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Effect of Plant Biostimulants on Growth, Yield and Some Mineral Composition of Broccoli Plants (Brassica oleracea var. Italica)

ABSTRACT

By offering biostimulants as substitutes for conventional chemical fertilization, increasing consumer awareness has helped to raise issues related to food safety and the environment. Creating a suitable plan for the deployment of conventional chemical fertilization alternatives in agriculture. So, this investigation was conducted at the College of Agricultural Engineering Sciences, University of Sulaimani, Kurdistan Region-Iraq to study the effect of two plant biostimulants, algae extract (Algaton-20) and plant bio-cozyme, on broccoli which was under greenhouse conditions. After two weeks from transplanting the biostimulants were applied through soil at different concentrations (0 ml.L-1, 1ml.L-1 and 2 ml.L-1). The experiment was laid out according to Randomized Complete Block Design (RCBD) with three replications. The results revealed that algae extract and bio-cozyme at 2 ml.L-1 gave the maximum number lateral head, lateral head weight, plant yield, total marketable yield, phosphorus and iron. The interaction effect of algae extracts and bio-cozyme showed that number of leaves per plant and head diameter at 1 ml.L-1 algae extract and bio-cozyme, percentage of dry matter at 1 ml.L-1 algae extract without bio-cozyme, content of chlorophyll in leaves and number lateral head at 1 ml.L-1 algae extract and 2 ml.L-1 bio-cozyme, and head diameter at 1 ml.L-1 of both algae extract and bio-cozyme were the best. Also, main head weight, lateral head weight, plant yield, total marketable yield and iron (674.46 g, 208.58 g, 883.04 g, 42.05 ton.ha-1, 5.36 ppm, respectively) were the highest at 2 ml.L-1 combination of the two biostimulants. Moreover, nitrate and potassium (134.67 ppm, 336.13 ppm, respectively) were maximum at 2 ml.L-1 algae extract and 1 ml.L-1 bio-cozyme. Individually, 2 ml.L-1 of the two biostimulants were the best concentrations, but there was no a big difference between 1 and 2 ml.L-1 of the two biostimulants when they interacted together in most study measurements.

INTRODUCTION

Broccoli (*Brassica Oleracea* var. *Italica*) is an economical vegetable crop which belongs to *Brassicacea* family (Abou El-Magd *et al.* 2006; Branham and Farnham, 2018). It is well-known by phytochemicals and its highly valued by consumers, which have health-promoting and chemo-preventive effects (Latte *et al.* 2011). Some bioactive compounds have been found in Broccoli, especially glucosinolates and polyphenols (Valverde *et al.* 2015). They counteract cancer and other diseases in an effective way (Ordiales *et al.* 2017). Moreover, this crop is rich in minerals (Fe, Zn,

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Ca and Mg) and vitamins (E, A, B1, B2, B5 and B6) (Weber 2017). Thus, broccoli serves as a good healthy food (Rodriguez Casado 2016).

There is raising demand in the agricultural sector for new biostimulant products and a lot of researches are fulfilled in this industry section. Plant biostimulants, including different substances and microorganisms that enhance plant characteristics, such as growth, abiotic stress tolerance, efficiency nutrition and/or crop quality contents (Du Jardin 2015). Application of biostimulants could be regarded as a potent and sustainable nutritional crop supplementation which may mitigate the environmental problems arise from excessive fertilization (Halpern *et al*. 2015). Additionally, biostimulant compounds may also have a positive impact on biological and physical soil structure (Calvo *et al*. 2014). Biostimulants impact on nutritional uptake from the soil, physiological and plants' metabolic processes. Therefore, they affect yield production and resistance of plants to biotic and abiotic stress factors (Ertani *et al*. 2009). The application of biostimulants is beneficial to increase production of antioxidants in plants which decreasing sensitivity to stress conditions (Ertani *et al.* 2011).

There are many commercially available products which are currently applied on different crops in sustainable and organic agriculture (De Pascale *et al*. 2017). Essential biostimulants include protein based biostimulants and marine algae. Protein is a main constituent of biostimulants can be classified into two main groups such as amino acids origin from animal or plant source and protein hydrolysates consisting of a mixture of peptides (Calvo *et al.* 2014). The application of biostimulants consisting of amino acids and the combinations of seaweed extracts with amino acids enhances the nutritional uptake by plants from the soil (Nardi *et al.* 2016), improves sprouting (Carvalho *et al*. 2013), increasing vegetative growth (DongLin and Yasunori 2003), enhance the chlorophyll content (Thirumaran *et al*. 2009), as well as the amount of production (Shehata *et al.* 2011). In this regard, the goal of this study is to determine the effects of various doses of two commercial biostimulants, algae extract and plant bio-cozyme on yield and quality of broccoli plant.

MATERIALS AND METHODS

The experiment was conducted inside a plastic house at the research farm of the College of Agricultural Engineering Sciences, University of Sulaimani, Sulaymaniyah city, Kurdistan Region-Iraq, during the growing season of 2021/2022. The experiment was started by ploughing, rolling and then plotting the soil inside the plastic-house. Three bridges were made, an even drip irrigation system set on them, then they mulched with black polyethylene. The broccoli (Jassmine F1) seedlings were planted on 1st Nov. 2021 according to Randomized Complete Block Design (RCBD) with three replications per treatment. In each replication, six seedlings were planted on the bridges with 40 cm distance and the experimental unit area was (2.1 cm^2). The replications were divided and arranged on 9 treatments and their combinations two biostimulants, including algae extract (0 $ml.L^{-1}$, 1 $ml.L^{-1}$ and 2 $ml.L^{-1}$) and plant bio-cozyme (0 $ml.L^{-1}$, 1 $ml.L^{-1}$ and 2 $ml.L^{-1}$). The ingredients of them are shown in (Table 1). After two weeks from transplanting, the algae extract and bio-cozyme were applied and repeated four times with 15-day intervals. The main physical and chemical characteristics of the soil was analyzed (Table 2).

Table (1): algae extract and bio-cozyme are composed of different substances

Algae extract	Bio-cozyme
Total nitrogen (N): 7.68% W/V	Organic biostimulant %100 that enhances a plant's ability to absorb and utilize nutrients
Phosphorous pentoxide (P_2O_5) water-soluble: 3.84% W/V	
Potassium oxide (K_2O) water-soluble: 12.8% W/V	Derived from natural occurring cytokinin, hormones, enzyme, vitamin, amino acid and micronutrients
Molybdenum (Mo) water-soluble: 0.38% W/V	
Seaweed extract %20	

Table (2): The physical and chemical characteristics of the experiment soil location

Soil properties	Units	The values
Sand	g.kg^{-1}	430.69
Silt		251.56
Clay		317.75
Texture sandy clay loam		Sandy clay loam
EC	d.ms^{-1}	1.03
pH		7.68
Organic matter	g.kg^{-1}	30.01
Total nitrogen		10.17
Available phosphorus		0.04
Soluble potassium		0.07

Measurements

The studied parameters were the number of leaves per plant, plant height, head diameter, number lateral head, main head weight, plant yield and total marketable yield tons per hectare, and also some other chemical parameters including nitrate, phosphorus, potassium and irone content. Three plants from each experimental unit have been chosen. Moreover. the content of chlorophyll in leaves was detected by using a digital chlorophyll meter (SPAD 502 PLUS). In addition, to determine main head dry weight, they were kept in an oven at 65°C until the weight became constant, then percentage of the dry weight of main heads were calculated depending on the formula:

Percentage of dry matter (%) = head dry weight/head fresh weight ×100.

Nitrate analysis

Nitrate and the extraction process of the samples were carried out according to (Cataldo *et al.* 1975).

Mineral quantification

Phosphorus, potassium and iron were quantified according to (Elmer, 1996).

Statistical analysis

The measured data were analysis using two-way ANOVA-CRBD by computer XLSTAT software version 2019.2.2. Duncan's multiple ranges test at $P \leq 0.05$ was applied to compare the means.

RESULTS AND DISCUSSION

The results were shown in figure (1) confirmed that the two biostimulants, algae extract and bio-cozyme were not effective to induce plant height and the number of leaves per plant. Besides, effects of algae extract and bio-cozyme biostimulants individually resulted in different content of chlorophyll when they were applied to broccoli plants in comparison with control plants. Algae extract at 1 ml.L^{-1} rendered the densest (77.01 SPAD) chlorophyll to the leaves. In contrast, content of chlorophyll in leaves was the lowest (73.29 SPAD) in plants without algae extract (Figure 1A). Content of chlorophyll in leaves was also different with reference to application of bio-cozyme (Figure 1B). Increasing bio-cozyme concentration from 1 ml.L^{-1} to 2 ml.L^{-1} elevated content of chlorophyll in leaves, and 2 ml.L^{-1} bio-cozyme gave the best (77.81 SPAD) content of chlorophyll in leaves. The broccoli plants without bio-cozyme application and the ones with 1 ml.L^{-1} bio-cozyme application showed the lowest (74.41 and 74.87 SPAD, respectively) content of chlorophyll in leaves. Besides, the analyzed data confirmed that the two biostimulants, algae extract and bio-cozyme, were not effective to induce plant height and the number of leaves per plant. Similar plant height and the number of leaves per plant were observed among the plants were supplemented with the two biostimulants and those without supplementation. This is supported by Bulgari *et al.* (2015) who mentioned that the biostimulant application often increases color of leaves by stimulating chlorophyll content. Moreover, Sharma *et al.* (2014) observed that the plant responses to biostimulants treatment manifested in increasing chlorophyll content. Increasing chlorophyll might be due to that biostimulants contain cytokinin which enhance cell division and stimulate chlorophyll biosynthesis. Betaines, which are plant biostimulants, decelerated chlorophyll degradation and delay leaf senescence. Furthermore, Richardson and Simpson (2011) explained that chlorophyll

contents are crucial principle to determine the plant health because chlorophyll contents are directly associate with the physiological activities that manufacture food.

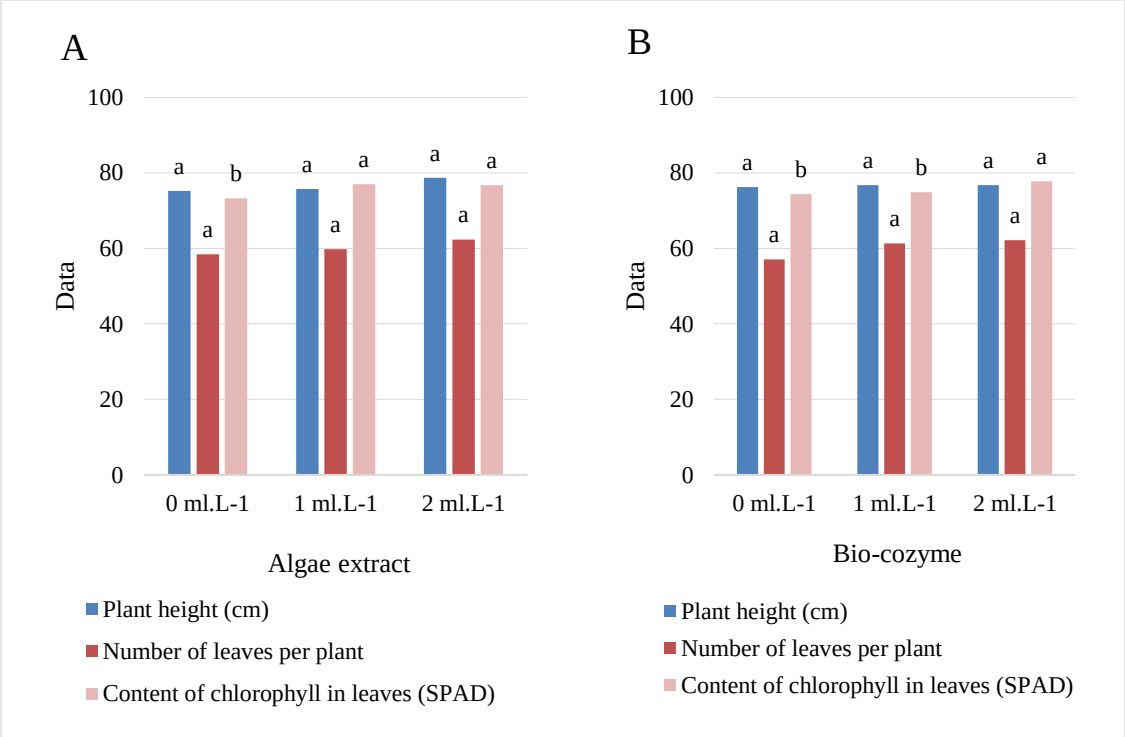

Figure (1): Effect of biostimulants, (A) Algae extract and (B) Bio-cozyme on plant height (cm), number of leaves per plant and content of chlorophyll in leaves (SPAD) of broccoli plant. The same letter on the columns displays that there are not significant differences according to Duncan's multiple range test at (P≤ 0.05).

Figure (2) explained that algae extract and bio-cozyme biostimulants were not different significantly with percentage of dry matter and head diameter, although they were significantly improved number of lateral head of broccoli plants. Algae extract at both concentrations 1 and 2 $ml.L^{-1}$ increased number lateral head (9.11 and 9.22, respectively), but the plants were not supplemented with algae extract produced minimum (7.22) number lateral head (Figure 2A). In addition to bio-cozyme biostimulants, gave rise to maximum (9.56) number lateral head at 2 $ml.L^{-1}$ (Figure 2B). Whereas, lateral head number was the lowest (7.56) in control plants. Although, the two biostimulants concentrations were unsuccessful to increase percentage of dry matter and head diameter in the present study. The results of number lateral head agree with Ouda and Mahadeen (2008) who explained that the number of heads was enhanced when organic and conventional fertilizers applied. Manea and Abbas (2018) mentioned that the positive effect of biostimulants such as seaweed and humic acid on number lateral head were detected as well. Khan *et al.* (2009) demonstrated that the reason for superiority of the biostimulants to promote better number lateral head is likely caused by auxins, cytokinin, and other nutrients occur in the biostimulants that enhanced cell division and enlargement. Mattner *et al.* (2013) reported that there are many reports about beneficial effects of seaweed extracts on shoot system and yield of broccoli plant.

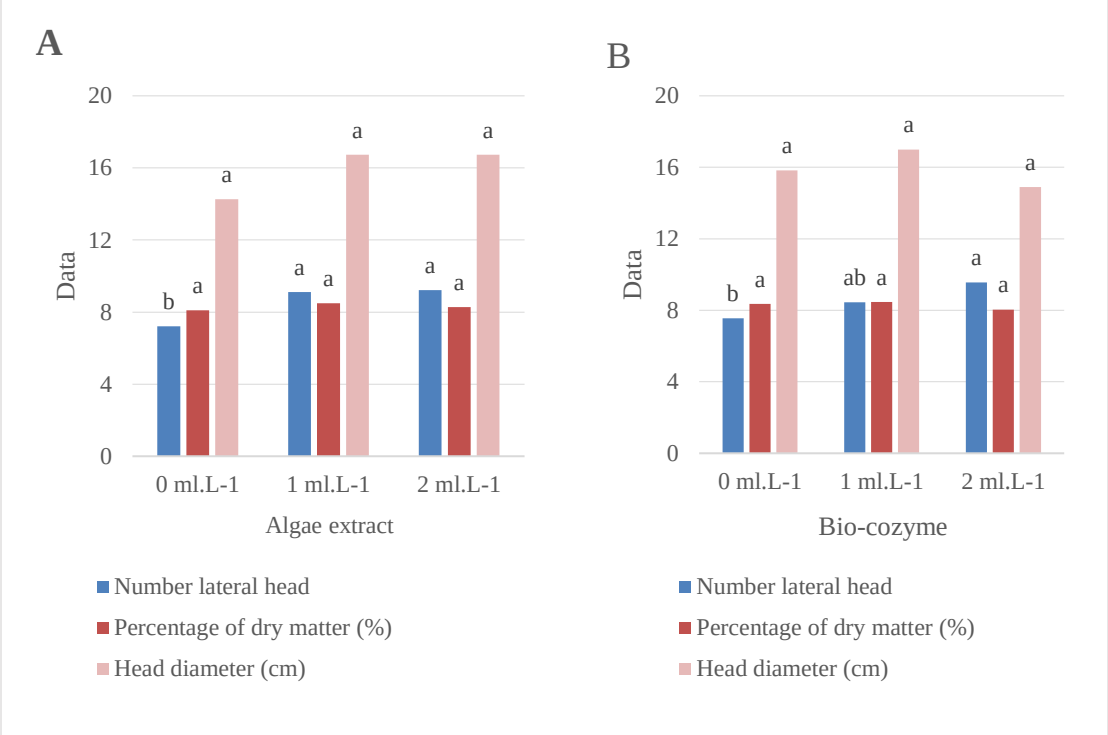

Figure (2): Effect of biostimulants, (A) Algae extract and (B) Bio-cozyme on number lateral head, percentage of dry matter and head diameter (cm) of broccoli plant. The same letter on the columns displays that there are not significant differences according to Duncan's multiple range test at (P≤ 0.05).

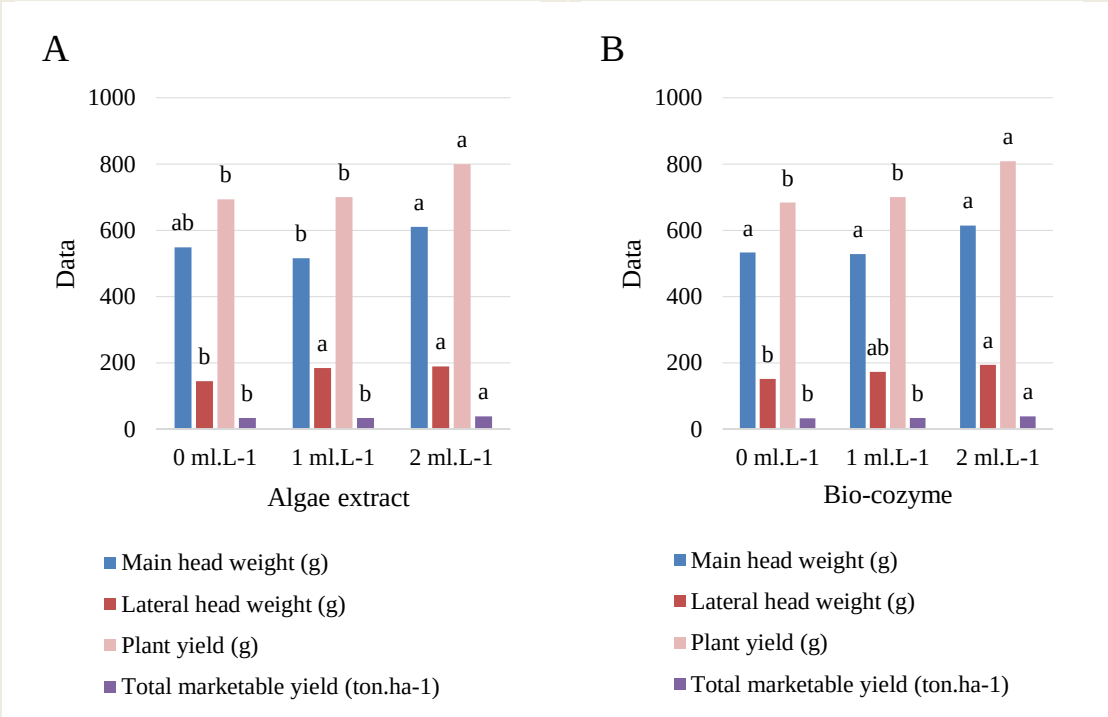

Figure (3): Effect of biostimulants, (A) Algae extract and (B) Bio-cozyme on main head weight (g), lateral head weight (g), plant yield (g) and total marketable yield (ton.ha^{-1}) of broccoli plant. The same letter on the columns displays that there are not significant differences according to Duncan's multiple range test at (P≤ 0.05).

As it was depicted in Figure (3), various main head weight, lateral head weight, plant yield and total marketable yield were recorded owing to independent effect of algae extract and bio-cozyme biostimulants application to broccoli. Algae extract at 2 ml.L^{-1} was the best dose for obtaining the highest main head weight (610.47 g), lateral head weight (188.82 g), plant yield (799.30 g) and total

marketable yield (38.06 ton.ha^{-1}) (Figure 3A). Contrastingly, algae extract at 0 and 1 ml.L^{-1} gave the lowest results. Further, bio-cozyme at 2 ml.L^{-1} made a significant difference relative to control in lateral head weight (193.49 g), plant yield (807.97 g) and total marketable yield (38.47 ton.ha^{-1}). However, no effect of bio-cozyme on main head weight was observed. Hawall Al-jaf *et al.* (2019) explained that the biostimulants such as humic acid gave the best results on main head and total yield of broccoli.

Different chemical parameters of broccoli were measured, such as nitrate, phosphorus, potassium and iron content (Figure 4). Effects of 2 ml.L^{-1} of algae extract on nitrate, phosphorus and iron content gave the maximum values (125.33, 61.42 and 5.06 ppm, respectively) compared to the control which gave the minimum values of nitrate (115.11 ppm) and iron (4.17 ppm). Potassium content was not different at algae extract concentrations (Figure 4A). While, bio-cozyme concentrations did not bring about higher nitrate content (Figure 4B), even increasing bio-cozyme concentrations to 2 ml.L^{-1} decreased nitrate content to the lowest value (115.89 ppm). Inversely, the highest bio-cozyme concentration at 2 ml.L^{-1} led to a maximum (61.32, 321.54 and 5.05 ppm) phosphorus, potassium and iron content, respectively. In this respect, Fahrmand *et al.* (2014) found that the growth and yield quality of plants stimulated by biostimulants as humic acid through processes of metabolism including photosynthesis, cell respiration, synthesis of protein, water and nutrient uptake, and enzyme activities. Further, Khaled and Fawy (2011) found that the humic acid increased nutrient uptake with foliar and soil applications.

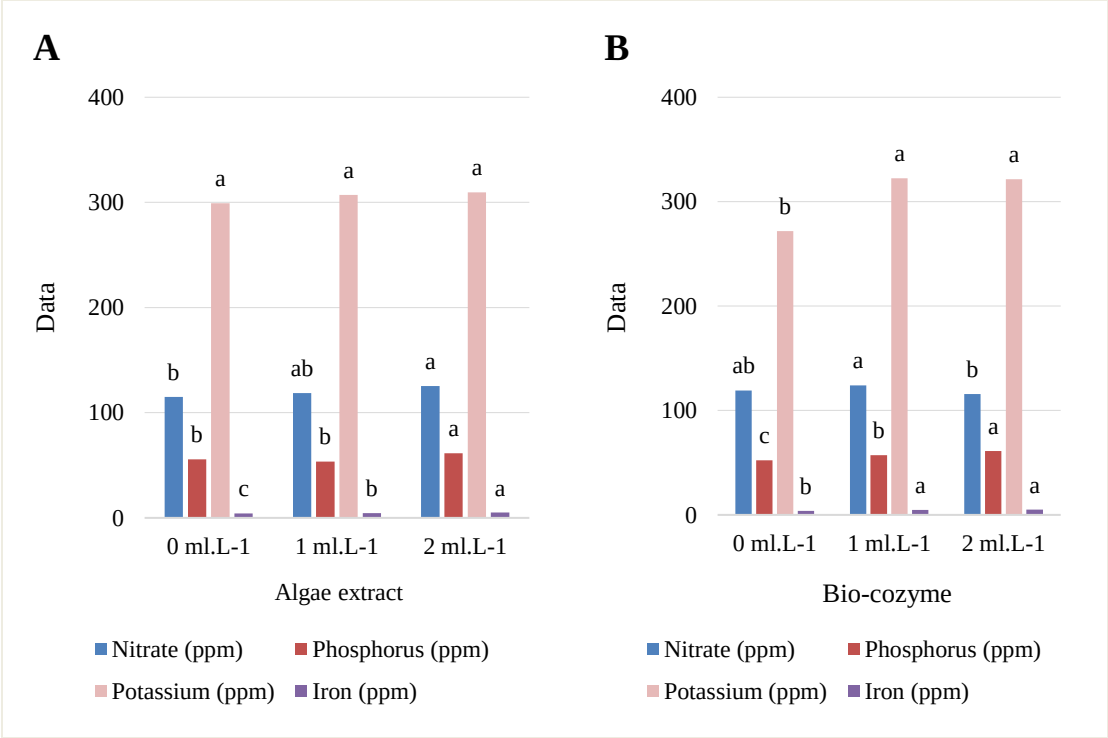

Figure (3): Effect of biostimulants, (A) Algae extract and (B) Bio-cozyme on nitrate (ppm), phosphorus (ppm), potassium (ppm) and iron (ppm) of broccoli plant. The same letter on the columns displays that there are not significant differences according to Duncan's multiple range test at (P≤ 0.05).

The interaction effect of the two biostimulants on number of leaves per plant, content of chlorophyll in leaves, number lateral head and percentage of dry matter of broccoli plant were significant (Table 3). Combination of 1 ml.L^{-1} algae extract and bio-cozyme was the best for producing the highest (66.50) number of leaves per plant. Also, the maximum (82.13 SPAD) content of chlorophyll in leaves and (10.33) number lateral head were observed at the combination of 1 ml.L^{-1} algae extract and 2 ml.L^{-1} bio-cozyme. Similarly, (10.33) number lateral head was obtained at the interaction of 2 ml.L^{-1} algae extract and bio-cozyme. Percentage of dry mater was outstanding (8.63%) at the interaction of 1 ml.L^{-1} algae extract and 0 ml.L^{-1} bio-cozyme. In general, the plants without using of algae extract and bio-cozyme, or without algae extract application and 1 ml.L^{-1} bio-cozyme

produced the lowest number of leaves per plant, content of chlorophyll in leaves, number lateral head and percentage of dry matter. Besides, no significant plant height was achieved when the two biostimulants concentrations interacted. Kim and Chojnacka (2015) indicated that the biostimulants contain cytokinin which enhanced photosynthesis activity and this might improve plant growth properties. Mattner *et al*. (2013) explained that biostimulants significantly increased stem diameter of broccoli due to stimulation of broccoli establishment and growth under greenhouse conditions and open field.

Table (3): Interaction effects of biostimulants (A) Algae extract and (B) Bio-cozyme on plant height, number of leaves per plant, content of chlorophyll in leaves, number lateral head and percentage of dry matter of broccoli plant

Algae extract ($ml.L^{-1}$)	Bio-cozyme ($ml.L^{-1}$)	Plant height (cm)	Number of leaves per plant	Content of chlorophyll in leaves (SPAD)	Number lateral head	Percentage of dry matter
0 $ml.L^{-1}$	0 $ml.L^{-1}$	74.67 a	52.17 b	74.47 bc	6.67 b	8.26 ab
	1 $ml.L^{-1}$	74.83 a	57.50 ab	70.93 c	7.00 b	8.37 ab
	2 $ml.L^{-1}$	76.33 a	65.67 a	74.47 bc	8.00 ab	7.67 b
1 $ml.L^{-1}$	0 $ml.L^{-1}$	74.67 a	55.50 ab	71.32 c	8.00 ab	8.63 a
	1 $ml.L^{-1}$	80.83 a	66.50 a	77.57 ab	9.00 ab	8.47 ab
	2 $ml.L^{-1}$	71.83 a	57.50 ab	82.13 a	10.33 a	8.37 ab
2 $ml.L^{-1}$	0 $ml.L^{-1}$	79.33 a	63.67 a	77.43 ab	8.00 ab	8.20 ab
	1 $ml.L^{-1}$	74.67 a	60.00 ab	76.11 bc	9.33 ab	8.57 a
	2 $ml.L^{-1}$	82.17 a	63.33 a	76.83 ab	10.33 a	8.08 ab

*The Duncan's multiple range test indicates that means with the same letter are not statistically different at ($P \leq 0.05$).

Supplementation of broccoli plants with the combination of the two biostimulants showed different head diameter, main head weight, lateral head weight, plant yield and total marketable yield of broccoli (Table 4). Accordingly, the plants were supplemented with a combination of 1 $ml.L^{-1}$ algae extract and bio-cozyme together with the plants with 2 $ml.L^{-1}$ algae extract and bio-cozyme showed the highest (17.87 cm and 17.83 cm, respectively) head diameter. Furthermore, the best main head weight (674.46 g), lateral head weight (208.58 g), plant yield (883.04 g), total marketable yield (42.05 $ton.ha^{-1}$) were achieved with the dual interaction of algae extract and bio-cozyme at 2 $ml.L^{-1}$. Whereas, the plants without the two biostimulants led to the lowest lateral head weight (119.24 g), plant yield (613.48 g) and total marketable yield (29.21 $ton.ha^{-1}$). Drobek *et al.* (2019) explained that using of biostimulants in agriculture is a way of increasing the growth, yield characteristics and sustainable crops. Petrozza *et al.* (2012) displayed that biostimulants have the potential for significant contributions in yield and quality of commercial crops in modern agriculture. Tejada *et al.* (2018) indicated that increasing yield often related to enhancing the quality of vegetables or fruit, and it is important in organic agriculture where conventional fertilizers cannot be used. Grabowska and Kunicki *et al.* (2009) observed that using biostimulants by foliar applications at the transplanting stage and soil application before head formation increased the early yield in the autumn cultivation by %17 of broccoli under greenhouse conditions.

Table (4): Interaction effects of biostimulants (A) Algae extract and (B) Bio-cozyme on head diameter, main head weight, lateral head weight, plant yield and total marketable yield of broccoli plant

Algae extract ($ml.L^{-1}$)	Bio-cozyme ($ml.L^{-1}$)	Head diameter (cm)	Main head weight (g)	Lateral head weight (g)	Plant yield (g)	Total marketable yield ($ton.ha^{-1}$)
0 $ml.L^{-1}$	0 $ml.L^{-1}$	15.75 ab	494.26 b	119.24 c	613.48 b	29.21 b
	1 $ml.L^{-1}$	16.26 ab	558.94 ab	134.73 bc	693.67 b	33.03 b
	2 $ml.L^{-1}$	10.80 b	593.47 ab	178.55 abc	772.03 ab	36.76 ab
1 $ml.L^{-1}$	0 $ml.L^{-1}$	16.25 ab	485.43 b	175.57 abc	661.00 b	31.48 b
	1 $ml.L^{-1}$	17.87 a	485.93 b	183.60 abc	669.53 b	31.88 b
	2 $ml.L^{-1}$	16.07 ab	575.48 ab	193.35 ab	768.83 ab	36.61 ab
2 $ml.L^{-1}$	0 $ml.L^{-1}$	15.50 ab	618.33 ab	158.78 abc	777.11 ab	37.01 ab
	1 $ml.L^{-1}$	16.87 ab	538.62 ab	199.12 ab	737.74 ab	35.13 ab
	2 $ml.L^{-1}$	17.83 a	674.46 a	208.58 a	883.04 a	42.05 a

*The Duncan's multiple range test indicates that means with the same letter are not statistically different at ($P \leq 0.05$).

Analysis of nitrate, phosphorus, potassium and iron content explained that effect of the two biostimulants was significantly affected on nutrient element contents in broccoli (Table 5). In this context, nitrate and potassium reached the highest values (134.67 and 336.13 ppm, respectively) at the interaction of 2 $ml.L^{-1}$ algae extract and 1 $ml.L^{-1}$ bio-cozyme. Meanwhile, phosphorus content was the best (65.18 ppm) in those plants were only treated without (0 $ml.L^{-1}$) algae extract and 2 $ml.L^{-1}$ bio-cozyme. Regarding iron content, 2 $ml.L^{-1}$ combination of the two biostimulants gave the maximum value (5.36 ppm). Whereas, the lowest nitrate (109.67 ppm), phosphorus (45.12 ppm), potassium (257.63 ppm) and iron (3.31 ppm) were achieved in the plants without application of the two biostimulants (control plants). Kałużewicz *et al.* (2018) reported that the biostimulants significantly influenced the content of nitrogen, phosphorus, potassium, magnesium and iron in broccoli. According to Colla *et al.* (2015) protein hydrolysate applications increased nitrogen assimilation in plants may be on account of the positive effects of protein hydrolysates on the production of carbon skeletons and energy supply needed for amino acid biosynthesis.

Table (5): Interaction effects of biostimulants (A) Algae extract and (B) Bio-cozyme on nitrate, phosphorus, potassium and iron of broccoli plant

Algae extract ($ml.L^{-1}$)	Bio-cozyme ($ml.L^{-1}$)	Nitrate (ppm)	Phosphorus (ppm)	Potassium (ppm)	Iron (ppm)
0 $ml.L^{-1}$	0 $ml.L^{-1}$	109.67 c	45.12 d	257.63 c	3.31 e
	1 $ml.L^{-1}$	115.67 c	56.93 bc	316.54 a	4.22 cd
	2 $ml.L^{-1}$	120.00 bc	65.18 a	323.04 a	4.98 ab
1 $ml.L^{-1}$	0 $ml.L^{-1}$	118.67 bc	48.18 d	282.10 b	3.90 d
	1 $ml.L^{-1}$	121.67 bc	54.94 c	314.37 a	5.18 a
	2 $ml.L^{-1}$	115.67 c	57.75 bc	324.87 a	4.82 ab
2 $ml.L^{-1}$	0 $ml.L^{-1}$	129.33 ab	63.18 ab	275.58 bc	4.52 bc
	1 $ml.L^{-1}$	134.67 a	60.08 abc	336.13 a	5.30 a
	2 $ml.L^{-1}$	112.00 c	61.02 abc	316.72 a	5.36 a

*The Duncan's multiple range test indicates that means with the same letter are not statistically different at ($P \leq 0.05$).

CONCLUSION

The results of the current study confirmed that the two biostimulants, algae extract and bio-cozyme were effective to enhance some characteristics of broccoli plant. So, 2 $ml.L^{-1}$ algae extract gave rise to significant results of most study parameters. Additionally, 2 $ml.L^{-1}$ bio-cozyme was the best and

made difference in some of the measurements. Besides, the effect of the two biostimulants were convincing when they interacted together, and they produced significant differences in all study parameters with exception of plant height.

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تأثير المحفزات الحيوية النباتية على نمو وحاصل وبعض المحتويات المعدنية للبروكولي

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الكلمات المفتاحية:
بروكلي، المحفزات الحيوية، مستخلص الطحالب، Bio-cozyme

الخلاصة

من خلال تقديم المحفزات الحيوية كبدائل التسميد الكيميائي التقليدي، أسهمت في زيادة وعي المستهلك في إثارة القضايا المتعلقة بسلامة الأغذية والبيئة و وضع خطة مناسبة لنشر بدائل الاسمدة الكيمائية التقليدية في الزراعة. لذلك، تم إجراء هذا البحث في كلية علوم الهندسة الزراعية، جامعة السليمانية، إقليم كوردستان العراق، لدراسة تأثير محفزين بيولوجيين نباتيين، مستخلص الطحالب (ألجاتون -20) والمحفز الحيوي النباتي (Bio-cozyme)، على نبات البروكلي تحت ظروف البيت البلاستيكي. بعد أسبوعين من الزراعة، تمت اضافة المحفزات الحيوية بتراكيز مختلفة (0 مل.لتر-1، 1 مل.لتر-1، 2 مل.لتر-1). نفذت التجربة وفق تصميم القطاعات العشوائية الكاملة (RCBD) بثلاثة مكررات.

أظهرت النتائج أن مستخلص الطحالب والـ (Bio-cozyme) عند مستوى 2 مل.لتر-1 أعطى اكبر عدد للاقراص الجانبية و وزن القرص الجانبي و حاصل النبات و إجمالي الحاصل التسويقي ونسبة الفوسفور والحديد. كما أظهر تأثير التفاعل بين مستخلصات الطحالب والـ (Bio-cozyme) أن عدد الأوراق لكل نبات و قطر القرص الرئيسي عند اضافة 1 مل.لتر-1، النسبة المئوية للمادة الجافة عند 1 مل.لتر-1 مستخلص الطحالب بدون الـ (Bio-cozyme)، محتوى الكلوروفيل في الأوراق والاقراص الجانبية عند 1 مل.لتر-1 مستخلص الطحالب و 2 مل.لتر-1 والـ (Bio-cozyme)، و قطر القرص الرئيسي عند مستوى 1 مل.لتر-1 عند التداخل مستخلص الطحالب والـ (Bio-cozyme) الأفضل. أيضا، كان وزن القرص الرئيسي، الوزن الجانبي للقرص، محصول النبات، إجمالي الحاصل التسويقي و الحديد (674.46 جم، 208.58 جم، 883.04 جم، 42.05 طن هكتار-1، 5.36 جزء في المليون، على التوالي) هي الأعلى عند 2 مل.لتر-1 من تداخل المنشطات الحيوية. علاوة على ذلك، كانت النترات والبوتاسيوم (134.67 جزء في المليون، 336.13 جزء في المليون، على التوالي) اعطت اعلى نسبة عند 2 مل.لتر-1 من مستخلص الطحالب و 1 مل.لتر-1 من (Bio-cozyme). بشكل فردي، وكان اضافة 2 مل.لتر-1 من المحفزات الحيوية المستخدمة هما من أفضل التراكيز، ولكن لم يكن هناك فرق كبير بين 1 مل.لتر-1 و 2 مل.لتر-1 للمحفزين الحيويين عند التداخل بينهما في معظم القياسات.