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Effect of biofertilizers, seaweed extract and inorganic fertilizer on growth and yield of lettuce (*Lactuca sativa* var. longifolia L.)

Biyogübre, deniz yosunu ve inorganik gübrelerin marulda (Lactuca sativa var. longifolia L.) bitki gelişimi ve verimi üzerine etkisi

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ABSTRACT

Due to the excessive use of inorganic fertilizers, the structure of the soil is deteriorated, and the product quality is adversely affected. For this reason, the use of environmentally friendly organic fertilizers in production has increased in recent years. A field experiment was conducted in a plastic greenhouse in the Research Center Field at General Directorate of Agriculture, Dohuk Governorate, Northern region of Iraq during autumn season of 2020 to examine the effect of soil application of the biofertilizer (Corabac) with three levels (0, 5 and 10 g L^{-1}) and foliar spraying with the seaweed (algal extract) at three concentrations (0, 4 and 8 g L^{-1}) and the inorganic fertilizer at two concentrations (0 and 1 g L^{-1} (N:P:K 10:10:10)) compared with control (without treatment) on growth and yield of lettuce crop (*Lactuca sativa* L.). The obtained data indicated that the application of the three fertilizers and their interactions significantly induced vegetative growth parameters like stem length and leaf number per plant but did not positively affected leaf area (cm^2) and chlorophyll content (%). Head weight (kg), yield (kg m^{-2}), and total yield (ton ha^{-1}) were also notably increased due to soil addition of biofertilizer and foliar spraying of the algal extract and inorganic fertilizer as compared to the control. Biofertilizer, seaweed and inorganic fertilization affected the N, P and K content in lettuce leaves. For a sustainable production in greenhouse lettuce, it is possible to obtain higher head weight and therefore higher yield by using seaweed and biofertilizers, which are considered environmentally friendly.

Key Words: Algae extract, Biofertilizer, Chemical fertilization, Organic fertilizer, Plant nutrition

ÖZ

İnorganik gübrelerin aşırı kullanımı nedeniyle toprağın yapısı bozulmakta ve ürün kalitesi olumsuz etkilenmektedir. Bu nedenle üretimde çevre dostu organik gübrelerin kullanımı son yıllarda artış göstermiştir. Çalışma 2020 yılı sonbahar yetiştirme döneminde Irak'ın Kuzey Bölgesi, Dohuk Valiliği, Tarım Genel Müdürlüğü Araştırma Merkezi arazisinde bulunan plastik serada yürütülmüştür. Denemede topraktan uygulanan biyogübre (0 , 5 ve 10 g L^{-1}) (Corabac) ile yapraktan uygulanan deniz yosunu (alg özü) (0, 4 ve 8 g L^{-1}) ve inorganik gübrenin (0 ve 1 g L^{-1} (N:P: K 10:10:10)) marulda (Lactuca sativa L.) verim ve bazı büyüme parametreleri üzerine etkisini incelenmek amacıyla gerçekleştirilmiştir. Elde edilen veriler, üç gübrenin ve bunların interaksiyonlarının, baş ağırlığı, verim, gövde uzunluğu, yaprak sayısı gibi parametreleri önemli ölçüde arttırdığı, ancak yaprak alanı (cm^2) ve klorofil içeriğini (%) azalttığını göstermiştir. Biyogübre, deniz yosunu ve inorganik gübreleme yapraklardaki N, P ve K içeriğini etkilemiştir. Sera marul üretiminde sürdürülebilir bir üretim için, çevreye dost olduğu düşünülen deniz yosunu ve biyogübre kullanılarak daha yüksek baş ağırlığı ve dolayısıyla daha yüksek verim elde edilmesi mümkündür.

Anahtar Kelimeler: Deniz yosunu, Biyogübre, Kimyasal gübreleme, Organic gübreleme, Bitki besleme

Introduction

Lettuce (*Lactuca sativa* L.) is an important member of Astaraceae family which is categorized under annual leafy herbs. It is consumedas a famous salad crop, occupying the world largest land devoted for salad crops production. Lettuce is very valuable from nutritional aspect since it contains a considerable amount of minerals, vitamins, antioxidants and phytochemical compounds which possess anti-carcinogenic function (Norman, 1992; Hanafy et al*.,* 2000; Masarirambi *et al.,* 2012).

The rapid increase in the world population, on the other hand, along with the decrease in agricultural lands and the loss of yields due to various stress factors bring the problem of nutrition and food safety to the agenda. In order to get maximum yield and best quality from the plants, it is essential to carry out a correct fertilization program for plants as well as providing convenient ecological factors for each type. However, as well as getting maximum yield, it is quiet crucial to get a sustainable agricultural production and save environment and human health while establishing this fertilization programme (Söylemez, 2021). There is an intensive use of inorganic fertilizers in order to increase the yield obtained from the unit area. Excessive use of inorganic fertilizers disrupting the soil structure, causing environmental pollution, and adversely affecting product yield and quality. Therefore, researchers have focused on the use of environmentally friendly organic fertilizers such as biofertilizer and seaweed, which are thought to be healthier. Biofertilizers are natural substances containing the living cells of several species of beneficial bacteria and fungi which are applied as a substitute for minimizing application of harmful inorganic fertilizers. The strength of biofertilizer products is proved from their ability to give optimum growth and productivity for plant. They enhance the soil fertility and soil microbial activity via different biological processes due to they contain of microorganisms that convert the nourishing elements from complex forms into simple obtainable forms. (Ekta et al., 2017; Shinde et al., 2018; Cevheri et al., 2021). Ahmed et al. (2000) showed that lettuce plants received *Azotobacter sp*. as a biological fertilizer possessed better vegetative components like height of the shoot, leaf number and total fresh weight. Menamoa and Woldeb (2013) have demonstrated that treating romaine lettuce with the biofertilizer cyanobacteria caused an enhancement in the leaf number, leaf area, leaf length, leaf fresh weight, leaf dry weight and the root dry weight of lettuce. González et al. (2019) showed that treating lettuce plants with EM (effective Microorganisms) at 10 mL m^{-2} at 0 and 15 DAT (day after tretment) was the best in term of growth and productivity of the crop. The influence of biofertilizers on broccoli performance has been studied and the results displayed that biofertilization gave the optimum foliage traits such as plant height, number of leaves per plant, chlorophyll content and leaf area comparing to control (Lateef et al., 2019).

Seaweed extracts are defined as bio-stimulants derived from non-blooming marine algae that have not true leaves, shoots, and foliage. These extracts are common in containing a high content of sterols, polysaccharides, N-containing compounds (e.g., betaines), numerous nourishing elements and a number of plant growth-promoting substances like auxins, cytokinins, gibberellins and brassinosteroids (Khan et al. 2009; Craigie 2011). When provided for plants, seaweed extracts significantly ameliorated plant outgrowth and yield and resulted in increased tolerance to hard exogenous stresses via raising the metabolic paths driving to production of antioxidants within plant cell (Thirumaran et al., 2009). Mohamed and Zewail (2016) reported that the biggest head diameter and the premium fresh and dry weight and total yield were recorded by 200% organic fertilizer (T5) and seaweed extract at 3 ml/l. Chrysargyris et al. (2018) displayed that foliar spraying of seaweed extract *Ascophyllum nodosum* remarkably improved growth and quality of cut lettuce. Yusuf et al. (2019), showed that the Maxicrop extract significantly enhanced shoot biomass, root biomass and leaf chlorophyll content by 66, 47 and 9%, respectively. It has been found that the seaweed concentrate Kelpak® and effective microorganisms caused a magnitude increment in the growth and yield of cabbage in term of fresh leaves, fresh head weight, stem diameter and head polar diameter (Satekge et al., 2016).

The aim of this study is to determine the effects of biofertilizers, seaweed extract and inorganic fertilizers on growth, development and yield of lettuce in order to reduce environmental pollution and production costs.

Materials and Methods

Materials

The study was conducted in a greenhouse (500 m^2) in Research Center Field at General Directorate of Agriculture, Dohuk Governorate, Northern region of Iraq during autumn season of 2020. Al-Fajr variety of lettuce was used as plant material.

The plowing land of plastic house was carried out then the soil was well softened. The organic manure (1500 kg per 500 m^2) was added to the soil for improving soil fertility and second plough was given to the land for the aim of mixing the organic matter with the soil particle then the land was amended. The land of plastic house was divided into ten lines each line comprised two lines of lettuce according to the distribution system of the drip irrigation. Each line consisted of 18 parts which compartmented into a line. The plants were planted with space 60 cm between narrow rows, 80 cm between wide rows and 30 cm in rows. The land was well irrigated and the doors were closed for a few days so the field of plastic house was disinfected.

The seeds of lettuce were sown in multiplate pods on October 1st 2020. The young lettuce plants were transplanted on November 6th 2020 at distance of 30 cm between plants with 60 cm spacing between terraces. In the study, we used biofertilizer, seaweed (algae) extract and inorganic fertilizer. The plants were harvested on December 15.2020. The microorganisms contained in biofertilizers have the potency to convert nutrient elements from complex form to obtainable form via different biological processes. There are many species of microorganisms, like predominant populations of lactic acid, yeasts, smaller numbers of photosynthetic bacteria, bacteria, actinomycetes and many other types of organisms selected for preparation of biofertilizers (Woodward, 2003). The Corabac product contains selected strains of bacteria such as *Azotobacter* and *Bacillus megaterium*. Table 1 illustrates the chemical and physical properties of Corabac fertilizer (Saleem and Ali, 2021).

Table 1. Physical and chemical properties of Corabac fertilizer

Content of material availability	Units	Amounts
Azotobacter *Bacillus megaterium*	%	1.0
Gardened cultured	%	2.0
Riolete granules	%	97.0

Seaweed extracts are rich in complex polysaccharides, fatty acids, plant hormones, various vitamins and nutrient elements that assist plant to exhibit high performance and productivity (Battacharyya et al., 2015). Bioactive compounds contained in algae were showed excellent ability to promote plant growth. These compounds positively influence metabolic activities such as photosynthesis, biosynthesis of nucleic acid and nutrient absorption (Tarraf et al., 2015). Table 2 shows chemical analysis and amino acid content of algal extract (Tarraf et al., 2015).

Table 2. Chemical analysis and amino acid content of algal extract

Content of material availability	Units	Amounts
Amino acid	%	16.00
Nitrogen	%	10.24
Phosphorus	%	3.40
Potassium	%	1.50

Methods

The study comprised of soil drenching of the biofertilizer (Corabac) at three concentrations (0, 5 and 10 g L^{-1}) and foliar sprayed of seaweed extract (Alga Seaweed) (algae) at three concentrations (0, 4 and 8 ml L^{-1}) and inorganic fertilizer applied at two rates (0 and 1 g L^{-1}) of NPK (10:10:10) and their interactions. The first spray and soil drenching was done on November 25th 2020 with ten days' interval between each spray. The second spray was done on October 5th 2020 and the third one was performed on October 15th 2020. The following parameters were measured:

Vegetative growth parameters

The plants were harvested by cutting at the soil level on 30.01.2021. The leaves were counted by separating them from the stem. The plant was weighed on a precision scale for head weight. Leaf area was measured by leaf area meter. Chlorophyll index was determined from inner, midle and outer leaves by Chlorophyll Meter, SPAD-502, Konica Minolta after 45 days from transplanting. Stem length was determined by measuring bar from full-physiologically grown stems of plants.

Yield per square meter was calculated by equation 1 given below;

$$\text{Yield} = \frac{\text{Yield of experiment unit (kg)}}{\text{Area of experimental unit}} \qquad (1)$$

Mineral contents in leaves

The total nitrogen percentage was measure based on the modified method of Kjeldahl and the analysis was attained by using the Microkjeldahl equipment (A.O.A.C., 1980) that cited by Black (1965). The Phosphorus content (%) was determined in leaves of lettuce depending upon the colorimetric methods and the spectrophotometer equipment was utilized for this purpose (Matt, 1970). Potassium percentage (%) was measured according to flame method by use of Flame photometer instrument (A.O.A.C., 1970 and Al -Sahaf, 1989).

Statistical analysis

The factorial Randomized Complete Block Design (RCBD) was used for treatment arrangement and the study included 18 treatments (2 × 3 × 3 = 18), each treatment was replicated three times and each treatment was represented by 14 plants. The data were analyzed using (SAS, 2010) program.

Results and Discussion

Vegetative growth parameters

Number of leaves

According to the results showed in the Table 3, effect of soil application of biofertilizer and foliar spraying of seaweed extracts and inorganic fertilizer were significant on number of leaves of lettuce. Seaweed treatments significantly increased the number of leaves compared to the control. While the maximum number of leaves (56.00) was obtained from the 4 g L^{-1} seaweed dose, it was in the same statistical group with the 8 g L^{-1} dose (55.72). Also, the number of leaves increased significantly with the increase of biofertilizer doses. The highest number of leaves (57.06) was obtained from the dose of 10 g L^{-1} of biofertilizer, and the lowest number of leaves (53.83) was determined in the control application (0 g L^{-1}). But, application of inorganic fertilizers adversely affected the number of leaves. The most number of leaves was in control application (0 g L^{-1} of inorganic fertilizer) with 56.82 .

All of interactions had a significantly influence on leaf number. Application of inorganic fertilizer (0 g L^{-1}) and seaweed (0 g L^{-1}) gave the most leaf number (57.11 pieces). The least leaf number (50.22 pieces) was taken from application of inorganic fertilizer (1 g L^{-1}) and seaweed (0 g L^{-1}). The peak mean number of leaf for biofertilizer * seaweed interaction (58.33 and 58.00 pieces) was counted in plants supplied with 4 and 8 g L^{-1} algae extract and 10 g L^{-1} bio fertilizer, while the lowest value was seen in the control application (52.00 pieces). For the interference between biofertilizer and inorganic fertilizer, 10 g L^{-1} of biofertilizer and no inorganic dose resulted in the highest leaf number per plant (59.56).

Table 3. Effect of biofertilizer, seaweed and inorganic fertilizer on number of leaves per plant of lettuce

Inorganic fertilizer	Biofertilizer (g L^{-1})	Seaweed (g L^{-1})			Chemical* Biofertilizer	**Inorganic fertilizer**
		0	4	8		
With	0	49.67 g	55.67 b-e	52.00 fg	52.44 d	**53.44 b**
With	5	51.33 fg	53.33 ef	55.33 c-e	53.33 cd	
With	10	49.67 g	56.67 b-d	57.33 a-d	54.56 bc	
Without	0	54.33 d-f	53.00 ef	58.33 a-c	55.22b	**56.82 a**
Without	5	57.00 a-d	57.33 a-d	52.67 e-g	55.67 b	
Without	10	60.00 a	60.00 a	58.67 ab	59.56 a	
Seaweed (g L^{-1})		**53.67 b**	**56.00 a**	**55.72 a**	**Biofertilizer (g L^{-1})**	
Inorganic *Seaweed	With	50.22 d	55.22 bc	54.89 c		
	Without	57.11 a	56.78 ab	56.56 a-c		
Biofertilizer *Seaweed	0	52.00 c	54.33 b	55.17 b	**0**	**53.83 b**
	5	54.17 b	55.33 b	54.00 bc	**5**	**54.50 b**
	10	54.83 b	58.33 a	58.00 a	**10**	**57.06 a**

Different letters indicate significant differences at P<0.05 by Duncan test.

The triple combinations among the three factors at 10 g L^{-1} of biofertilizer plus 4 g L^{-1} of algal extract plus no inorganic rate and at 10 g L^{-1} of biofertilizer plus 0 g L^{-1} of algal extract plus no inorganic rate produced the greatest average value (60.00) in comparison with other treatments.

Leaf area

The leaf area of lettuce exhibited no improvement in attribution to the individual doses of biofertilizer, the inorganic fertilizer and algal extract. Seaweed doses had a negative effect on leaf area and the largest leaf area was measured with 38.01 cm^2 in the control application containing 0 g L^{-1} seaweed. The same effect was also true for biofertilizer that resulted in the maximum leaf area of 38.04 cm^2 at 5 g L^{-1} that was not differed from 37.91 produced by 0 g L^{-1} dose. Application of inorganic fertilizer to lettuce suppressed leaf area and the largest leaf area was seen in control.

Concerning the dual interaction between seaweed and inorganic fertilizer, there were significant differences in leaf areas and the highest leaf area (39.74 cm^2) was measured in plants that took 0 g L^{-1} doses of both fertilizers. The combination of 0 g L^{-1} biofertilizer and 8 g L^{-1} algae extract with 5 g L^{-1} biofertilizer and 0 g L^{-1} seaweed resulted in the highest leaf area (39.89 cm^2). The inorganic fertilizer-biofertilizer interaction had important effect on leaf area. The largest leaf area (39.66 cm^2) was obtained from the inorganic dose (1 g L^{-1}) plus biofertilizer at level of (5 g L^{-1}).

Table 4. Effect of biofertilizer, seaweed and inorganic fertilizer on leaf area (cm^2) of lettuce

Inorganic fertilizer	Biofertilizer (g L^{-1})	Seaweed (g L^{-1})			Inorganic* Biofertilizer	**Inorganic fertilizer**
		0	4	8		
With	0	34.28 hi	37.53 f	39.27de	37.03 c	**36.91 b**
With	5	34.05 hi	41.79 c	43.13 b	39.66 a	
With	10	40.49 d	33.84 i	27.80 g	34.04 d	
Without	0	38.97 e	36.83 f	40.50 d	38.79 b	**37.81 a**
Without	5	45.09 a	35.67 g	28.50 j	36.40 c	
Without	10	35.15 gh	39.97de	39.58 de	38.23 b	
Seaweed (g L^{-1})		**38.01 a**	**37.62 a**	**36.46 b**	**Biofertilizer (g L^{-1})**	
Inorganic *Seaweed	With	36.27 c	37.72 b	36.73 c		
	Without	39.74 a	37.51 b	36.20 c		
Biofertilizer *Seaweed	0	36.63d	37.21 cd	39.89 a	**0**	**37.91 a**
	5	39.60 a	38.73 b	35.82 e	**5**	**38.04 a**
	10	37.82 c	36.91 d	33.69 f	**10**	**36.14 b**

Different letters indicate significant differences at P<0.05 by Duncan test.

On the other hand, the complex (triple) dose of the fertilizers affected the leaf area of lettuce. The peak leaf area (45.09 cm^2) was found in plants treated with no inorganic fertilizer, algal extract at (0 g L^{-1}) and biofertilizer at (5 g L^{-1}) whereas the smallest leaf area (27.80 cm^2) was measured for leaves of plants given inorganic fertilizer + algal extract at (8 g L^{-1}) + biofertilizer at (10 g L^{-1}) in comparison with the other treatments (see Table 4).

Chlorophyll content

The soil drenching of biofertilizer and foliar feeding of inorganic fertilizer and seaweed caused no amelioration in the chlorophyll content of lettuce. The individual levels of the three factors were not supreme in enhancing chlorophyll content of leaves and the chlorophyll content located between (31.03 %) and (31.55 %). Similarly, the double levels of fertilizers drove to no remarkable increment in the chlorophyll content in comparison with control. The triple interaction inorganic fertilizer * algal extract * biofertilizer at no inorganic fertilizer and biofertilizer along with algal extract at level of (8 g L^{-1}) gave the maximum chlorophyll content (32.43 %) surpassing other treatments as shown in the Table 5.

Table 5. Effect of biofertilizer, seaweed and inorganic fertilizer on chlorophyll content (%) of lettuce

Inorganic fertilizer	Biofertilizer (g L^{-1})	Seaweed (g L^{-1})			Inorganıc* Biofertilizer	**Inorganic fertilizer**
		0	4	8		
With	0	32.07 ab	30.30 ab	32.43 a	31.60 a	**31.34 a**
With	5	30.10 ab	31.07 ab	31.47 ab	30.88 a	
With	10	31.37 ab	31.93 ab	31.30 ab	31.53 a	
Without	0	32.27 ab	31.23 ab	31.00 ab	31.50 a	**31.03 a**
Without	5	31.57 ab	30.90 ab	30.83 ab	31.10 a	
Without	10	29.17 b	32.40 a	29.93 ab	30.50 a	
Seaweed (g L^{-1})		**31.09 a**	**31.31 a**	**31.16 a**	**Biofertilizer (g L^{-1})**	
Inorganic *Seaweed	With	31.18 a	31.10 a	31.73 a		
	Without	31.00 a	31.51 a	30.59 a		
Biofertilizer *Seaweed	0	32.17 a	30.77 a	31.72 a	**0**	**31.55 a**
	5	30.83 a	30.98 a	31.15 a	**5**	**30.99 a**
	10	30.27 a	32.17 a	30.62 a	**10**	**31.02 a**

Different letters indicate significant differences at P<0.05 by Duncan test.

Stem length

The data displayed in the Table 6 indicates effects on stem length of lettuce in ascription of foliar feeding of inorganic fertilizer and seaweed extracts and soil addition of biofertilizer. The singular impact of the biofertilizer and the algal extract was not significant on stem length and it changed between 13.34 and 14.14 cm. For inorganic fertilizer dose the longest stem length was recorded 14.21 cm.

Table 6. Effect of biofertilizer, seaweed and inorganic fertilizer on stem length (cm) of lettuce

Inorganic fertilizer	Biofertilizer (g L^{-1})	Seaweed (g L^{-1})			Inorganic* Biofertilizer	**Inorganic fertilizer**
		0	4	8		
With	0	13.83 ab	15.38 a	14.10 ab	14.43 a	**14.21 a**
With	5	12.87 b	14.90 ab	14.43 ab	14.07 ab	
With	10	13.73 ab	14.47 ab	14.17 ab	14.12 ab	
Without	0	13.23 ab	13.00 b	12.77 b	13.00 b	**13.30 b**
Without	5	12.80 b	12.80 b	13.17 ab	12.92 b	
Without	10	13.60 ab	14.30 ab	14.00 ab	13.97 ab	
Seaweed (g L^{-1})		**13.34 a**	**14.14 a**	**13.77 a**	**Biofertilizer (g L^{-1})**	
Inorganic *Seaweed	With	13.48 b	14.91 a	14.23 ab		
	Without	13.21 b	13.37 b	13.31 b		
Biofertilizer *Seaweed	0	13.53 ab	14.18 ab	13.43 ab	**0**	**13.72 a**
	5	12.83 b	13.85 ab	13.80 ab	**5**	**13.49 a**
	10	13.67 ab	14.38 a	14.08 ab	**10**	**14.04 a**

Different letters indicate significant differences at P<0.05 by Duncan test.

Regarding the binary effect of factors, the dual interference between inorganic dose and seaweed extract dose, the average longest stem (14.91 cm) was owned by plants recieved seaweed at level of (4 g L^{-1}) along with inorganic dose. In state of interaction between biofertilizer and seaweed extract, the best stem length average (14.38 cm) was measured in plants supplied with biofertilizer at level of 10 g L^{-1} plus seaweed extract at level of 4 g L^{-1} in comparison with other treatments. In the inorganic fertilizer*biofertilizer interaction, the longest stem (14.43 cm) was obtained from control plants.

The complex interaction among the fertilizers was important. The longest stem (15.38 cm) was recorded from application of inorganic fertilizer dose + seaweed extract at 4 g L^{-1} + biofertilizer at 0 g L^{-1}.

Yield parameters

Head weight

The data analysis revealed significant variations in lettuce head weight owed to treating with bofertilizer, seaweed and inorganic fertilizer (Table 7). For individual doses, the seaweed exhibited supremacy over control and the heaviest head was 1.19 kg in plants treated with 8 g L^{-1} of the algal extract. The same superiority was found for the increased doses of biofertlizer in term of the biggest average head (1.19 kg) that was weighed for plants treated with 8 g L^{-1} of the algal extract. The single effect of inorganic fertilizer did not show efficacy in ameliorating the head weight.

In state of seaweed * inorganic fertilizer interaction, the most average head weight (1.22 kg) was measured for plant given 8 g L^{-1} algal extract and inorganic fertilizer compared with other treatments.

For seaweed * bio fertilizer interaction, the heaviest average of head weight (1.25 kg) was estimated for plants delivered algal extract at level of 8 g L^{-1} and biofertilizer at level of 10 g L^{-1} over other treatments whereas the highest average (1.22 kg) was possessed by plants given inorganic fertilizer dose plus biofertilizer at 10 g L^{-1} when compared with other treatments.

Relevant to triple interaction, the peak head weight average (1.27 kg) was determined in plants provided with the inorganic fertilizer and the algal extract at 8 g L^{-1} as well as biofertilizer at 10 g L^{-1}.

Table 7. Effect of biofertilizer, seaweed and inorganic fertilizer on head weight (kg) of lettuce

Inorganic fertilizer	Biofertilizer (g L^{-1})	Seaweed (g L^{-1})			Inorganic* Biofertilizer	**Inorganic fertilizer**
		0	4	8		
With	0	1.025 d	1.18 a-d	1.15 a-d	1.12 cd	**1.18 a**
With	5	1.09 b-d	1.23 ab	1.25 ab	1.19 a-c	
With	10	1.19 a-c	1.21 a-c	1.27 a	1.22 a	
Without	0	1.12 a-d	1.14 a-d	1.13 a-c	1.13 b-d	**1.14 a**
Without	5	1.06 cd	1.13 a-d	1.10 b-d	1.09 d	
Without	10	1.23 ab	1.15 a-d	1.23 ab	1.20 ab	
Seaweed (g L^{-1})		**1.12 b**	**1.17 ab**	**1.19 a**		
Inorganic *Seaweed	With	1.10 c	1.20 ab	1.22 a	**Biofertilizer (g L^{-1})**	
	Without	1.13 bc	1.14	1.16 a-c		
Biofertilizer *Seaweed	0	1.071 d	1.158 a-d	1.14 b-d	**0**	**1.12 b**
	5	1.075 cd	1.175 a-d	1.17 a-d	**5**	**1.14 b**
	10	1.208 ab	1.179 a-c	1.25 a	**10**	**1.21 a**

Different letters indicate significant differences at P<0.05 by Duncan test.

Yield

Results in the Table 8 indicated statistically significant differences in the yield (kg m^{-2}) in response to the treatment with the inorganic fertilizer, the biofertilizer and the seaweed along with their interactions. The sole dose of seaweed at 8 g L^{-1} led to produce the best average (4.75 kg m^{-2}) over control. The same efficiency was seen for the single dose of biofertilizer and the highest average (4.85 kg m^{-2}) was measured for plants treated with 10 g L^{-1} of the biofertilizer compared to other doses. The single rate of inorganic fertilizer has had no magnitude influence on yield (kg m^{-2}).

The twin interaction between all fertilizers improved yield. In inorganic fertilizer * seaweed interaction, the highest average yield (4.89 kg m^{-2}) was ascribed to inorganic dose plus 8 g L^{-1} of algal extract. The same effectiveness was given by seaweed * biofertilizer interaction and the highest average yield (5.00 kg m^{-2}) was resulted from seaweed at 8 g L^{-1} and biofertilizer at 10 g L^{-1} over the other treatments. About inorganic fertilizer * biofertilizer, the maximum average (4.89 kg m^{-2}) was yielded by plants received the inorganic fertilizer dose plus biofertilizer at 10 g L^{-1}.

In respect to the triple combination, the highest yield (5.07 kg m^{-2}) was in plants treated with inorganic fertilizer + algal extract at 8 g L^{-1} + biofertilizer at 10 g L^{-1}.

Table 8. Effect of biofertilizer, seaweed and inorganic fertilizer on yield (kg.m^2) of lettuce

Inorganic fertilizer	Biofertilizer (g L^{-1})	Seaweed (g L^{-1})			Inorganic* Biofertilizer	**Inorganic fertilizer**
		0	4	8		
With	0	4.10 d	4.70 a-d	4.60 a-d	4.47 cd	**4.70 a**
With	5	4.37 b-d	4.90 ab	4.99 ab	4.75 a-c	
With	10	4.77 a-c	4.83 a-c	5.07 a	4.89 a	
Without	0	4.47 a-d	4.57 a-d	4.53 a-d	4.52 b-d	**4.57 a**
Without	5	4.23 cd	4.50 a-d	4.40 b-d	4.38 d	
Without	10	4.90 ab	4.60 a-d	4.93 ab	4.81ab	
Seaweed (g L^{-1})		**4.47 b**	**4.68 ab**	**4.75 a**	**Biofertilizer (g L^{-1})**	
Inorganic *Seaweed	With	4.41 c	4.81 ab	4.89 a		
	Without	4.53 bc	4.56 a-c	4.62 a-c		
Biofertilizer *Seaweed	0	4.28 d	4.63 a-d	4.57 b-d	**0**	**4.49 b**
	5	4.30 dc	4.70 a-d	4.70 a-d	**5**	**4.57 b**
	10	4.83 ab	4.72 a-c	5.00 a	**10**	**4.85 a**

Different letters indicate significant differences at P<0.05 by Duncan test.

Mineral content

Nitrogen

The effect of seaweed and biofertilizer applications on the nitrogen content of the leaves was found to be significant, while the effect of inorganic fertilizer application was found to be nonsignificant. When the dose of the applied seaweed increased, the nitrogen content in the leaves also increased, and the highest nitrogen content was obtained from the application with 8 g L^{-1}. In the biofertilizer application, while the highest nitrogen content was obtained from the control application (0 g L^{-1}), 10 g L^{-1} biofertilizer application followed the control application. The effect of inorganic fertilizer application on nitrogen content was not observed and they were in the same group statistically.

Table 9. Effect of biofertilizer, seaweed and inorganic fertilizer on nitrogen content (%) of lettuce

Inorganic fertilizer	Biofertilizer (g L^{-1})	Seaweed (g L^{-1})			Inorganic* Biofertilizer	**Inorganic fertilizer**
		0	4	8		
With	0	1.87 c-e	2.06 a-c	2.17 ab	2.03 a	**1.82 a**
With	5	1.64 e	1.18 f	2.00 a-c	1.61 c	
With	10	1.92 b-e	1.64 e	1.92 b-d	1.83 b	
Without	0	2.20 a	2.08 a-c	2.09 a-c	2.12 a	**1.88 a**
Without	5	1.38 f	1.69 de	1.36 f	1.48 c	
Without	10	1.70 de	2.19 ab	2.27 a	2.05 a	
Seaweed (g L^{-1})		**1.78 b**	**1.81 b**	**1.97 a**	**Biofertilizer (g L^{-1})**	
Inorganic *Seaweed	With	1.81 b	1.63 c	2.03 a		
	Without	1.76 bc	1.98 a	1.91 ab		
Biofertilizer *Seaweed	0	2.04 ab	2.07 ab	2.13 a	**0**	**2.08 a**
	5	1.51 ef	1.43 f	1.68 de	**5**	**1.54 c**
	10	1.81 cd	1.91 bc	2.10 ab	**10**	**1.94 b**

Different letters indicate significant differences at P<0.05 by Duncan test.

As seen in Table 9, all bilateral interactions were found to be significant. In the inorganic fertilizer *seaweed interaction, the highest nitrogen content was obtained from the inorganic fertilizer plus 8 g L^{-1} seaweed application and the inorganic fertilizer-free plus 4 g L^{-1} seaweed application. In the biofertilizer *seaweed interaction, the highest nitrogen content was determined in the application of 0 g of the biofertilizer and 8 g L^{-1} of seaweed. The highest nitrogen content in the inorganic fertilizer*biofertilizer interaction was determined in the control application where the inorganic fertilizer and biofertilizer were 0 g L^{-1}. This application was followed by inorganic fertilizer 0 g L^{-1} plus biofertilizer 10 g L^{-1} and inorganic fertilizer plus 0 g L^{-1} biofertilizer applications.

In connection with the complex combination among fertilizers, the greatest average percentage (2.27 %) was determined in plants without the inorganic fertilizer dose and seaweed at (8 g L^{-1}) plus biofertilizer (10 g L^{-1}). The minimum average percentage (1.18 %) was obtained in application of inorganic fertilizer + biofertilizer (5 g L^{-1}) + algal extract (4 g L^{-1}).

Phosphorus

The effect of inorganic fertilizer, seaweed and biofertilizer applications on phosphorus content was found to be statistically significant (Table 10). The highest phosphorus content (0.29%) was obtained from the plants applied with inorganic fertilizers while the maximum phosphorus content (0.30 %) was found in plants recieved seaweed at 8 g L^{-1}.

In biofertilizer * seaweed interaction, the highest phosphorus content (0.37%) was obtained from biofertilizer at 10 g L^{-1} and seaweed at 4 g L^{-1}. All binary interactions on phosphorus content were significant. In the inorganic fertilizer * seaweed interaction, the highest phosphorus content (0.34 %) was determined in the plants applied with inorganic fertilizer and 8 g L^{-1} seaweed. In the biofertilizer * seaweed interaction, the highest phosphorus content (2.03 %) was determined in the application of 10 g L^{-1} biofertilizer + 4 g L^{-1} seaweed. In the inorganic fertilizer * biofertilizer interaction, the highest phosphorus content (0.37 %) was determined in the application of inorganic fertilizer and 10 g L^{-1} biofertilizer.

In the triple interaction, the highest phosphorus content was (0.42%) obtained in application of inorganic fertilizer + 10 g L^{-1} biofertilizer and 0 g L^{-1} seaweed, while the lowest phosphorus content (0.12%) was detected in application of inorganic fertilizer + 4 g L^{-1} seaweed and 0 g L^{-1} biofertilizer.

Table 10. Effect of biofertilizer, seaweed and inorganic fertilizer on phosphorus content (%) of lettuce

Inorganic fertilizer	Biofertilizer (g L^{-1})	Seaweed (g L^{-1})			Inorganic* Biofertilizer	**Inorganic fertilizer**
		0	4	8		
With	0	0.33 d	0.12 k	0.32 d	0.26 d	**0.29 a**
With	5	0.19 h	0.17 i	0.36 c	0.24 e	
With	10	0.42 a	0.33 d	0.35 c	0.37 a	
Without	0	0.21 g	0.35 c	0.39 b	0.32 b	**0.28 b**
Without	5	0.29 e	0.21 g	0.14 j	0.21 f	
Without	10	0.28 e	0.40 ab	0.24 f	0.31 c	
Seaweed (g L^{-1})		**0.29 b**	**0.26 c**	**0.30 a**	**Biofertilizer (g L^{-1})**	
Inorganic *Seaweed	With	0.31 b	0.22 d	0.34 a		
	Without	0.26 c	0.32 b	0.26 c		
Biofertilizer *Seaweed	0	0.27 d	0.24 f	0.36 ab	**0**	**0.29 b**
	5	0.24 ef	0.19 g	0.25 e	**5**	**0.23 c**
	10	0.35 b	0.37 a	0.29 c	**10**	**0.34a**

Different letters indicate significant differences at P<0.05 by Duncan test.

Potassium

The effect of inorganic fertilizer and seaweed applications on potassium content was insignificant, but the effect of biofertilizer application was significant (Table 11). The highest potassium content (5.32 %) was determined in the application of 10 g L^{-1} biofertilizer. The effect of bilateral interactions on potassium content was found to be significant. In the inorganic fertilizer * seaweed interaction, the highest potassium content (5.33 %) was determined in the 0 g L^{-1} application of inorganic fertilizers and seaweed. In biofertilizer * seaweed application, the highest potassium content (5.52 %) was obtained from (0 g L^{-1}) dose of seaweed and (10 g L^{-1}) dose of Corabac. In the inorganic fertilizer * biofertilizer interaction, the highest potassium content (5.47 %) was found in 0 g L^{-1} application of inorganic fertilizer and 10 g L^{-1} application of biofertilizer. The triple interaction was found to be statistically significant, and the highest potassium content was obtained from the application where inorganic fertilizer and seaweed were 0 g L^{-1}, and biofertilizer was 10 g L^{-1}. The lowest potassium content was determined in the application of 1 g L^{-1} inorganic fertilizer + 0 g L^{-1} seaweed + 5 g L^{-1} biofertilizer.

Table 11. Effect of biofertilizer, seaweed and inorganic fertilizer on potassium content (%) of lettuce

Inorganic fertilizer	Biofertilizer Corabac (g L^{-1})	Seaweed (g L^{-1})			Inorganic fertilizer * Corabac	**Inorganic fertilizer**
		0	4	8		
With	0	5.62 a-c	5.03 a-d	4.75 b-d	5.13 ab	**4.98 b**
With	5	4.19 d	4.87 b-d	4.80 b-d	4.62 b	
With	10	5.09 a-d	5.06 a-d	5.37 a-c	5.17 a	
Without	0	4.81 b-d	4.81 b-d	5.42 a-c	5.01 ab	**5.15 a**
Without	5	5.21 a-c	5.03 a-d	4.65 cd	4.96 ab	
Without	10	5.96 a	5.67 ab	4.77 b-d	5.47 a	
Seaweed (g L^{-1})		**5.15 a**	**5.08 a**	**4.96 a**	**Biofertilizer Corabac (g L^{-1})**	
Inorganic fertilizer *Seaweed	With	4.97 a	4.99 a	4.97 a		
	Without	5.33 a	5.16 a	4.95 a		
Corabac *Seaweed	0	5.21 ab	4.92 ab	5.09 ab	**0**	**5.07 ab**
	5	4.70 b	4.94 ab	4.72 b	**5**	**4.79 b**
	10	5.52 a	5.36 ab	5.07 ab	**10**	**5.32 a**

Different letters indicate significant differences at P<0.05 by Duncan test.

The insignificant impact of the three factors on chlorophyll content and leaf area could be due to good soil fertility of the plastic house after field preparation and addition of organic manure. The improved stem length and leaf number and yield traits could attribute to the effect of seaweed extracts, which contain numerous beneficial molecules such as complex polysaccharides, plant growth regulators, fatty acids, betaine-like compounds, different vitamins and a variety of macro and micronutrients that help the plant to grow optimally with better yield (Battacharyya et al., 2015).

Furthermore, the beneficial influence may have resulted from the activity of microorganisms contained in the biofertilizer product.

It is enriched with *Azotobacter and Bacillus megaterium* microbial agents that increase soil fertility via decomposition of organic material into organic matter that acts like a nutrient, thereby increasing nutrient availability for plant as well as their works as regulators of the suitable progress of plant outgrowth and productivity (Bhat et al., 2015). On the other hand, the inorganic fertilizer NPK may also has contributed to ameliorated foliage growth and yield of lettuce through providing N, P, and K required for plant growth and productivity. The results agree with Ahmed et al., (2000) on effect of biofertilizer on lettuce plants, Chrysargyris et al. (2018) on effect of seaweed on cut lettuce and Nermadodzi et al. (2017) on the influence of NPK fertilizer on baby spinch.

Bender Özenç and Şen (2017) reported that the application of liquid seaweed to grafted and ungrafted tomato plants under greenhouse conditions positively affects plant growth. In addition, they reported that liquid seaweed applications increased the availability of nutrients, optimizing their delivery to the needed tissues, and the increased yield is due to this effect. Kiraci et al. (2013) reported that the use of microbial fertilizers in organic carrot cultivation increased the quality. Crouch et al. (1990) investigated the effect of liquid algae extract on growth and nutrient content of lettuce and it was noted that algae extract increased the product amount and the amount of Ca, K, Mg in the leaves. Crouch and Staden (1992) applied seaweed extract from soil and leaves to tomato seedlings and reported that soil application during the seedling period and foliar application during the flowering period gave better results, and the algae extract increased the plant fresh weight and root weight. Abetz and Young (1983) reported increased yield and head diameter in lettuce and cauliflower plants treated with seaweed. The results of our study are consistent with the literature reported above.

Conclusion

In recent years, consumers prefer that the product they consume is grown organically and that environmentally friendly techniques are used during production. According to the results obtained, it caused an increase in head weight, yield and nitrogen content depending on the increasing doses of seaweed applied, while it caused a decrease in leaf area. In addition, seaweed application increased the leaf number and phosphorus content compared to the control. The effects of seaweed applications on chlorophyll, stem length and potassium content were found to be statistically insignificant.

With the application of biofertilizer, the number of leaves, yield, head weight, phosphorus and potassium contents increased, and the amount of nitrogen decreased compared to the control. The effects of biofertilizer applications on chlorophyll content and stem length were found to be insignificant.

While inorganic fertilizer application increased the stem length and phosphorus content, decreased the number of leaves and leaf area. The effects of inorganic fertilizer application on chlorophyll content, head weight, yield per square meter, nitrogen and potassium content were found to be insignificant.

Therefore, it is recommended that application of biofertilizer, seaweed and inorganic fertilizer, especially in combination, is essential for optimum production of lettuce crop with more scientific research to be carried out in this field.

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