



Marine Science Center-University of Basrah

Mesopotamian Journal of Marine Sciences

Print ISSN: 2073-6428

E- ISSN: 2708-6097

www.mjms.uobasrah.edu.iq/index.php/mms



Effect of Bio-fertilization with Two Types of Algae on Some Physiological Characteristics of Date Palm *Phoenix dactylifera* Seedlings

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Article info.

✓ Received: 30 September 2025

✓ Accepted: 4 January 2026

✓ Published: 29 June 2026

Key Words:

Algae

Bio-fertilizer

Chara vulgaris

Date palm

Enteromorpha intestinalis

Methanolic extracts

Phoenix dactylifera

Abstract - Recently, Biofertilizers, including algae, emerge as harmless and ecofriendly fertilizers. This study investigated a comparison between two algal methanolic extracts; *Enteromorpha intestinalis* and *Chara vulgaris*. Methods involved the foliar spraying of the two extracts separately on Date palm leaves, measuring several parameters in the leaves. Results showed a low 325.5mg/100g, moderate 14.31mg/g and significant 15.40mg/g increment of proteins, carbohydrates, and phenols, respectively, comparing to control in the leaves sprayed with *E. intestinalis* extract, whereas, spraying leaves with *C. vulgaris* extract raised the amount of carbohydrates significantly 16.98mg/g, and the phenols moderately 15.89mg/g, while reduced the proteins content 325.5mg/100g comparing to control. Additionally, both algal extracts reduced the total chlorophyll and carotenes pigments 5.529 mg/100 g, 6.82mg/100g and 0.020mg/100 g, 0.022mg/100 g comparing to control applied in the experiments. It could be concluded the possibility of utilizing algal extract as alternatives to chemical fertilizer or environmentally friendly and harmless natural stimulants to enhance the Date palm growth. Nevertheless, extraction and spraying methods should be selected appropriately.

تأثير التسميد الحيوي بنوعين من الطحالب على بعض الخصائص الفسيولوجية لشتلات نخيل التمر

Phoenix dactylifera

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المستخلص - تضمنت الدراسة الحالية استخلاص نوعين من الطحالب طحلب الكارا وطحلب الانتيرومورفا استخلاصا كحوليا وبحث تأثيراتهما على نمو وفسلجه نبات نخيل التمر. وقد اظهرت نتائج الرش الورقي ثنائيا في الاستجابة من قبل النبات. اذ ارتفع تركيز الفينولات بشكل ملحوظ في اوراق النخيل عند المعاملة بمستخلص طحلب الانتيرومورفا 15.4 mg/g، بينما كان الارتفاع متوسطا 14.31 mg/g وضعيفا 325.5 mg/100g بالنسبة للكربوهيدرات والبروتينات على التوالي في اوراق النبات المعاملة بنفس المستخلص. كما سجل مستخلص الكارا ارتفاعا واضحا لتركيز الفينولات 15.89 mg/g ومتوسطا بالنسبة للكربوهيدرات 16.98 mg/g، على العكس من البروتينات التي لوحظ انخفاضها 325.5 mg/100g خلال الرش الورقي للنبات عند استخدام نفس المستخلص. بالإضافة الى ذلك ادى مستخلص كلا الطحالب الى خفض الكلوروفيل الكلي وصبغة الكاروتينات 5.529 mg/100g و 6.82 mg/100g و 0.020 mg/100g و 0.022 mg/100g على التوالي بالمقارنة مع معاملة السيطرة. استنتجت الدراسة أنه بالإمكان استخدام المستخلصات الطحلبية كبديل للأسمدة الكيميائية او محفزات طبيعية صديقة للبيئة وغير مضره لتعزيز نمو نبات النخيل، مع ضرورة اختيار طريقة الاستخلاص والرش المناسبين للنبات

Introduction

In order to address the requirements of nutrition of the ever-increasing population of world, pesticides and chemical fertilizers are applied on large scale to increase production of crop. However, the agrochemicals injudicious use has resulted in pollution of environment leading to hazards of public health. Moreover, agriculture soils are frequently losing their physical properties and quality as well as their chemical nutrients imbalance and biological health. Biofertilizers use has currently arised as ecofriendly alternative and cost effective than fertilizers of chemical-based (Kumar *et al.*, 2021).

Biofertilization is a sustainable practice of agriculture includes bio-fertilizer using to upsurge the soil nutrient content and organic matters, accomplishing in higher productivity. Algae are biobased fertilizers and correct environmentally friendly for pollution-free applies of agriculture. Microalgae are more effective to soil than macroalgae, but macro alga gives the best results in aquatic media of mega scaled in addition to the availability for the rapid huge bulk reproduction from microalga in the laboratory. With clay soil, microalga recorded the utmost fertility levels of soil than a sandy one. Both algae can be used for remove of heavy metal similarly (Ammar *et al.*, 2022).

In the arid and semi-arid regions of the world, Date palm (*Phoenix dactylifera* L.) represents the main fruit crop, which adapts naturally to the water scarcity and extreme heat and prevents further desertification, as well as producing high nutritional value fruits (Saadaoui *et al.*, 2019). El-Mahdy *et al.*, (2017) concluded that spraying Sewy date palm with algae extract, along with silicon and boron, three times at growth start during each season, and after 45 days later, was very effective on yield increasing and improving physical and chemical characteristics of fruit. A study conducted by Ibrahim *et al.*, (2018) showed that the water extract irrigation treatment of blue-green algae resulted in a significant increase in the plant height, plant survivor rate, and the leaves number of the acclimatized plants; whereas the control showed the lowest values.

The addition of extracellular products extract of the cyanobacteria to the nutrient medium, which is half the MS salts strength, improved the multiplication of shoot in *Phoenix dactylifera* trees cv. (Barhee) (Hashem *et al.*, 2018). The bio stimulants use is not limited to spraying on the leaves, but can also be used in the plant tissue cultures laboratories for the cultivated plant tissue with positive results (Ati and Al-Kabi, 2022). Furthermore, Seaweed (Tecamin Algae) extract and Urea spraying is an effective in nourishing the Zahdi variety. It contributes to chemical content increase, and enhances the growth of date palm trees. Therefore, it is recommended as an effective and a complementary method for fertilization of Zahdi cultivar. (Murad and Al-Dulaimy, 2021). As a result of foliar spraying with the alga *Dunaliella* sp extract in the study conducted by Ati *et al.*, (2023), the content of plant leaves pigments is affected by the extract type; the aqueous extract had an effect in the chlorophyll pigments increasing compared to the alcoholic extract of the same algae. Moreover, a combination of potassium nitrate and algae extract spraying on date palms, especially at higher concentrations, increasing yield and improving the quality of fruit of Barhee date palm under the study conditions (Alebidi *et al.*, 2021).

Current objective is the study of utilizing two algal extracts (*E. intestinalis* and *C. vulgaris*) as an effective and safe biofertilizers for growth promoting of *Phoenix dactylifera* , and to determine the optimal concentration that achieves the best results in terms of seedling growth and development, for widespread application in palm farms.

Materials and Methods

A factorial laboratory experiment was applied according to a completely randomized design (CRD) with a three factors; extract type, algae type and concentration with three replicates, in the Palm Research Center laboratories at the University of Basrah and the College of Science - Department of Biology laboratories. Methanolic extract (70%), at a concentration of 200 mg/L were prepared in addition to a control treatment without algae. Thus, the experimental units were 36 seedlings (two years) treated twice, with two weeks between the first and second sprays.

Preparation of alcoholic extracts:

Two algal species *Enteromorpha intestinalis* and *Chara vulgaris* were acquired from (Algal laboratory /Biology department / College of Science/ University of Basrah). According to Harbone (1984) method, the extracts were prepared; with a weight of 100 gm. of algae, each separately, 500 ml of ethanol was added at a concentration of 98%, mixed well and left for 24 hours at laboratory temperature ($25^{\circ}\text{C}\pm 2$), after which the extract was filtered. Using Whatman No 1 filter paper, the filtrate was concentrated in a rotary evaporator at 40°C to remove the solvent and left to dry in a petri dish at laboratory temperature to completely remove the solvent until a highly viscous concentrate was obtained. This material is 50 ml of distilled water to make the concentrations used in the study.

Extraction and Estimation of Chemical Properties

1. Plant pigments in leaves

Plant pigments in leaves were estimated according to the Howertiz method described in Ati *et al.*, (2023). The results were calculated according to the following equations:

chlorophyll a (mg. L^{-1}) = $12.7 \times \text{O.D. (663)} - 2.69 \times \text{O.D. (645)}$

chlorophyll b (mg. L^{-1}) = $22.9 \times \text{O.D. (645)} - 4.68 \times \text{O.D. (663)}$

The carotene concentration was calculated as follows: $X = \frac{EY}{e \cdot 100} \times 1000 \text{ mg}$

Where:

O.D: Optical density reading of the extracted chlorophyll

E: Instrument reading at a wavelength of 480 nm

X: Number of milligrams of carotene in 1 cm³ of solution

Y: Final volume of the solution after dilution with acetone

e: Carotene constant 2300

The results were then converted to units of mg/100 g.

2. Total Soluble Carbohydrates

Total soluble carbohydrates were estimated by the Howertiz method described in Ati *et al.*, (2023) using the phenol-sulfuric acid method. to determine the standard curve for glucose, 200 mg of glucose was dissolved in 1 liter of distilled water as the glucose base solution. Five concentrations (0, 10, 20, 40, and 80) mg/L were prepared from this solution, the absorbance of the resulting color was then measured at 490 nm using a Shimadzo UV-1700 Spectrophotometer.

3. Total Soluble Proteins:

Total soluble proteins in the leaves were extracted and estimated according to the Herbert *et al.*, (1971) method described in Al-Hamoud *et al.*, (2023). Standard protein solutions (bovine

serum albumin (B.S.A.) and sample solutions were mixed thoroughly, then the resulting solutions were left to stand for 30 minutes and measured using a spectrophotometer at a wavelength of 600 nm , several concentrations of the standard protein solution were prepared by diluting the original solution: 0, 100, 200, 300, and 400 mg/L.

4. Total soluble phenols:

Total soluble phenols in the leaves were extracted and estimated according to the Melo *et al.*, (2005) method described in Ati *et al.*, (2024).

Statistical Analysis:

Randomized experimental design was applied. The data of statistical analysis was carried out by variance ANOVA analysis using SPSS version 26 with T-test. The means differences were determined by the least significant difference test (LSD) ($p < 0.05$).

Results and Discussion:

Algae Classification

Division : Chlorophyta

Class: Charophyceae

Order: Charales

Family : Characeae

Genus: *Chara*

species : *Chara vulgaris* (Linnaeus , 1753).

Division : Chlorophyta

Class: Ulvophyceae

Order : Ulvales

Family: Ulvaceae

Genus: *Enteromorpha (Ulva)*

Species : *Enteromorpha intestinalis* (Linnaeus, 1753)

Proteins:

Regarding with the protein content of palm leaves, *E. intestinalis* extract had the highest effect in protein rising after 4 weeks from spraying comparing with zero time (Control), whereas reducing impact was shown by *C. vulgaris* However, analysis showed no significance between the two algae at probability ≤ 0.05 , as demonstrated in Figure 1, as well as between the two concentrations, and 0.5 % was the best, as in Figure 2.

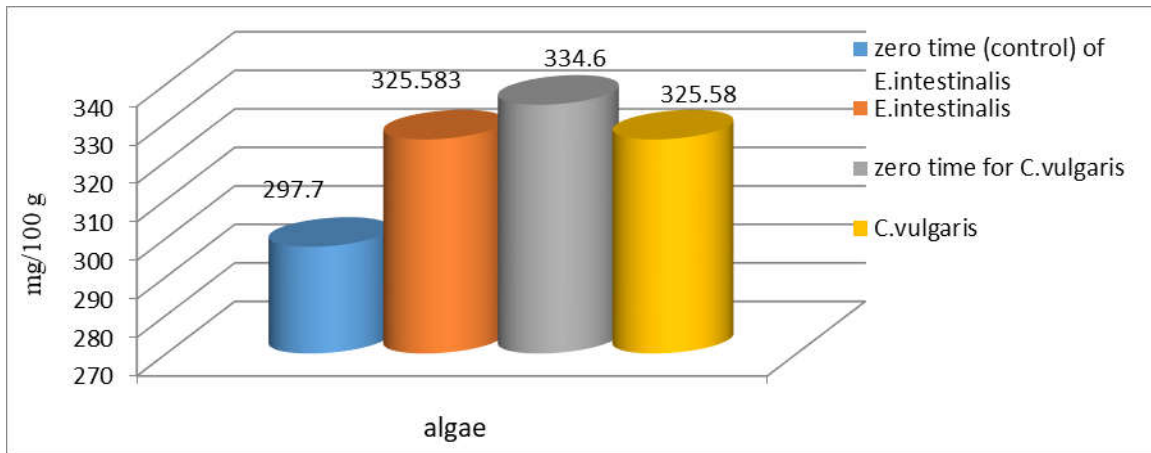


Figure 1: The effect of algal extracts of *E. intestinalis* and *C. vulgaris* on the proteins content of palm leaves

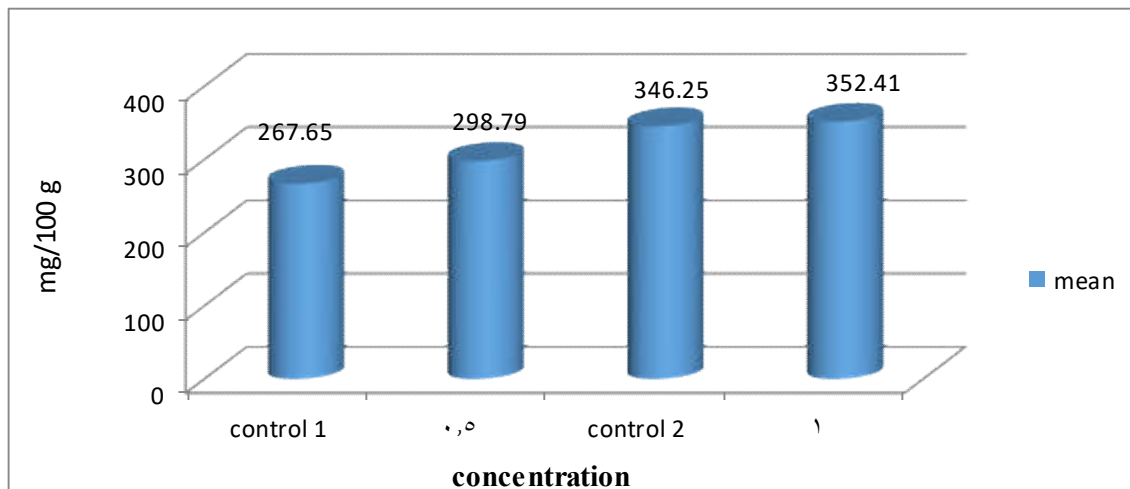


Figure 2: The effect of the two concentrations on leaves protein content

Carbohydrates:

Despite the highest impact of *C. vulgaris* extract on rising carbohydrates content of palm leaves, both algae showed this impact. No significance between the two algae was shown at probability ≤ 0.05 , Figure 3. Nevertheless, the differences between the two concentrations were significant and concentration 0.5% was the best, whereas 0.1% caused a decreasing impact, as shown by Figure 4.

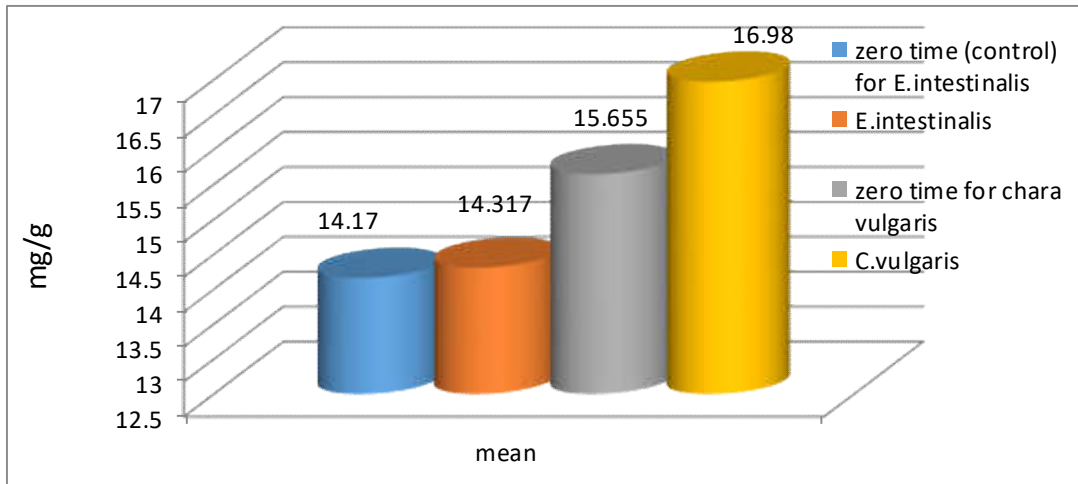


Figure 3: The effect of algal extracts *E. intestinalis* and *C. vulgaris* on the carbohydrates content of the palm leaves

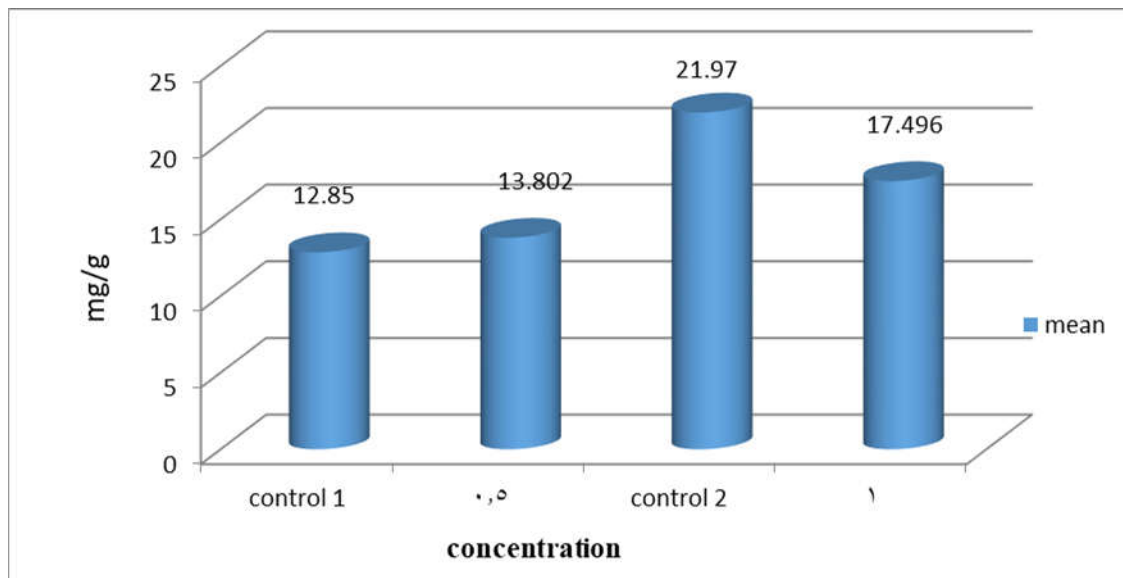


Figure 4: The effect of the two concentrations on carbohydrates content of palm leaves.

Phenols:

Both algae elevated leaves phenols content, and significant differences existed between them at probability ≤ 0.05 , as illustrated by Figure 5. The two concentrations also had a closely impact, but with no significant differences between them, Figure 6.

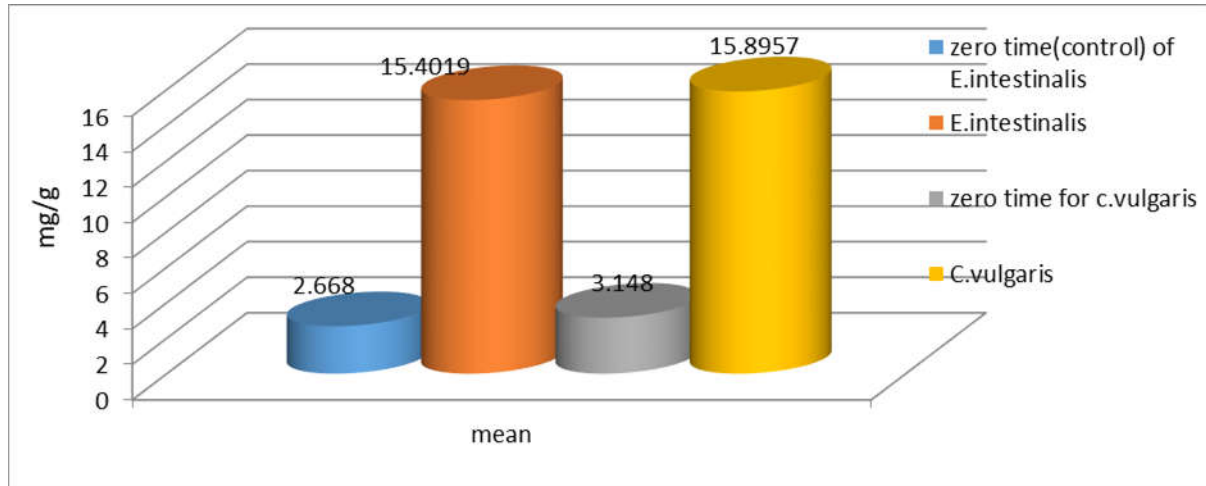


Figure 5: The effect of algal extracts *E. intestinalis* and *C. vulgaris* on phenols content of palm leaves

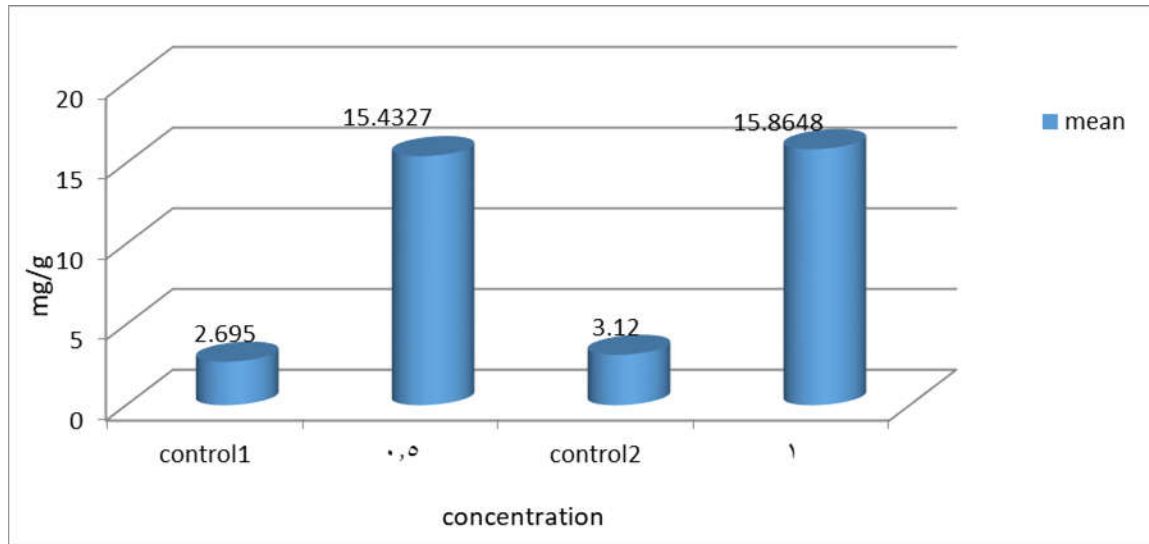


Figure 6: The effect of the two concentrations on phenols content

Total chlorophyll:

Conversely, both algae with the two concentrations had reducing effect on the total chlorophyll content of leaves, with no significant differences, as shown in Figure 7.

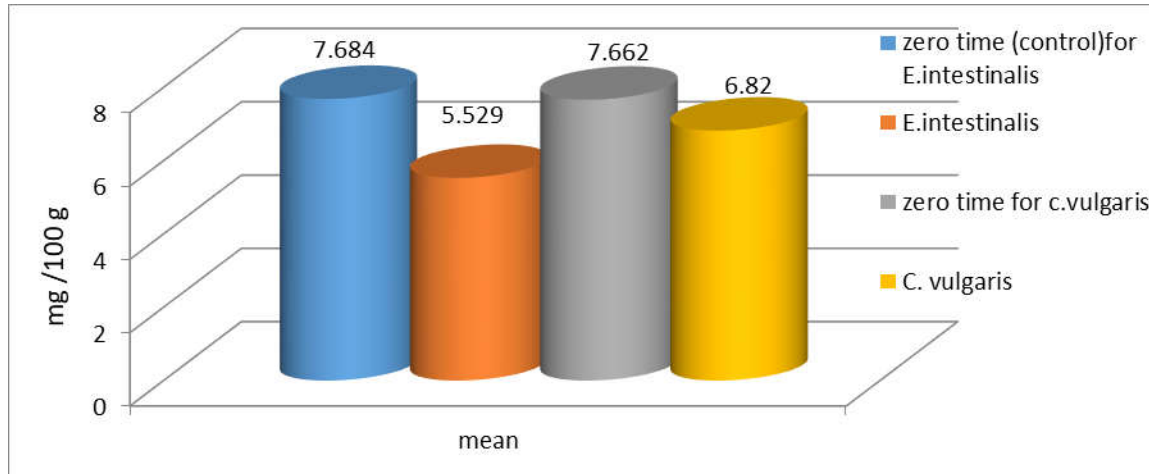


Figure 7: The effect of algal extracts *E. intestinalis* and *C. vulgaris* on total chlorophyll content of palm leaves

Carotenes:

Leaves carotenes had been reduced also as a reversely effect by algal extract, with no significant differences observed, as found in Figure 8.

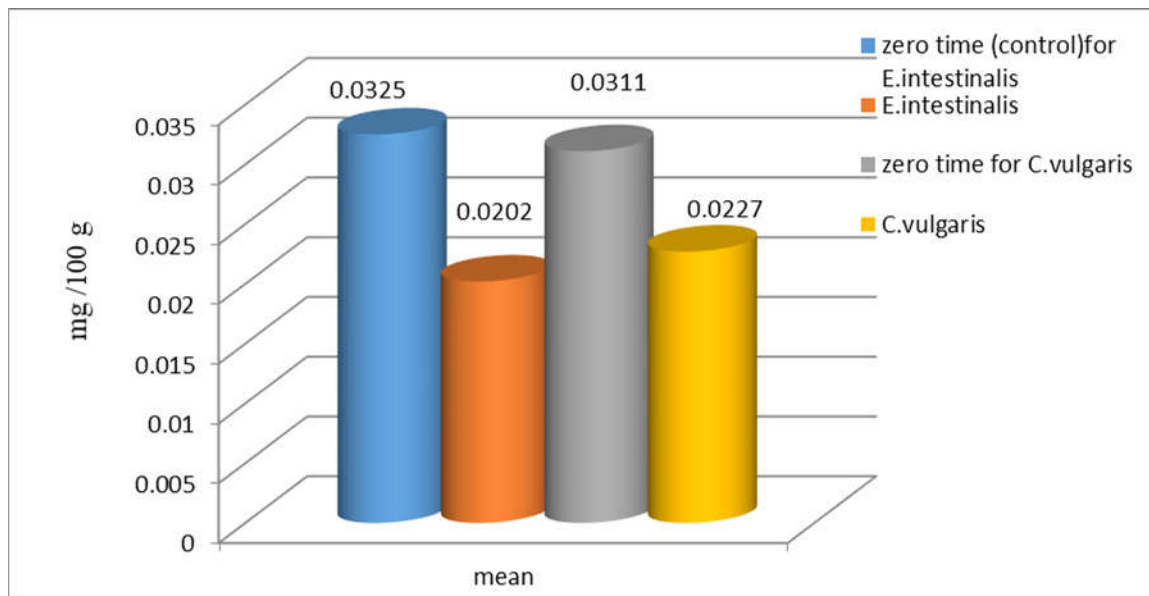


Figure 8: The effect of algal extracts *E. intestinalis* and *C. vulgaris* on carotenes content of palm leaves

In the current study, the low, moderate and high increment of carbohydrates, proteins, and phenols respectively in the leaves sprayed with *E. intestinalis* extract probably attributed to the variety of nutrients, minerals and ions in alga, which may act as growth stimulants in the plant. This was expressed previously; Mathur *et al.*, (2015) suggested that the plant growth enhancement may be due to the presence of various growths promoting factors in the extract of *E. intestinalis*, which improved the seed germination, root and shoot length, carbohydrates, protein, pigments and phenols content of the Glycine max plant. Furthermore, the foliar application of *E.*

intestinalis and *Ulva flexuosa* liquid extracts enhanced the physiology and overall growth of Hot Pepper plant, may associated with occurrence of several phytohormones such as auxin, cytokinin and gibberellins, macro and micro nutrients such as Cu, Mn, Fe, Zn, Co, K, Mg and Na detected in the crude extracts of marine algae liquid fertilizer (Azzam *et al.*, 2022). Other studies showed that the *E. intestinalis* extract exhibits favorable properties, such as nutrients content and a lower heavy metals content than the raw biomass and phenolics content, and the detection of NO₃⁻, PO₄³⁻, and K⁺ which considered as main plant nutrients, and the additional content of Ca²⁺, Mg²⁺, SO₄²⁻, and F⁻ make the extracts an interesting liquid organic fertilizer (Nekvapil *et al.*, 2021). Moreover, methanolic extract of *E. intestinalis* exhibited significant antibacterial activity as a result of the phenolic chemicals that are present (Deepak *et al.*, 2024).

Likewise, present findings showed that spraying leaves with extract raised the amount of carbohydrates moderately and significantly in phenols. The moral superiority of the double addition of the fungus *Trichoderma harzianum* and the alga *Chara* sp increased nitrogen, phosphorous, and potassium concentrations in the wheat plant, in addition to the number of grains of a spike and the weight of 1000 grains of an enzyme (Al-Ziadi and Al-Jubouri, 2023).

Furthermore, *Chara* extract contains phenolic and terpene compounds as the main active compounds (Al-Maphregy and Al-Magdamy, 2024). However, chemical composition of *C. vulgaris* were investigated (Shaker *et al.*, 2023) showed that carbohydrates were higher in *Chara* than in *Enteromorpha*, whereas in the proteins situations is opposite. This, in the present study, might be explained of disability of *Chara* extract to raise the proteins content in the plant leaves in contrast to *Enteromorpha*.

Conversely, pigments were affected negatively by both extracts, possibly attributed to the small residue of methanol in the extracts that destroyed parts of the chlorophylls and carotenes in the plant leaves.

Conclusions

According to algal extract application in our recent investigation, some critical compounds could be raised as a result of algal rich content of nutrients and minerals. However; the solvents types and the way of fertilizing could increase or decrease these materials.

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