Simple and Economical Methods for Producing a Nano-Algae Fertilizer and its Efficacy for Germination of Cucumber Seeds

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ABSTRACT

The purpose of this study was to investigate and evaluate the effect of three different seaweed extracts (alkali extract, acid extract, neutral extract) on germination parameters of cucumber seeds. The first extract was treated with potassium hydroxide solution (Alg. K), the second was treated with nitric acid (Alg. N), and a third one was treated with potassium hydroxide and nitric acid (Alg. KN). Green algae (Ulva sp) were used to prepare these extracts. Four concentrations of Alg. K, Alg. N, and Alg. KN (0.1, 0.2, 0.3, 0.4 %) were used to construct priming treatments in a completely randomized design with three repetitions. The cucumber (*Cucumis Sativa*) seeds were soaked three hours in treated seaweed extracts then put on wet filter sheets in Petri dishes to germinate for three to four days at 28°C. Comparing Alg. K treatments to Alg. N and Alg. KN treatments, the results showed that Alg. K treatments greatly boosted germination parameters. The most effective way to improve germination parameters are to prime cucumber seeds with 0.3% Alg. K, according to statistical analysis of the data collected. Alkaline extract method was the most efficient and fastest in terms of the specifications of algae fertilizer from the smell and content of organic matter and potassium.

Keywords: Alkali extract, Acid extract, Neutral extract, Algae, Germination.

INTRODUCTION

In order to meet global food needs, more than 50% of food is produced to face the expanding global population (Mittal *et al.*, 2020). Newly developed, enhanced management techniques and policies will be able to accomplish this goal (Siddiqui *et al.*, 2015). Nano materials enable a wide range of agricultural uses. As a result, scientists have integrated nanotechnology into seed preparation to improve the germination process and seedling growth (Maroufi *et al.*, 2011 and Imtiaz *et al.*, 2023).

Egypt has many types of marine algae, which have not been exploited optimally despite their extreme importance yet. Algae is regarded as an essential source of important natural substances, such as vitamins, minerals, pigments, proteins, lipids, polysaccharides, antioxidants, and phytohormones (Bhuyar *et al.*, 2020). It also offers an environmentally friendly and non-toxic method and saves energy and raw materials (El-Sheekh *et al.*, 2022 and Elkhateeb *et al.*, 2024). Previous studies have shown improved germination and increased productivity when crops are grown in plots treated with seaweed fertilizers or when seaweed fertilizer is used as a liquid spray (Rathore *et al.*, 2009 and Divya *et al.*, 2015).

Fertilization is a key farmland management strategy used in agricultural production to change the nutrient requirements of crops (Jia *et al.*, 2022). On the other hand, excessive mineral fertilizer use over time can deteriorate the soil and pollute the ecosystem (Hossain *et al.*, 2022 and Zhou *et al.*, 2024). Organic fertilizers improve soil fertility (Seleem *et al.*, 2022), crop yield, plant growth simulation, microbial biomass, raise the organic carbon content of the soil, improve aggregate stability and improve the biological processes of the soil (Diacono & Montemurro, 2011 and Bhunia *et al.*, 2021).

Germination is one crucial phase of a plant's life cycle, which mainly uses water or solutions containing substances (Shukla *et al.*, 2019 and Sherif *et al.*, 2022). When the dry seed absorbs water, continues with the biochemical preparative process and the elongation of the embryonic axis, and typically ends when the radicle emerges from the seed. For plants and crops to successfully establish and thrive, a germination test is a crucial component. The viability or germination potential of a non-dormant seed is ascertained by it. The purpose of seed lot germination is to ascertain the seed's homogeneity, and germination capacity, and measure the germination rate of the seed lot (Ghaleb *et al.*, 2022).

Cucumbers are a well-liked vegetable; they belong to the family (Cucurbitaceae). Their crisp and refreshing quality has made them a popular crop and food around the globe. They are a great low-calorie, highly hydrating dietary choice because of their high-water content (Chakraborty & Rayalu, 2021 and Ejaz & Bahadur, 2024).

The objective of this study were to investigate the effect of extraction solutions, from seaweed on cucumber seed germination and determine the most effective extraction method, prepare a low-cost organic

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fertilizer from algae, determine the most effective extraction method, evaluate the ability of fertilizer to provide balanced nutrition to the plants, providing an organic alternative to chemical fertilizers that may be harmful to the environment and public health, highlighting algae as a sustainable and renewable source of organic fertilizer, and improving soil fertility and quality of agricultural products.

MATERIAL AND METHODS

Collecting seaweeds

Sea lettuce (Ulva sp.) green algae was collected from El-Shatby beach, Alexandria, Egypt (31°, 21`N; 29°, 91`E) in July 2023. The samples were kept in plastic bags containing a little seawater to protect the seaweeds from drying out and transported to 403 soil fertility and plant nutrition lab, faculty of agricultural, Alexandria University, Egypt. The samples washed with fresh water to remove the sand and salt content and they were air-dried. After that, they were dried in the oven at 75°C for 3 days. The dried seaweeds were grounded and used for the preparation of seaweed extracts.

Preparation of seaweed extracts

a) Alkali extract (Alg. K): seaweed powder (25g) was extracted with 150ml of potassium Hydroxide (KOH) solution (5M) then the extract was put on the hot plate until it boiled. Then, it was cooled to room temperature for 72 hours. The supernatant was utilized as a seaweed extract with a ratio (1:6).

b) Acid extract (Alg. N): seaweed powder (250g) was extracted with Nitric acid (HNO_3) (20%) then the extract was put on the hot plate until it boiled. Then, it was cooled to room temperature for 72 hours. The supernatant was utilized as a seaweed extract with a ratio (1:6).

c) Neutral (Alkali and Acid) extract (Alg. KN): seaweed powder (25g) was extracted with 150ml potassium Hydroxide solution (5M). Then, the extract was put on the hot plate. After that, we added Nitric acid (HNO_3) until we got an extract with pH=7. The extract was evaporated until it reached a volume of 1500 ml. It was cooled to room temperature for 72 hours. The supernatant was utilized as a seaweed extract with a ratio (1:6).

Identification of Fertilizer

pH was determined using a pH meter, calcium and magnesium were determined using a titration method, nitrogen was determined using a Kjeldahl method, phosphorus was determined using a Spectronic, potassium was determined using a Flame photometer, organic matter (O.M %) was determined using wet oxidation, and organic acids were determined using GC-MS.

Cucumber germination experiment

Cucumber seeds with a local genotype (Barracuda F1) from Egypt were used. After being cleaned with distilled water, the seeds were immersed for three hours at 28°C with tap water (Control) and differing concentrations of K, N, and KN (0.1%, 0.2%, 0.3%, and 0.4%) (3 seeds/dish). On moist filter paper, seeds were germinated in 39 Petri dishes. The germination rate was measured 36 hours later. Root length, shoot weight, and fresh root weight were assessed 72–96 hours later. After that, all treatments were dried in the oven at 75°C for 72 hours and then the dry weight was measured. Relative germination rate (RGR), relative root length (RRL), relative root fresh weight (RRFW), relative fresh weight (RFW), relative dry weight (RDW), relative moisture (RM) was calculated according to Leggatt *et al.* (1949); as follows (Fouad *et al.*, 2024):

$$\textbf{RGB (\%)} = \frac{\text{Seeds germinated in test sample}}{\text{Seeds germinted in control}} \times 100$$

$$\textbf{RRL (\%)} = \frac{\text{Mean root length in test sample}}{\text{Mean root length in control}} \times 100$$

$$\textbf{RRFW (\%)} = \frac{\text{Mean root fresh weight in test sample}}{\text{Mean root fresh weight in control}} \times 100$$

$$\textbf{RFW (\%)} = \frac{\text{Mean fresh weight in test sample}}{\text{Mean fresh weight in control}} \times 100$$

$$\textbf{RDW (\%)} = \frac{\text{Mean dry weight in test sample}}{\text{Mean dry weight in control}} \times 100$$

$$\textbf{RMC (\%)} = \frac{\text{Mean (fresh weight} - \text{dry weight) in test sample}}{\text{Mean (fresh weight} - \text{dry weight) in control}} \times 100$$

Statistical analysis

The treatments were set up according to the completely randomized design with three replicates. Statistical analysis of experimental data was carried out using CoStat Software package (CoHort, 2004). Differences between means of treatments were tested using the least square difference technique of Student Newman-Keuls at 5% significance level (LDS.05).

RESULTS AND DISCUSSION

Data in Table (1) showed that the highest percentage of organic matter recorded at Alg. K and extracts contains macronutrients. The highest total dissolved solids (TDS) were recorded at Alg. K compared to Alg. N and Alg. KN.

Table 1. Identification of extracts (Alg. K, Alg. N and Alg. KN)

Parameters	K	N	KN
Appearance	**Green, black (Liquid)**	**Yellow (Liquid)**	**Deep brown (Liquid)**
Oduor	**Seaweed**	**Un clear**	**Un clear**
pH value	14.3	<0.1	7.17
Density (Mg m^{-3})	1.23	1.16	1.2
CaO (%)	0.28	0.7	0.42
MgO (%)	0.1	0.9	0.8
P_2O_5 (%)	0.2	0.05	0.2
K_2O (%)	17.5	0.57	16.3
O.M (%)	46.3	5.16	12.75

The high pH in Alg. K was as a result of extraction with potassium hydroxide, while the pH decreased to less than 0.1 in Alg. N because the extraction was conducted by nitric acid. The highest percentage of calcium and magnesium was recorded at Alg. N because of the nitric acid dissolved organic calcium and magnesium, and calcium nitrate is considered the best soluble (solubility = 144 g / 100 g H_2O at 25 °C) (Haynes, 2015). The amount of calcium and magnesium present in the Alg. KN ranged in the middle between the amount present in Alg. K and Alg. N because the extraction was obtained by using both a solution of potassium hydroxide (5M) and nitric acid. The nitric acid dissolved the calcium and magnesium but in a smaller amount than the extraction that was done with nitric acid only. The high percentage of organic matter in Alg. K may related to the percentage of algae. Data in Fig. (1) showed that size of Alg. K components was in the nanoscale (100nm).

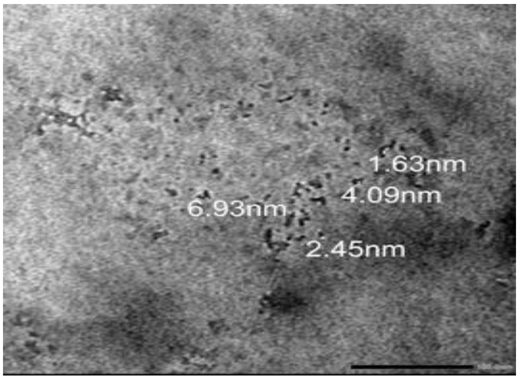

Fig. 1. Transmission electron micrographs of Alg. K components nanoparticles

The results depicted in Table (2) and Fig. (2) showed the presence of some essential organic acids like Butanoic acid, Lactic acid, and Oxalic acid.

Table 2. Analysis of some nutrients and plant hormones in Alg. K

Parameters	Result	Method
Appearance	Green black (Liquid)	-
Odour	Seawwed	-
pH value	14.3	pH meter
O.M (%)	46.3	Wet Oxidation
Water solubility (%)	100	-
N (ppm)	112	Kjeldahl
P_2O_5 (%)	0.2	Spectronic
K_2O (%)	17.5	Flame photometer
CaO (%)	0.28	Titration
MgO (%)	0.1	Titration
Oxalic acid	detected	GC-MS
Butanoic acid	detected	GC-MS
Lactic acid	detected	GC-MS

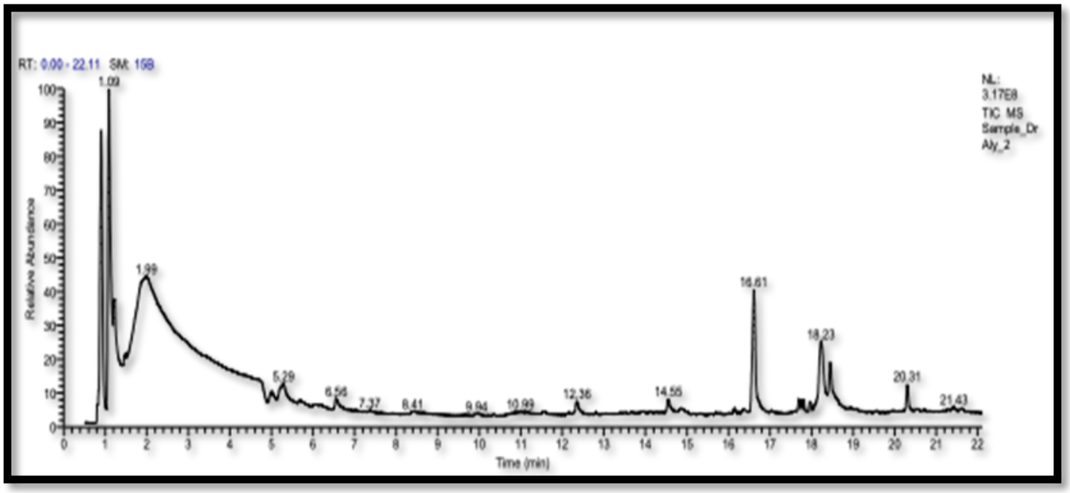

Fig. 2. GC-MS chromatograph of organic acids in Alg. K

Results in Table (3) indicated that the highest significant seed germination percentage (GP%) of primed cucumber seeds was recorded at 0.3% of Alg. K concentrations (200% relative to control) compared to other Alg. K treatments. Mean GP% of Alg. K treatments was significantly higher than Alg. N treatments or Alg. KN treatments. Also, it is notice that with increasing K concentration increased GP% up to 0.3% K compared to the control while GP decreased with increasing Alg. K concentration to 0.4%.

Table 3. Effect of priming with different Alg. K, Alg. N and Alg. KN concentrations on relative germination percentage (RGP, %) of cucumber seeds

Concentration (%)	Alg. K	Alg. N	Alg. KN
	RGP (%)		
0.1	125^{d}	150^{c}	100^{e}
0.2	125^{d}	125^{d}	100^{e}
0.3	200 ^{a}	125^{d}	50^{f}
0.4	175^{b}	125^{d}	25^{g}

Means with the same letters are not significantly different.

Data in Tables (4&5) and Figs. (3&4&5) explained that the means of root length (RL) and root fresh weight (RFW) were affected by all investigated treatments. The highest significant RL was recorded at 0.3% of K concentrations (237% relative to control). Alg. K treatments had significantly higher mean RL compared to Alg. N and Alg. KN. Mean RL values were increased with increasing Alg. K concentration up to 0.3%, while it was reduced at Alg. K 0.4%. Treating seeds with Alg. N 0.1 and Alg. KN 0.1 showed a slight increase in RL, but with increasing concentration, RL significantly decreased. Mean RFW was significantly lower for Alg. N and Alg. KN treatments compared to K treatments.

Table 4. Effect of priming with different Alg. K, Alg. N and Alg. KN concentrations on relative root length (RRL, %) of cucumber seeds

Concentration (%)	Alg. K	Alg. N	Alg. KN
	RRL (%)		
0.1	107.4^{e}	114.8^{d}	103.7^{e}
0.2	159.2^{c}	6.3^{f}	9.6^{f}
0.3	237^{a}	4.8^{fg}	7^{f}
0.4	192.6^{b}	2.6^{g}	1.5^{g}

Means with the same letters are not significantly different

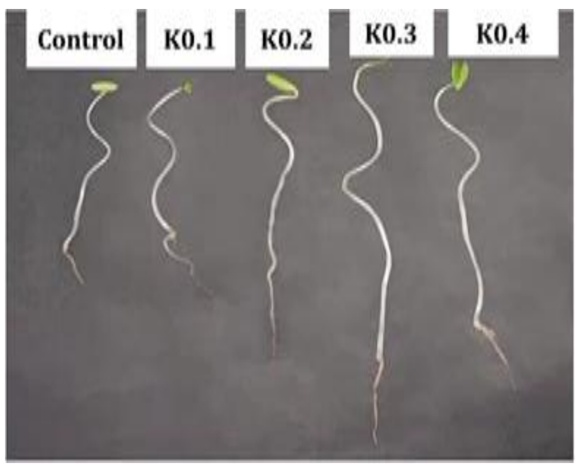

Fig. 3. Effect of priming with different K concentrations on germination parameters of cucumber seeds

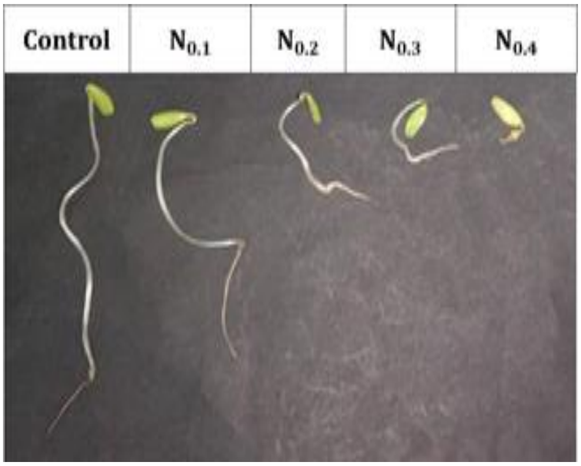

Fig. 4. Effect of priming with different N concentrations on germination parameters of cucumber seeds

Fig. 5. Effect of priming with different KN concentrations on germination parameters of cucumber seeds

Table 5. Effect of priming with different Alg. K, Alg. N and Alg. KN concentrations on relative root fresh weight (RRFW, %) of cucumber seeds

Concentration (%)	Alg. K	Alg. N	Alg. KN
	RRFW (%)		
0.1	157^{ab}	153.6^{b}	109^{c}
0.2	159^{a}	14.3^{e}	41^{d}
0.3	162.5^{a}	10.7^{e}	37.5^{d}
0.4	160.7^{a}	5.4^{f}	1.8^{f}

Means with the same letters are not significantly different.

Data in Tables (6&7) showed that the highest significant fresh weight (FW) was recorded at 0.3% of Alg. K concentrations (187.3% relative to control). Alg. K treatments had significantly higher mean FW compared to Alg. N and Alg. KN.

Table 6. Effect of priming with different Alg. K, Alg. N and Alg. KN concentrations on relative fresh weight (RFW, %) of cucumber seeds

Concentration (%)	K	N	KN
	RFW (%)		
0.1	115.6^{d}	131^{c}	101.4^{e}
0.2	155.4^{b}	91.7^{f}	50.7^{h}
0.3	187.3^{a}	58^{g}	17.8^{l}
0.4	186.7^{a}	50.3^{h}	11^{m}

Means with the same letters are not significantly different.

Table 7. Effect of priming with different Alg. K, Alg. N and Alg. KN concentrations on relative dry weight (RDW, %) of cucumber seeds

Concentration (%)	K	N	KN
	RDW (%)		
0.1	166.7^{h}	450^{c}	316.7^{g}
0.2	416.7^{d}	383.3^{e}	300^{g}
0.3	816.7^{a}	350^{f}	100^{m}
0.4	483.3^{b}	133^{l}	50^{n}

Means with the same letters are not significantly different.

The beneficial effects of Alg. K up to 0.3% can be attributed to its high content of organic matter and potassium. Potassium is essential for many plant growth processes, activating at least 60 different enzymes essential for plant growth (Van Brunt and Sultenfus, 1998). It is a basic requirement for protein and starch synthesis (Kadam *et al.,* 2011) and it plays an essential role in transporting water and nutrients through the xylem (Schwartzkopf, 1972 and Prajapati & Modi, 2012). Alg. K also contains the most important plant hormones, such as cytokinin, which promote cell division, transport products of photosynthesis, alter the activity of other enzymes, have a profound effect on physiological and biochemical processes (Wang *et al.,* 2023), and raise the level of photosynthetic protein (Sosnowski *et al.,* 2023). Gibberellin is one of the most significant plant hormones utilized to promote seed germination due to its ability to increase emergence and germination under a variety of environmental conditions (Sedghi *et al.,* 2010). It also works to raise the amount of different substances that are present in the cell and this causes the cell's osmotic pressure and insufflation to rise, as well as the amount of water that enters the cell and increases its expansion (Hedden & Thomas, 2006 and Kadhim, 2023). Auxins can extend stem cells and facilitate a wide range of plant development processes, including the formation of tropisms, lateral roots, vascular tissue differentiation, and the formation of the apical-basal axis during embryogenesis (Friml *et al.,* 2002, 2003; Heisler *et al.,* 2005; Abas *et al.,* 2006 and Serrano *et al.,* 2023). Alg. K also contains oxalic acid, which impacts the regulation of plant growth and development, the plant's defense mechanisms, the removal of heavy metals from the body (Li *et al.*, 2022), and Mun *et al.* (2024) showed the Butanoic acid had significantly positive effects on plant growth. Murindangabo *et al.* (2023) showed the Lactic acid control plant pathogens and diseases, reduce the use of chemical fertilizers, improve water-holding capacity and increase plant growth and yield.

Germination parameters decreased with increasing K concentration to 0.4% and this may be explained by excessive concentration from elements and pH hurting germination parameters.

Negative effects of Alg. N can be explained by the decrease in pH according to Deska *et al.* (2011). They concluded that the largest changes in seedling dry matter were induced by decreasing the pH of the growing medium, and that low pH of the medium had a negative influence on the green part length of the seedlings.

During the preparation of Alg. KN, potassium nitrate (KNO_3) may be formed at pH= 7, which slightly improved germination parameters at KN 0.1% but increasing concentration showed an adverse impact on germination parameters because of excessive concentration from KNO_3. The effect of KNO_3 on germination parameters agreed with results obtained by Fouad *et al.* (2024) who concluded that KNO_3 increased root and shoot length but at high concentrations, KNO_3 dampened plant growth.

CONCLUSION

Algae K method is the most efficient and simple to produce algae fertilizer in terms of the percentage of organic matter and potassium.

Nano algae were successfully used to prepare organic fertilizer investigated in this study. Priming cucumber seeds using alkaline extract with a concentration of 0.3% proved to significantly improve germination parameters. On the contrary, other extraction methods (algae KN or N) negatively affected the germination of cucumber seeds either because of low acidity or presence of nitrates.

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الملخص العربي

طرق بسيطة واقتصادية لإنتاج سماد الطحالب النانوي وفاعليته لإنبات بذور الخيار

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الهدف من هذه الدراسة هو تقييم تأثير ثلاثة مستخلصات سائلة تم تحضيرها من الطحالب الخضراء (المستخلص القلوي، المستخلص الحامضي، المستخلص المتعادل) على معايير إنبات بذور الخيار. تم تحضير المستخلص الأول باستخدام الطحالب ومحلول هيدروكسيد البوتاسيوم (Alg. K) ، والمستخلص الثاني تم تحضيره باستخدام الطحالب وحمض النيتريك (Alg. N) ، والمستخلص الثالث تم تحضيره باستخدام الطحالب وكل من محلول هيدروكسيد البوتاسيوم وحمض النيتريك (Alg. KN). تم تحضير التركيزات الأولية باستخدام ٤ تركيزات مختلفة من Alg. K, Alg. N, Alg. KN(٠،١، ٠،٢، ٠،٣، ٠،٤ ٪) ، تم تنفيذ التجربة وفقًا للتصميم الإحصائي العشوائي الكامل من ثلاث مكررات. تم نقع بذور الخيار في التركيزات السابقة لمدة ٣ ساعات ثم نبتت على أوراق ترشيح رطبة في أطباق بتري لمدة ٣-٤ أيام عند درجة حرارة ٢٨ درجة مئوية. أظهرت النتائج أن المعاملة Alg. K عززت بشكل كبير من معايير الإنبات مقارنة ب Alg. N وAlg. KN، ووفقًا للتحليل الإحصائي للبيانات، فإن نقع بذور الخيار باستخدام Alg. K ٠،٣٪ هو المعاملة الأكثر كفاءة لتعزيز إنبات بذور الخيار. وكانت طريقة المستخلص القلوي هي الأكفء والأسرع من حيث مواصفات سماد الطحالب من رائحة ومحتوي المادة العضوية والبوتاسيوم.

الكلمات المفتاحية: المستخلص القلوي، المستخلص الحامضي، المستخلص المتعادل، الطحالب، الإنبات.