts when soaking the seeds before cultivation led to negative results and a low percentage of the analysed traits. Treatment 3 showed the highest germination rates for *S. platensis* extract 88.220%, 2.51333, 5.58333 cm, 5.88333 cm, 11.47333 cm, 10110.2, 0.78533 g and 0.59200 g, while the lowest percentage of treatment 4 was 59.217%, 1.40667, 3.74000 cm, 3.38667 cm, 7.65000 cm, 5084.9, 0.78533 g and 0.37800 g, respectively. This study has shown that algae extracts have a positive effect on the germination of oat seeds and that it is possible to produce a commercial and environmentally friendly biofertilizer by using different concentrations of algae extracts.

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