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RESPONSE OF PARSLEY *(PETROSELINUM CRISPUM)* TO MICROALGAE *CHLORELLA VULGARIS* AND *ANABAENA SPHAERICA* AS BIO-FERTILIZERS

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Abstract

The current problems of mineral fertilizers in agricultural production were adverse impact of their production and use on environment, resulted in serious problems in the soil and contaminate the underground water. Many solutions were suggested to reduce the previous problem; one of them is using the algal biomass as bio fertilizers. This study aimed to investigate the effects of algal strain, concentration and their application methods on growth, macronutrients and heavy metals of parsley *(Petroselinum crispum)*. Pot experiment was carried out in the greenhouse of National Research Centre, Egypt. Algal strains used in this study were: *Chlorella vulgaris* and *Anabaena sphaerica*, with two concentration (1% and 2%) and two different application methods (soil and foliar application). Soil samples were collected from El-Gabal El-Asfar. This soil contaminated with sewage sludge. The obtained results showed that all treatments with algae had positive effect on fresh and dry weight of parsley plants as compared with control, indicating that nutrients and extra cellar substances excreted by algae had beneficial effect on plant growth. *Anabaena sphaerica* at concentration 2% gave the highest dry weight, N and P content of parsley plant under foliar application. Potassium had a different direction as it gave both types of algae at 2% concentration the highest content of K in parsley with soil application. *Anabaena sphaerica* gave better results for parsley growth and (N, P and K) contents than *Chlorella vulgaris*. Also, all bio-fertilizers treatment reduced heavy metals contents in parsley plants. Such results indicated that algae can be used to improve soil fertility. Collectively the results indicate the possibility of at least partial replacement of chemical fertilizer with bio fertilizers from microalgae biomass field growing of parsley.

Key words: Bio-fertilizers- *Chlorella vulgaris*- *Anabaena sphaerica* - algae - Parsley - growth and quality.

Introduction

High yields ensured by modern intensive agriculture technologies are achieved through extensive use of mineral fertilizers, especially nitrogen fertilizer are very damaging to environment. Bio-fertilizers are gaining significance in sustainable agriculture as a means of enhancing crop productivity, in an environmentally friendly and economically viable manner and reducing the polluting effect of synthetic fertilizers (Singh *et al.,* 2011a, 2011b). Both green algae and cyanobacteria are involved in the production of metabolites such as growth hormones, polysaccharides, antimicrobial compounds, etc., which play an important role in the colonization of plants (Nirmal *et al.,* 2018, Abd El-Aty *et al.,* 2014). The involvement of cyanobacteria and eukaryotic green microalgae in the mineralization, mobilization of organic and inorganic, major and micronutrients, production of bioactive compounds, (polysaccharides, growth hormones, antimicrobial compounds, etc.) can improve the plant growth and thus makes them suitable as bio- fertilizing options (Gayathri *et al.,* 2015; Hernández-Carlos and Gamboa-Angulo, 2011). Yehia I.A u Seif *et al.,* (2016) found that, all foliar application with algal extracts significantly increased all the studied parameter (chlorophyll, N, P and K contents of leaves and seed yield compared with control treatment. Salwan and Hamza (2020) showed that the combination of fungi with algae was effective and provoked carbon decomposition and soil aggregates formation by lowering pH and boosting bacterial biomass C as well as roots density which can be beneficial to soil ecology through the enhancement of the soil bio-characteristics and the potential for the corn growth promotion in the arid saline soil.

Vegetative growth of olive transplants was markedly enhanced as their root zoon was surrounded by pre-digested *Scenedesmus* bulk at the concentration equal to the recommended nitrogen dose. Nutrient concentrations and balance were also improved by the partial substitution of nitrogen by algal bulk (Abdel-Maguid *et al.,* 2004). Also freshwater microalgae such as *Chlorella vulgaris* have been shown to provide high amounts of macro- and micronutrients and proteins (Elarroussia *et al.,* 2016) and growth promoting factors, such as cytokines (Stirk *et al.,* 2002). Another work of Grzzesik *et al*., (2017) indicated that foliar application with *chlorlla* sp. caused an increase in growth and physiological performance of willow plants. Shaaban *et al*., (2010) showed that the best uptake, nutrient balance and dry matter accumulation was recorded with combined algal extract and micronutrient fertilizer treatment.

Moreover, Zaccaro *et al.,* (2001) reported that, foliar application of biochemical organic substances, which supply macro and micro nutrients, of increased demand. Fresh water algae contain high percentage of macro and micronutrients bounded in their major biochemical constituents and metabolites such as carbohydrates and proteins (Wake *et al.,* 1992).

Some algal strain show a high capacity for accumulation of heavy metals as results of tolerance mechanisms and many algae synthesize phytochelatins and metallothioneins that can form complexes with heavy metals and translocate them into vacuoles (Suresh, 2004) Recently, the use of aquatic plants especially micro and macro algae has received much attention due to their ability to absorption of metals and taking up toxic elements from the environment (Abd El-Aty *et al.,* 2013, Radwan *et al.,* 2020 and Matagi *et al.,* 1998). Similarly, Gougoulias *et al.,* (2018) revealed that the carbon mineralization maximised especially with a decline of the soil pH following algal biomass combinations in the soil. Faheed and Abd-El Fattah (2008) observed increases in germination, fresh and dry weights and pigment content of *Lactuca sativa* seedlings treated with *Chlorella vulgaris*. Recently, the use of aquatic plants especially micro and macro algae has received much attention due to their ability to absorption of metals and taking up toxic elements from the environment or rendering them less harmful (Matagi *et al.,* 2012).

Parsley *(Petroselinum crispum)* is herb belonging to the Apiaceae (formerly Umbelliferae) family. It is native to the Mediterranean region where it is found in the wild form. It is mostly grown outdoors and is sea son ally harvested (Navazio, 2012). Parsley is a leafy vegetable, rich in many biologically active compounds. Parsley *(Petroselinum crispum Mill)* contains flavonoids *(apiin and luteoli*n) and essential oils (apiol and myristicin) that are responsible for the medical uses (Pino and Fuentes, 1997). Some people take parsley by mouth for bladder infections (UTIs), kidney stones (nephrolithiasis), gastrointestinal (GI) disorders, constipation, diabetes, cough, asthma and high blood pressure. Some people apply parsley directly to the skin for dark patches on the face, cracked or chapped skin, bruises, tumors, insect bites and to stimulate hair growth. In foods and beverages, parsley is widely used as a garnish, condiment, food and flavoring. In manufacturing, parsley seed oil is used as a fragrance in soaps, cosmetics and perfumes. (Najla and Murshed, 2012). Parsley can also provide dietary sources of calcium (Ca), potassium (K), phosphorus (P), magnesium (Mg) and iron (Fe), as well as vitamin A, vitamin C and carotenoids (Mangels *et al.,* 1993; Pennington and Church, 1985).

Parsley may help to protect against cancer, diabetes and bone weakness. One cup of chopped parsley provides 1.230 percent of an individual's daily recommended vitamin K intake.

This work aimed to study the effect of different algal biomass (bio-fertilizer) as soil and foliar additive on growth, yield quality and some nutrient uptake of parsley plant and determine the ability of algae to reduce the accumulation of heavy metals in parsley plant.

Materials and Methods

Soil samples were collected from El-Gabal El-Asfar, Egypt. This soil contaminated with sewage sludge. Particle size distribution and soil texture along with soil moisture constants of the representative soil samples were determined according to Klute*,* (1986). Contents of organic matter and $CaCO_3$ as well as EC and pH were evaluated according to Black *et al.,* (1982). Total N and available P, K, total Cu, Pb, Cd and available Cu, Pb and Cd were also determined according to Jackson (1973). Some physical and chemical characterizations of the used soil were documented in Mahmoud *et al.,* 2019.

Isolation, Purification and culture condition of algal species

Anabaena sphaerica, N_2-fixing *Cyanobacterial* species and *Chlorella vulgaris*, green alga were isolated from phytoplankton community of the River Nile*,* purified and recultivated in a fresh algal nutrient medium BG11 (Carmichael, 1986). *Chlorella vulgaris* isolated in 100 % $NaNO_3$ (1.5 g/l), where $NaNO_3$ was excluded completely from *Anabaena sphaerica* media (Abd El-Aty, *et al.,* 2015).

The cultures were incubated at 24 ± 2 and 30 ± 2°C for *Chlorella vulgaris* and *Anabaena sphaerica* under continuous white fluorescent illumination (33.3 E/m^2/s). Cultures were shaken once daily to prevent clumping of algal cells.

Cultivation was carried out in sterilized conical flasks. The cultivation time differed from one strain to another depending on the optimum growth rate till reaching stationary phase which always ranged between (10-15) days.

Preparation of biomass for extract

At maximum growth, the algae were harvested by centrifugation at 3000 rpm for 15 min. Then, after removal of clear liquid, the pellet of algae was washed several times by distilled water till the effluents became almost transparent. The washed biomass was then dried in an oven at 40^{o}C until a constant weight was reached. The dried biomass was then ground into fractions. The algae were stored in an air tight container in a dry place. The preparation of algal extracts and the chemical composition of algal strain were previously described in Mahmoud *et al.,* 2019.

Pot experiments were conducted in the greenhouse of National Research Centre (NRC), Dokki, Giza, Egypt, to study the effect of algae (*Chlorolla vulgaris* and *Anabaena sphaerica*), on growth, yield quality and some nutrient uptake of parsley plant and determine the ability of algae to reduce the accumulation of heavy metals in parsley plant. Two algal concentrations were used (C1=1% and C2= 2%) and two methods of algal application (foliar and soil application).

The treatments were arranged in a randomized complete block design factorial, with two factors and three replications. Factors of the experiment were as follows: (A): algal strain and (B) method of application. Used plastic pots, 5kg capacity of air dried soil ten seeds of parsley *(Petroselinum crispum)* were sown in each pot by pressing them into soil to depth of 0.5 cm and the following treatments were applied: 1-Control, 2- *Chlorolla vulgaris* 1% as foliar application 3- *Chlorolla vulgaris* 2% as foliar application 4- *Anabena sphaerica* 1% as foliar application, 5- *Anabena sphaerica* 2% as foliar application, 6- *Chlorolla vulgaris* 1% as soil application, 7- *Chlorolla vulgaris* 2% as soil application 8- *Anabena sphaerica* 1% as soil application and 9- *Anabena sphaerica* 2% as soil application.

The pots were watered daily to 70% of the water holding capacity and then thinned out to 5 seedlings per pot after 10 days then treated by algae and allowed to grow for a period of 75 days.

The basic amounts of mineral fertilizers were applied (20 kg fed^{-1} ammonium sulphate, 100 kg fed^{-1} super phosphate and 50 kg fed^{-1} potassium sulphate) for each pots.

At harvesting, plants were carefully removed, washed with tap water, to remove any attached particles, rinsed twice with distilled water, fresh and dry weight were record. Nitrogen, phosphorus, potassium and trace elements (Cd, Pb, Cu) in plant were analyzed. All the analysis were determined using standard methods by Jackson (1973), Lindsay and Norvell (1978). Statistical analyses were conducted as described by Snedecor and Cochran (1982).

Results and Discussion

The effects of algal strain, its concentration and its application methods on fresh and dry weight of parsley are shown in table 1. The weight of fresh and dried parsley plants was increased using different algal strains at two concentrations and two methods of application. All addition of algae led to a positive effect on the fresh and dry weight of parsley plant.

Data indicated that soil application of *Chlorella vulgaris* caused positive effect of parsley fresh and dry weight compared with foliar application because the *Chlorella vulgaris* of high content of protein, amino acids, antioxidants, micro and macro elements and etc. (Stirk *et al.,* 2002). Its application has beneficial effect on plants growth and yield, the rate of increase in dry matter of parsely plants is 13% and 22% under soil application at C1 and C2 respectively compared with foliar application with *Chlorella vulgaris*. May be application of *Chlorella* biomass stimulates actual nitrogen fixation in the soil and caused a decrease in actual denitrification of the soil under parsley plant as well as increases the pool of bioavailability nitrogen in soil and increase the yield this result confirm with Kublnovskaya *et al.,* 2019, while using *Anabaena sphaerica* as foliar application gave highest fresh and dry weight.

Anabaena sphaerica gave the highest dry weight of parsley plant under foliar application at Cl concentration

Table 1: Effect of species, concentration and methods of addition algae on fresh and dry weight of the parsley plants.

Treatments	Weight of parsley plants (g)							
	Chlorella vulgaris				Anabaena sphaerica			
	Fresh		Dry		Fresh		Dry	
	C1	C2	C1	C2	C1	C2	C1	C2
Control	11.05	11.05	5.70	5.70	11.05	11.05	5.70	5.70
Foliar application	14.13	13.64	8.69	7.66	22.00	21.80	11.1	10.19
Soil application	16.27	17.65	9.90	9.40	16.73	16.80	9.50	8.10

recording an increase of 16.8% compared with soil application at the same concentration, while soil application gives the highest increase when using Chlorella vulgaris by concentration Cl recording 22.7% compared with foliar application. These results are harmony with Mahmoud *et al.,* 2019 who found that the highest result of spinach plants were found at Anabaena sphaerica under foliar application with extract concentrate 1%. Also, these results were in agreement with findings of Rajaa Kholssi *et al.,* (2018) who stated that treatments with filtrate of Chlorella soroiniana improved the total biomass by 22% and 51% as compared to control, indicating that nutrients and extra-cellular substances excreted by algae were pertinent to the beneficial effects on plant growth. These results are in harmony with those obtained by Brahmbhatt and Kalasariya (2015) they reported that addition of all algal extracts can enhance plant growth of radical.

Data in Fig. 1 pointed out that, *Anabaena sphaerica* gave the highest rate increase of dry weight of parsley under foliar application at concentration 1% followed by concentration 2% while under soil application, *Chlorella vulgaris* gave the highest rate of increase at concentration 1% followed by concentration 2%. These results are agree with Mahmoud *et al.,* 2019 who revealed that application of algal extracts enhanced the growth of spinach plants. The data showed little difference between C1 and C2 in dry weight under two strains of algae.

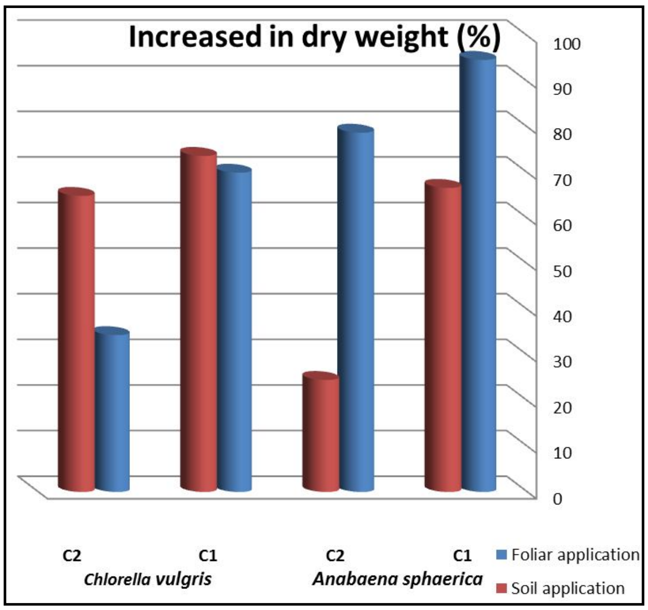

Fig. 1: Effect of algal species, concentration and method of application on the rate of increase in dry weight percentage of parsley plants compared with control.

Effect of the algal strain and methods of application on macronutrients (N, P and K) concentration (%) in parsley plants shown in the table 2. The data indicated that *Anabaena sphaerica* treatment gave the high content of N and P in parsley plant at C1 with foliar application while, *Chlorella vulgaris* treatment gave the high content of N and P in parsley plant at C2 with soil and foliar application (32.1-35.7 mg/pot). Potassium had a different direction as it gave both types of algae at C2 concentration the highest content of K in parsley with soil application. Similar trend was found by Adam (1999) who showed that the improvement of the nitrogen contents was observed in response to application of cyanobacteria as bio-fertilizers on seed and related processes of wheat, sorghum, maize and lentil. Ghallab and Salem (2011) found that the bio-fertilizers increased nutrients content in wheat plant. Also, Mahmoud *et al*., 2019 revealed that *Anabaena sphaerica* strain gave better results for spinach growth, macronutrients and protein contents than *Chlorella vulgaris* strain. The use of microalgae biomass as fertilizer has a number of advantages, as effect on phosphours content,the results indicated all treatment with algae effect on P content especially when use *Chlorella* sp. at C2 under foliar application. Therefore the rate of release of bio-available phosphorus from microalgae cells in the soil is closer to the rate of its uptake by plants, which allows for a more complete uptake of the phosphorus applied to the soil reducing the unproductive losses this element and the risk of eutrophication (Mulbry *et al.,* 2005).

Data in Figs. 2, 3 and 4 shows that N, P and K uptake in parsley plants under two strains of algae (*Anabaena sphaerica* and *Chlorella vulgaris*) by different method application all nutrients uptake of parsley treated with

Table 2: Effect of algal species, concentration and method of addition on macronutrients concentration (%) in parsley plants.

Treatments	Macronutients concentration (%)											
	Chlorella vulgaris						Anabaena sphaerica					
	N%		P%		K%		N%		P%		K%	
	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2
control	00.46	00.46	00.22	00.22	10.40	10.40	00.46	00.46	00.22	00.22	10.40	10.40
Foliar application	10.85	20.86	00.22	00.42	10.70	20.70	30.23	20.16	00.30	00.19	20.30	30.40
Soil application	20.62	20.31	00.30	00.38	10.50	30.10	20.46	20.16	00.19	00.16	30.10	40.40

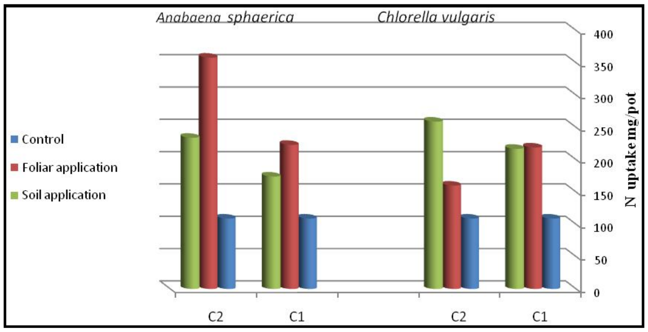

Fig. 4: Effect of algae species, concentration and method of application on K uptake in parsley plants.

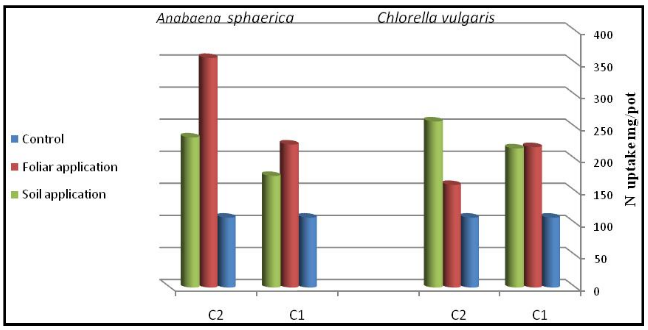

Fig. 2: Effect of algal species, concentration and method of application on N uptake in parsley plants.

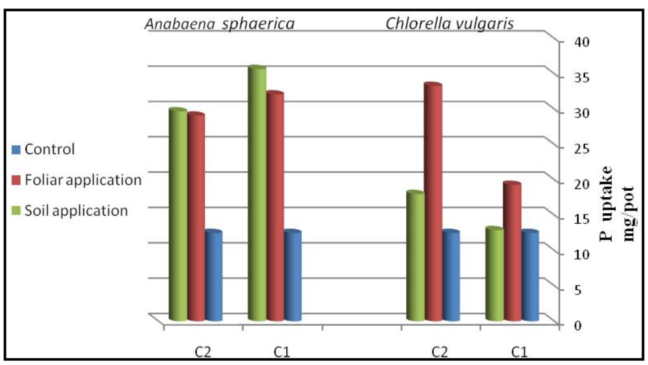

Fig. 3: Effect of algae species, concentration and method of application on P uptake in parsley plants.

algae were increased compared to control according this results showed the inoculation of algae increase the availability of nutrient in soil and their translocation in plant (Coppens *et al.,* 2016) and Abu Seifa *et al.,* (2016) showed that, all foliar application with algal exracts significantly increase all studied parameter (cholophyll, N, P and K) content of leaves. The highest uptake was recorded when *Anabaena sphaerica* added with concentration C1 by foliar application for N uptake, *Chlorella vulgaris* added with C2 concentration by soil application recorded the highest uptake of P uptake and when *Anabaena sphaerica* added with C2 concentration by foliar application for K uptake. Although the highest increase of P and K uptake was observed when *Anabaena sphaerica* added at extract concentrated C1 by foliar application. Similar trend was found by this corresponds to (Bloemberg *et al.,* 2000) who reported that microbiological fertilizers are important to environment friendly stainable agricultural practices. (Goel *et al.,* 1999) found that the bio-fertilizers include mainly the nitrogen fixing, phosphate solubilizing and plant growth promoting microorganisms.

Also, Mahmoud *et al.,* 2019 reported that *Anabaena sphaerica* strain gave better results for spinach growth, macronutrients and protein contents than *Chlorella vulgaris* strain.

Data in table 3 demonstrate of two concentrations from different algae strains, by different method application and effect on the heavy metal content in parsley. It clears that all treatments reduced the heavy metal content compared with control. *Chlorella vulgaris* gave the best results where it gave the largest rate of reduce in relation to Pb and Cu while *Anabaena sphaerica* gave the largest percentage of reduced for pb may be the cyanobacterial species such as *Anabaena* have the ability to form side pores for chelating micronutrient. These results are agree with Ali *et al.,* (2009) they found that foliar application more enhanced in reduced of heavy metals concentration in plant when used *Anabaena sphaerica* compared soil application while *Chlorella vulgaris* has positive effect in soil applied. Also, Mahmoud *et al*., 2019 reported that all bio-fertilizers treatment reduced heavy metals contents in spinach plants.

Conclusions

According to the results obtained in this study, it

Table 3: Effect of algal species, concentration and method of addition on heavy metals content (ppm) in parsley plants.

Treatments	Heavy metal content (ppm)											
	Chlorella vulgaris						Anabaena sphaerica					
	Cd		Pb		Cu		Cd		Pb		Cu	
	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2
control	2.7	2.7	1.48	1.48	9.9	9.9	2.7	2.7	1.48	1.48	9.9	9.9
Foliar application	0.85	1.02	0.96	1.16	8.0	8.9	2.1	2.3	0.52	0.56	9.7	9.2
Soil application	1.02	1.53	1.12	1.36	7.0	7.2	2.3	3.6	0.56	0.59	9.4	9.1

may be concluded that:

· Strong stimulating effect on growth was recorded in the treatments of plants with *Chlorella vulgaris* and *Anabaena sphaerica*.

· *Anabaena sphaerica* strain gave better results for parsely growth and (N, P and K) contents than *Chlorella vulgaris* strain.

It is recommended to farmers that organic fertilizer such *Chlorella vulgaris* and *Anabaena sphaerica* should be included in technology for planting parsely.

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