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NANNOCHLOROPSIS OCULATA MICROALGA FOR FERTILIZATION: BIOGUIDED FRACTIONATION OF N-HEXANE EXTRACT BY STIMULATING GROWTH ACTIVITY FOR *CUCURBITA MOSCHATA*

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Abstract

Biological fertilizers have ability to supply the main nutrient elements from unavailable form to available ones during biological processes and provide favorable conditions for germination and initial growth. Microalgae are the cheap and best source of nitrogen, which does not cause soil and water pollution. Buttemut squash (*Cucurbita moschata*) is a type of pumpkin from a group of horticultural annual plants with high agribusiness prospects, which, as many other crops is affected by the use of synthetic fertilizers .In this work, we tested the germination activities of fractions of extracts of the Microalgae *Nannochloropsis oculata* from the Moroccan coast (El Jadida). *Nannochloropsis oculata* is a unicellular small green alga belonging to the Eutigmatophyceae. We chose this microalga for our study, based on previous studies the proved its efficiency as a fertilizer. After maceration with solvents with increasing polarity n-hexane, ether, chloroform and water the extracts were tested for their stimulating activity on *Cucurbita moschata* seed germination. After these preliminary tests, we selected the n-hexane extract of this microalga for fractionation, where we collected eight fractions per gradient of Dichloromethane/Ethyl acetate/Methanol. The resulting fractions of the n-hexane extract were then tested for stimulating activity on Cucurbita moschata growth parameters such as length, weight and number of plants. The overall results show that the fractions from n-hexane extract of *Nannochloropsis oculata* has important fertilizing activity on the studied crop.

Keywords:*Nannochloropsis oculata, extraction, fractionation, Cucurbita moschata, germination, bio fertilizer.*

Introduction

The transition to a more sustainable food production system is becoming increasingly necessary in light of the growing global population, the resource scarcity, and environmental protection. To increase agricultural yields, boost nutrient use efficiency, and reduce nutrient losses, innovative nutrient recycling technologies, green fertilizers, and advanced cultivation practices must be implemented [1-2]. Biofertilizers are gaining traction in sustainable agriculture as a means of increasing crop production while reducing the polluting effects of conventional fertilizers in an environmentally friendly and economically viable manner [3]. Recent studies show the use of green microalgae as a biofertilizer, as well as their function in soil fertility, seed germination, plant growth, nutritional value, and crop yield [4-5]. Microalgae have the potential to prevent nutrient losses through a gradual release of N, P and K, as an organic fertilizer, which is attained for the plant requirements [6]. Because of their environmentally friendly properties, these marine-based resources can be used as biofertilizers. Since they are fuel independent, cost efficient, and readily available, algal bio-fertilizers would be the best alternative to nitrogenous chemical fertilizers. Microalgae are the cheapest and most effective source of N_2 that does not pollute the soil or water [7-8]. Microalgae constitute an important source for compounds with a wide range of applications that can be harnessed for commercial use, or comprise of three major macromolecules namely carbohydrate, protein, lipid, and therefore they have long been employed as a promising biomaterial for the production of various industrially important co-products. Based on the processing methods, different products could be obtained from the microalgal biomass. It is essential to provide a comprehensive view on utilization of algae in various sectors like food, pharmaceutic, agriculture, fuel and environment [9-11]. Application of microalgae extract as an organic biostimulant is fast becoming an accepted practice in horticulture. Microalgae are reported effective fertilizer in many corps including vegetables, trees, flowering plants and grain corps [12]. Additionally, the utilization of microalgae as a liquid biofertilizer has been shown to increase germination rate and plant height of plants [13]. Furthermore, Microalgal biomass is a rich source of macro elements in the agriculture, such as nitrogen, phosphorus, potassium, calcium that implemented for improving vegetative growth and yield, it was highlighted it could be used as a source for good green agriculture practice [14]. In the present short communication, we screened the n-hexane extract fractions of Moroccan marine microalgae *Nannochloropsis oculata* for the activity of germination of *Cucurbita moschata* seed.

The aim of this study was to assess the potential of this microalga as an organic slow-release fertilizer for this type of pumpkin crop.

Materials and Methods

Microalgae culture conditions

Nannochloropsis oculata microalga was grown in sterile natural seawater enriched with F/2 medium nutrients [15]. Batch cultures were performed in 5L flasks agitated by air bubbling at 25 °C, under continuous illumination with 150 µmol m^{-2} s^{-1}. The growth was monitored by measuring the optical density at 680 nm, every five days, using F/2 medium as the blank. The biomass of each strain was harvested at the stationary phase by centrifugation at (1000 RCF for 10 min) and then the wet biomass was freeze-dried and stored at -20° until extraction [16].

Extract preparation

In contrast to terrestrial crops, extracting useful compounds from microalgae is difficult since their cell walls can be made up of many layers that provide structural rigidity. Cell walls can be composed of several polysaccharide layers, including sporopollenin, which makes the cells extremely resistant to extraction [17]. The extracts of microalgae *Nannochloropsis oculata* obtained from the maceration extraction successively with solvents of increasing polarity: hexane (H), ether (E), chloroform (C) and water (W) [18].

Fractionation and biological screening

Fractionation the extracts of the *Nannochloropsis oculata* microalgae was done on an open silica gel column with gradient solvent of Dichloromethane/Ethyl acetate/Methanol. Fractions obtained were tested for the next two biological activities.

* Plant growth and yield

The various tests were carried out in situ and in triplicates for *Cucurbita moschata* seeds, in the region of Ouled Said – Settat (Morocco). Plus, the control, the extracts were added with the seedlings at a percentage of 50% (v / v).

The plant growth (length, fresh weight and dry weight of root and shoot/plant) and yield (flower/plant; fruit/plant) were taken after termination of experiments. Shoot length was taken from the point of emergence of the root to the shoot apex. While root length was recorded from root emergence to longest root and both were recorded in centimeter (cm). Fresh weight of roots and shoots were recorded in gram (g). After taking fresh weight, roots and shoots were dried in a hot air oven at 80°C for 48 hour and their dry weights were recorded.

Results and discussion

Microalgae extracts

Firstly, four extracts H, E, C and W were obtained from successive Soxhlet extractions with solvents of increasing polarity: Hexane, Ether, Chloroform and Water. Once the extracts were obtained, we determined their color and yield relative to the initial amount of dry microalgae. Data for samples obtained are given in the table 1. , the yield values of hexane, ether, chloroform and water were, respectively 42.34% , 5.92 %, 1.43 % , 5.76% , with the highest value belonging to the hexane extract with a percentage of 42.34. The different extracts of *Nannochloropsis oculata* microalgae, were further screened for their growth-promoting effects on *Cucurbita moschata*, the data in table 2 showed that the extracts had variable effects (both negative and positive) on the root and the shoot elongation and weight. Overall, treatment by the hexane extract resulted in maximum increase in all the studied parameters in comparison with untreated control, with shoot length of 270.1 cm and root length of 64.5 cm. The fresh weights had also noticeably increased to reach 288 g and 70 g for the plants shoot and root. The dry weights have known a slight rise, from 8g to 10.2 g in the roots dry weight and from 30.2 g to 35.9 g in shoots dry weight. Based on these results and the ones from table 1 we chose to proceed in the fractionation and screening of the hexane extract of the *Nannochloropsis oculata* microalga.

Fractionation and biological screening

The fractionation of the n-hexane extract has given the results presented in table 3. The eight fractions E1, E2, E3, E4, E5, E6, E7, and E8 are listed along with their colors and yields which are, respectively 2.30%, 12.26%, 1.49%, 4.29%, 3.44%, 24.00%, 20.56%, 8.31%. The effect of the n-hexane fractions on the different growth parameters is shown in Table 4. In comparison with the control, the fractions showed positive and negative results. The fractions E1 and E2 had positive effects on all the studied parameters, where the highest values occurred for plants treated with fraction E1. The other fractions didn't have a significant effect on the parameters. E5 didn't affect the values of the parameters where they stayed the same as the control. The treatment by the E3 had the most significant decrease in the growth parameters.

Effect of microalgal biomass on Cucurbita moschata plant performance

* The plant's length

The faster plant growth is confirmed by comparison with the control plant which in this case belonged to the E2 fraction with a shoot length of (266.1±12.9 cm) and root length of (57.5±1.3 cm).

* Dry and fresh weight

The graphs in figure 2 represents the effects of the different fractions of the n-hexane extract on the dry and fresh weight of the root and shoot of the studied plant. As the figure, shows the two fractions E1 and E2 were the ones to actually affect the weights of the plants .If we compare the control plant weights with the weight of the plants treated with these two fractions we could notice an increase of more than 20% in the root and shoot weight.

* Yield

As the graphs in figure 3 shows, the highest yield belonged to the plants treated with the E2 fraction.

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There was no significant difference in the final yield for the other fractions treatment.

Conclusion

Algal biofertilizers offer additional benefits such as biocontrol of plant pathogens, reduced use of chemicals and minimized greenhouse gas emissions, besides their use as nutrient supplements. This study reveals that the use of the n-hexane extract of the *Nannochloropsis oculata* microalgae, to some extent can increase the growth and production of the pumpkin crop *Cucurbita moschata*. Growth parameter of this crop increased with the application of E1 and E2 extracts of the studied microalgae.

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Table 1. The different extracts of *Nannochloropsis oculata* with yield and color.

Extract	Color	Yield (%)
H	Green - black	42.34
E	Green – yellow	5.92
C	Green	1.43
W	Green - dark	5.76
Marc (*)	White	44.4

(*) (After evaporation)

Table 2. Effects of the different extracts of *Nannochloropsis oculata* microalgae on the growth parameters of *Cucurbita moschata.*

Extract	Length (cm)		Fresh weight (g)		Dry weight (g)		Number/Plant	
	Root	shoot	Root	shoot	Root	shoot	Root	shoot
H	64.5±1.8	270.1±10.5	70.7±2.1	288.6±9.9	10.2±0.8	35.9±1.4	26.5±1.2	23.1±0.9
E	46.5±1.2	204.4±12.9	60.3±2.1	217.5±10.0	7.1±0.1	29.2±1.3	27.1±1.4	23.1±0.9
C	54.9±1.3	206.4±12.9	59.7±1.8	209.1±10.1	8.5±0.2	30.1±1.4	26.1±1.2	23.1±0.9
W	57.4±1.2	265.5±12.9	61.9±1.9	210.6±8.7	8.2±0.6	31.2±1.1	25.8±1.1	23.1±0.9
Control	57.5±1.3	266.1±12.9	61.4±2.8	208.4±12.7	8.1±0.6	30.2±1.7	26.4±1.3	23.1±0.9

Table 3. Fractionation n-hexane extracts of *Nannochloropsis oculata* microalgae**.**

Fraction	color	Yield (%)
E1	Yellow	2.30
E2	Black green	12.26
E3	Black green	1.49
E4	Yellow green	4.29
E5	Green	3.44
E6	Yellow green	24.00
E7	Black green	20.56
E8	Black green	8.31

Table 4. Effects of the different fractions of the n-hexane extract on the growth parameters of *Cucurbita moschata*.

Extract	Length (cm)		Fresh weight (g)		Dry weight (g)		Number/Plant	
	Root	shoot	Root	shoot	Root	shoot	Root	shoot
E1	64.5±1.8	270.1±10.5	70.7±2.1	288.6±9.9	10.2±0.8	35.9±1.4	26.5±1.2	23.1±0.9
E2	70.5±1.	280.1±11.5	74.3±1.8	298.1±4.9	11.2±0.8	38.2±1.3	30.5±1.5	25.1±1.2
E3	44.3±1.3	200.1±10.8	58.3±1.8	206.2±9.5	6.3±0.1	27.4±1.5	26.0±1.1	20.1±0.7
E4	57.5±1.3	265.1±11.5	60.3±2.2	207.6±11.4	8.0±0.7	31.1±1.7	25.9±1.4	23.4±0.8
E5	55.4±1.5	266.1±12.9	61.4±2.8	208.4±12.7	8.1±0.6	30.2±1.7	26.4±1.3	23.1±0.9
E6	46.5±1.2	204.4±12.9	60.3±2.1	217.5±10.0	7.1±0.1	29.2±1.3	27.1±1.4	22.2±0.8
E7	54.9±1.3	206.4±12.9	59.7±1.8	209.1±10.1	8.5±0.2	30.1±1.4	26.1±1.2	23.4±0.7
E8	57.4±1.2	265.5±12.9	61.9±1.9	210.6±8.7	8.2±0.6	31.2±1.1	25.8±1.1	22.9±0.8
Control	57.5±1.3	266.1±12.9	61.4±2.8	208.4±12.7	8.1±0.6	30.2±1.7	26.4±1.3	23.1±0.9

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Figure 1. Effects of the different fractions the n-hexane extract on the root and shoot length (cm).

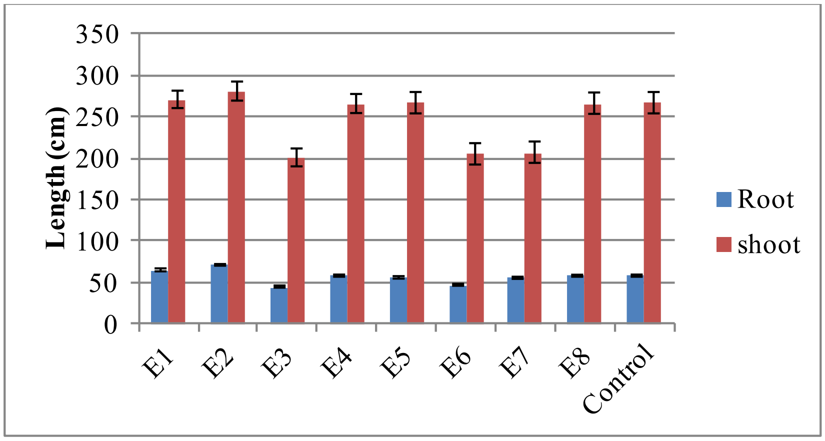


Figure 2. Effects of the different fractions the n-hexane extract on dry and fresh weight of plants (g).

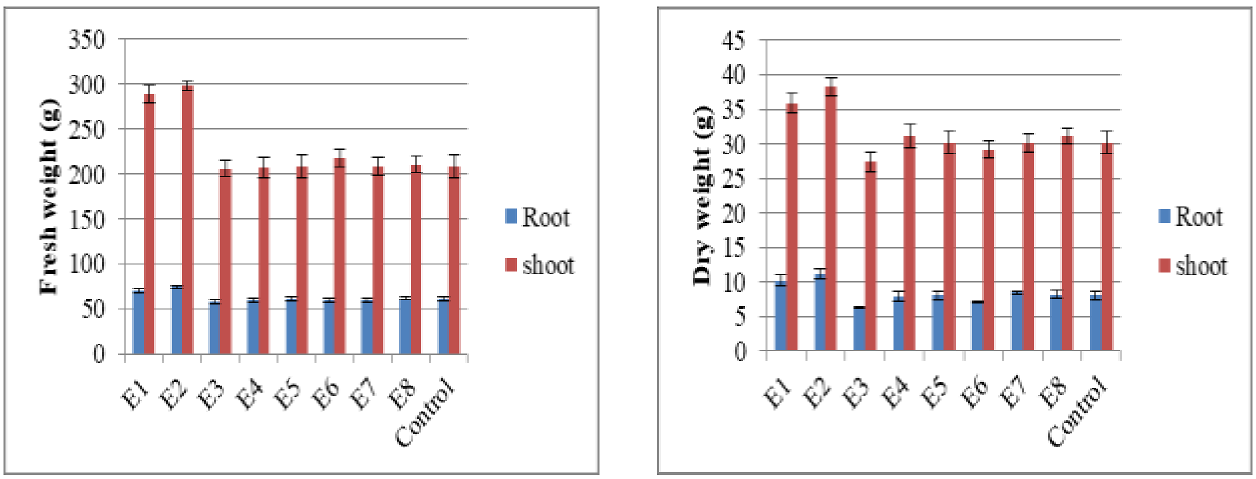
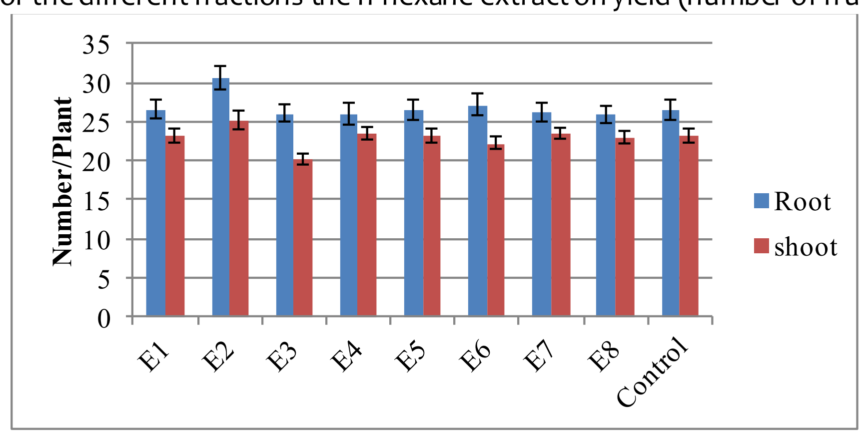


Figure 3. Effects of the different fractions the n-hexane extract on yield (number of fruit or flower/plant).



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