

Nitrogen Sources and Algae Extract as Candidates for Improving the Growth, Yield and Quality Traits of Broccoli Plants

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ABSTRACT

Increasing consumer awareness has contributed in establishing food safety issues and environmental concerns through introducing organic and bio-approaches as substitutes for the traditionally chemical fertilization. However, the efficiency of these fertilizers seemed to be relatively low when compared with the traditional mineral fertilizers. This study postulates that algae extracts of *Chlorella sp.* might stimulate the growth of plants. To examine this assumption, a field study was conducted for two successive seasons (2015/2016 and 2016/2017) using different N-sources e.g. organic (compost), bio (*Azotobacter chroococcum* and *Azospirillum lipoferum*) and mineral (ammonium nitrate) fertilizers with or without algal extracts of *Chlorella sp.* to investigate their efficiency for improving the growth parameters and yield components of broccoli plants grown on a sandy loam soil. Results reveal that the highest increases in uptake of NPK were attained for the application of "compost + ammonium nitrate + biofertilizer". Such improvements resulted in concurrent increases in plant height, number of leaves per plant, leaf area; shoot fresh weight, chlorophyll a and b, curd weight and diameter, vitamin C, TSS, total phenol and antioxidants content in broccoli heads and the outcome head yield. Moreover, the combination between different N-sources and foliar spray of alga extract resulted in further significant increases in NPK uptake by plants and this in turn improved significantly, the abovementioned growth parameters and yield components. Accordingly, our results support the hypothesis indicating that algae extracts improved significantly the growth parameters and yield components of broccoli plants grown on a sandy loam soil.

Keywords: broccoli, N-sources, compost, biofertilizer, *Azotobacter*, *Azospirillum*, algal extracts, *Chlorella sp.*, growth, head yield.

INTRODUCTION

Broccoli (*Brassica oleracea*) is an economically vegetable crop (Branham *et al.*, 2017) that contains a number of bioactive compounds, in particular glucosinolates and polyphenols (Valverde *et al.*, 2015). These compounds are beneficial against cancer and other illnesses (Ordiales *et al.*, 2017). Moreover, this crop is rich in minerals (Weber, 2017), vitamins A, B12 and C (Decoteau *et al.*, 2000); besides, its stem and leaves contain carotenoids which have anti-allergic, anti-cancer and anti-obesity bioactivities (Pedroza *et al.*, 2018). Thus, broccoli serves as a good food (Jeffery *et al.*, 2003; Rodriguez-Casado, 2016).

Managing N-inputs is one of the important factors affecting broccoli growth and yield development (Schellenberg *et al.*, 2009; Luna *et al.*, 2018) especially in light textured soils (Abd-Alrahman *et al.*, 2016). Although, the traditional farming is still dependent on mineral N-fertilization for vegetables production because these inorganic forms are easily absorbed and utilized by vegetables (Shams, 2012); however, significant losses might occur in mineral N-forms either through ammonia volatilization or nitrate leaching (Peng *et al.*, 2015; Kadyampakeni *et al.*, 2018). This might contribute effectively in N- pollution of the groundwater (Smith *et al.*, 2016) and consequently raises concerns about environmental sustainability (Kadyampakeni *et al.*, 2018). Thus, increasing consumer awareness has contributed in establishing food safety issues and environmental concerns (Worthington, 2001) through introducing organic and bio-approaches as substitutes for the traditionally mineral fertilization (Abbas *et al.*, 2011; Farid *et al.*, 2014 and 2018). Such approaches were used successfully in the production of several vegetable crops e.g. pepper (Abdalla *et al.*, 2001), cantaloupe (Adam *et al.*, 2002), squash (Rizk *et al.*, 2003), potatoes (Abou-Hussein *et al.*, 2002; Salem *et al.*, 2010), sweet pepper (Shams, 2011), snap beans (Morsy *et al.*, 2017). In this concern, the organic amendments not only enrich soils with nutrients which release upon its

decomposition, but also improve the physical, chemical and biological characteristics of the amended soils (Farid *et al.*, 2014; Bassouny and Abbas, 2019). However, the efficiency of these fertilizers seemed to be relatively low when compared with the traditional mineral fertilizers (Yildirim *et al.*, 2011). In this concern, the outcome head yield of broccoli obtained after application of 80 Mg organic amendments per hectare did not exceed 75% of the corresponding yield obtained for the application of the full dose of N as mineral fertilizers (Ouda and Mahadeen, 2008). Likewise, biofertilization improves the head yield; however, such increases stand at about 10 % below the increases occurred due to the application of the mineral N-fertilizer (Yildirim *et al.*, 2011). Thus, there is an actual need to increase the efficiency of N-use by plants. This might take place through introducing symbiotic algae (Quispe *et al.*, 2016) which improve the efficiency of N use efficiency by plants e.g. *Chlorella sp.* (Qin *et al.*, 2018). Moreover, this alga is thought to be beneficial to plant growth because of its ability to produce a variety of valuable compounds such as polyunsaturated (Lin *et al.*, 2018).

Algae extracts can be used foliarly to improve vegetable growth as they contain nutrients such as N, P, K, Ca, Mg and S as well as Zn, Fe, Mn, Cu, Mo and Co, some growth regulators, polyamines, vitamins (Zhang and Ervin, 2004 and Papenfus *et al.*, 2013), cytokinins, auxines, abscisic acid, vitamins and nutrients (Chojnacka and Kim, 2013). This study postulates that the algae extracts of *Chlorella sp.* might stimulate the growth of broccoli plants while, on the other hand, improve its efficiency to utilize soil N. To what extent can the organic, bio- and mineral fertilizers stand successfully for the production of broccoli grown on a sandy soil in presence of *Chlorella sp.* extract was the aim of the current study. Parameters defining quality (e.g. diameter, weight, and height of the heads) (Ordiales *et al.*, 2017) were considered in this study.

The current study aims at evaluating the efficiency of using different N-sources e.g. organic, bio and mineral fertilizers with or without alga extracts of *Chlorella sp.* on

the growth parameters and yield components of broccoli plants grown on a sandy loam soil for two successive seasons.

MATERIALS AND METHODS

Materials:

Surface soil samples (0-30 cm) were collected from the Experimental Farm of the Environmental Studies &

Research Institute (ESRI), EL-Sadat City University. These samples were air dried, sieved to pass through 2-mm sieves and then analyzed for their particle size distribution as well as the chemical characteristics as outlined by Sparks *et al.* (1996) and Klute (1986).

Table 1. Physical and chemical analysis of the soil.

Particle size distribution (%)				pH*	EC**	CaCO ₃	Organic matter			
Sand	Silt	Clay	Texture		dS m ⁻¹		g kg ⁻¹			
70.0	26.0	4.0	Sandy loam	7.2	4.52	5.5	8.0			
Available NPK (mg kg ⁻¹)			Cations (mmole L ⁻¹)			Anions (mmole L ⁻¹)				
N	P	K	Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺	C O ^{- -}	H CO ₃ ⁻	Cl ⁻	SO ₄ ⁻
25	14	80	16.1	9.8	17.1	2.2	0.0	8.0	23.0	14.1

*pH of 1:2.5 w:v water soil suspension and **EC of paste extract

Seeds of broccoli (*Brassica oleracea var. italica* cv. Waltham 29) were obtained from Modesto Seed Co. Inc., Modesto, California.

Preparation of Alga Cells Extract: A fresh slurry of the microalgae *Chlorella vulgaris* (contains about 10% water)

was washed with distilled water, re-concentrated by centrifugation, frozen then re-melted at room temperature. Afterwards, the melted slurry was centrifuged at 5000 rpm to obtain a clear cell sap. Major components and nutrient contents of the algal extract are shown in Table 2.

Table 2. Major chemical composition and elemental contents of *Chlorella vulgaris* cells extract

Fats %	7.3	Macro-elements %	Micro-elements (ppm)
Carbohydrates %	12.8	N 7.1	Fe 245.0
Protein %	44.6	P 0.66	Mn 131.2
Amino acid composition (g/100g protein)			
Arginine 6.9	Methionine 1.3	K 2.15	Zn 111.5
Histidine 2.0	Phenylalanine 5.5	Ca 0.18	Cu 28.0
Isoleucine 3.2	Threonine 5.3	Mg 0.34	
Lucien 9.5	Tryptophan 1.5	Na 0.04	
Lysine 6.4	Valine 7.0		

Source El-Fouly *et al.* (1992)

The compost manure: Compost manure was obtained from Faculty of Agriculture, Benha University, Egypt. Chemical composition of compost manure is presented in Table3.

Table 3. Chemical analysis of compost

Parameter	Unit	Value
pH	-	8.11
EC (1:5 extract)	dS m ⁻¹	8.21
Organic matter	%	21.57
Organic-C	%	12.54
Total-N	%	1.21
C:N ratio	-	10.36 : 1
Total-P	%	0.91
N-NH ₄	mg kg ⁻¹	274.7
N-NO ₃	mg kg ⁻¹	50.1

Biofertilizers: A biofertilizer solution containing 750 mL of N₂- fixing free living bacterial cultures (*Azotobacter chroococcum* and *Azospirillum lipoferum*) was prepared in the Microbiology Department, Faculty of Agriculture, Benha University, Egypt. Roots of broccoli transplants were dipped for about 10 minutes in a solution of the biofertilizers together with 10g Arabic gum as an adhesive material before transplanting.

The field study

A field experiment was carried out at the Experimental Farm of the Environmental Studies and Research Institute, EL-Sadat City University for two successive seasons i.e. 2015/2016 and 2016/2017 to study the implications of amending soils with five different N fertilizers sources in combination with the foliar spray of alga cells

extract to maximize the yield of broccoli and improve its quality. This experimental were carried out in a split plot design where the main plot comprises the following N-treatments (compost at a rate of 216 kg N ha⁻¹ (N1), 216 kg N ha⁻¹ + biofertilizers (N2), compost at a rate of 108 kg N ha⁻¹ + 108 kg N ha⁻¹ ammonium nitrate (N3), compost at a rate of 108 kg N ha⁻¹ + 108 kg N ha⁻¹ ammonium nitrate + biofertilizer (N4), 216 kg N ha⁻¹ as ammonium nitrate). The subplots received foliar application of alga cells extract at four different rates (0 (F1), 2.5 (F2) and 5 mL L⁻¹ (F3) alga cells extracts in distilled water). Each plot included 4 ridges and the plot area was 11.2 m² and the treatments were conducted in three replicates. Within the first week of October (during both seasons of study), all plots were transplanted with broccoli at 30 cm apart from each other on one side of ridges (80 cm wide and 3.5 m long) in presence of water. All plants received PK fertilizers at the recommended doses of the Egyptian Ministry of Agriculture i.e. 63 kg P ha⁻¹ as calcium super phosphate, 70 g P kg⁻¹ (16 % P₂O₅), and 96 kg K ha⁻¹ as potassium sulphate, 400 g K kg⁻¹ (48 % K₂O). Other agricultural practices were followed as usual. Plants were sprayed with alga cells extract at the following time period: 30, 45 and 60 days after transplanting.

Data collection

Plant growth parameters

Five plants were sampled from each plot the 60 days after transplanting to estimate the following growth parameters i.e. Plant height, number of leaves, leaf area (the 5th true from plant top was measured by using laser leaf area meter) and foliage fresh weight per plant.

Yield, yield components and yield quality

At the physiological maturity growth stage (85 days after transplanting), total yield of broccoli heads (ton per hectare) broccoli head weights and diameters were estimated for each treatment.

Chemical characteristics of broccoli plants and the quality traits

Chemical characteristics of broccoli were determined according to the standard methods described by AOAC (2000) i.e. chlorophyll a and b were determined colorimetrically after being extracted with ethanol, total soluble solids (TSS) was determined by the refractometer, ascorbic acids (vitamin C) was determined by titration in presence of 2, 6-di-chlorophenolindophenol as an indicator.

Samples of broccoli shoot and head were taken from each plot, oven dried at 70° C for 48 h, weighed, ground, and then digested in a mixture of conc. sulphuric and perchloric acids (2:1 ratio) as mentioned by Page *et al.* (1982). N, P, K and B were determined in the plant digests according to AOAC (2000) as follows: : N by micro-Keldahl, P by spectrophotometer (Jenway 6705 UV/Vis) using ammonium molybdate and ascorbic acid reagents, K by flame photometer (Jenway pfp7) and B using Inductively Coupled Plasma Spectrometry (Ultima 2 JY Plasma). Total phenols (mg g⁻¹ F.W) and antioxidant (μ mol g⁻¹ F.W) were determined according to Lu *et al.* (2007) by the Folin-Ciocalteu spectrophotometer. The enzymes activity of dehydrogenase (DHA) was measured in the rhizosphere using the method of Schinner *et al* (1997) at 7, 30 and 60 days from transplanting.

Statistical analysis

The obtained were subjected to the analysis of variance as a factorial experiment in a split plot design and Duncan test was considered to differentiate between means according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Results

Growth parameters of broccoli as affected by the application of N-fertilizer sources and alga cell extract either solely or in combinations

Data presented in Table (4) show that the highest increases in plant growth parameters i.e. plant height, number of leaves per plant, leaf area, shoot fresh weight, chlorophyll A and B, attained in both the growing seasons, were due to the application of 108 kg N ha⁻¹ as compost manure + 108 kg N ha⁻¹ as ammonium nitrate + biofertilizer (N4). The treatment 108 kg N ha⁻¹ as compost manure + 108 kg N ha⁻¹ as ammonium nitrate (N3) came after N4 recording significant increases in plant height and leaf area as compared to the 216 kg N ha⁻¹ as ammonium nitrate (reference treatment). Although, application of 216 kg N ha⁻¹ as compost manure (N1) recorded significant reductions in plant height, number of leaves and leaf area in both seasons, however, its application with the biofertilizer (N2) improved significantly the plant height, plant fresh weight and chlorophyll B. Moreover, such increases seemed to be comparable with those attained due to the application of the reference treatment.

Table 4. Growth parameters of broccoli as affected by the application of N- sources and alga cell extract either solely or in combinations

Nitrogen sources (N)	Foliar spray rate (F)															
	First season				Second season				First season				Second season			
	F0	F1	F2	Mean	F0	F1	F2	Mean	F0	F1	F2	Mean	F0	F1	F2	Mean
	plant height (cm)								number of leaves per plant							
N1	43.70i	46.14efg	46.35def	45.40D	42.65j	46.55efgh	46.87defgh	45.36D	14.98h	16.86efg	17.13ef	16.32D	15.45i	17.12def	17.25def	16.61D
N2	44.06hi	46.58cdef	47.23cde	45.96C	43.79ij	46.44efgh	48.59bcd	46.27C	16.12g	17.24def	17.20def	16.85C	15.94hi	17.06ef	18.2bc	17.07C
N3	45.35fgh	47.97bcd	49.29ab	47.54B	46.06fgh	48.27cde	50.27b	48.20B	16.15g	18.20bc	18.55ab	17.63B	16.26gh	17.3def	17.43cde	17.00C
N4	45.62efgh	48.21bc	50.59a	48.14A	45.82gh	50.06bc	52.82a	49.57A	16.49fgh	18.09bcd	19.41a	18.00A	16.58fgh	18.31b	19.20a	18.03A
N5	44.58ghi	47.10cde	47.00cdef	46.23C	45.11hi	47.81def	47.55defg	46.82C	15.95g	17.41cdef	17.61bcd	16.99C	16.94efg	17.69cde	17.87bcd	17.50B
Mean	44.66C	47.20B	48.09A		44.69C	47.83B	49.22A		15.94B	17.56A	17.98A		16.23C	17.50B	17.99A	
	chlorophyll a (mg/100g FW)								Leaf area (cm ²)							
N1	44.52h	63.64ef	68.42de	58.86C	46.83j	71.87cdefg	68.42defg	62.37D	3628g	4133ef	4279cde	4013D	3783fg	3929efg	3935efg	3882D
N2	51.45gh	73.12cde	76.64cd	67.07B	51.45ij	63.49fgh	82.62bc	65.85C	3725g	4308cde	4276cde	4103C	3821fg	3842fg	404def	3901C
N3	62.04efg	82.62bc	90.30ab	78.32A	55.85hij	76.64cde	71.25cdefg	67.91C	4091ef	4596bc	4620bc	4436B	3826fg	418cde	4373c	4126B
N4	64.97def	82.23bc	95.79a	81.00A	60.65ghi	90.07ab	95.79a	82.17A	4223def	4893ab	5172a	4763A	3761g	469.7b	5024a	4494A
N5	55.85fgh	73.57cde	71.25cde	66.89B	64.88efgh	73.57cdef	79.76bcd	72.74B	3886fg	4524cd	4194def	4201C	3895fg	3935efg	4224cd	4018C
Mean	55.77C	75.04B	80.48A		55.93B	75.13A	79.57A		3910.6B	4490.8A	4508A		3817C	411.7B	431.9A	
	chlorophyll b (mg/100g FW)								shoot fresh weight (g)							
N1	30.67i	39.34efgh	42.13defg	37.38D	31.28h	42.52defg	42.09def	38.63C	4268g	5827def	6111cde	5402C	5056h	6194cdef	6316cde	5855C
N2	35.67hi	43.27cdef	44.22cde	41.05C	35.67gh	38.47efg	48.48bc	40.87BC	5278f	6171cd	6128cde	5859B	5209gh	5775efgh	708.1b	602.2BC
N3	37.48fgh	48.48bc	53.33b	46.43B	36.15gh	44.22cde	47.62bcd	42.66B	5601ef	6741ab	6890ab	641.1A	5389gh	6408bcd	6883bcd	612.7BC
N4	38.84efgh	49.06bc	60.15a	49.35A	37.26fg	53.31b	60.15a	50.24A	5690def	6487bc	7135a	643.7A	5550fgh	7075b	825.5a	696.0A
N5	36.21ghi	42.02defg	47.62bcd	41.95C	39.75efg	42.02def	46.99cd	42.92B	5439f	6102cde	6110cde	588.4B	587.7defg	6240cdef	676.3bc	629.3B
Mean	35.77C	44.43B	49.49A		36.02C	44.11B	49.07A		5255.5B	6265.5A	6475.5A		541.6C	638.8B	700.0A	

N-source (N): 216 kg N ha⁻¹ as compost manure (N1), 216 kg N ha⁻¹ as compost manure + biofertilizer(N2), 108 kg N ha⁻¹ as compost manure + 108 kg N ha⁻¹ as ammonium nitrate (N3), 108 kg N ha⁻¹ as compost manure + 108 kg N ha⁻¹ as ammonium nitrate + biofertilizer (N4) and 216 kg N ha⁻¹ as ammonium nitrate (N5). Foliar spray rate with alga cell extract (F): F0, F1, F2 corresponding to 0, 2.5 mL L⁻¹ and 5 mL L⁻¹ alga cells extract in distilled water, respectively.

In case of spraying plants with the algal extract, results reveal that this extract improved significantly plant growth parameters as compared with the control treatment (only distilled water). Moreover, increasing the dose of application from 2.5 mL L⁻¹ (F1) to 5 mL L⁻¹ (F2) resulted

in further significant increases in the plant height and chlorophyll b during both seasons of study. Although, increasing the rate of application increased the other growth parameters, however, many of these increases i.e.

number of leaves, leaf area and shoot fresh weight occurred in the second season only.

The highest increases in plant growth parameters were attained by the treatment N4F2, which recorded significant increases in plant height, number of leaves, leaf area and shoot fresh weight during the second season only, while the increases in chlorophyll b were significant during both seasons of study. The treatments “N3F2” and N4F1 came second recording significant improvements in all the investigated plant growth parameters. Likewise, N5F2 recorded comparable; however, insignificant increases with N3F2 and N4F1

N-source (N): 216 kg N ha⁻¹ as compost manure (N1), 216 kg N ha⁻¹ as compost manure + biofertilizer(N2), 108 kg N ha⁻¹ as compost manure + 108 kg N ha⁻¹ as ammonium nitrate (N3), 108 kg N ha⁻¹ as compost manure + 108 kg N

ha⁻¹ as ammonium nitrate + biofertilizer (N4) and 216 kg N ha⁻¹ as ammonium nitrate (N5). Foliar spray rate with alga cell extract (F): F0, F1, F2 corresponding to 0, 2.5 mL L⁻¹ and 5 mL L⁻¹ alga cells extract in distilled water, respectively.

Yield, yield components and quality of head broccoli as affected by the application of N-fertilizer sources and alga cell extract either solely or in combinations

The treatment that received 108 kg N ha⁻¹ as compost manure + 108 kg N ha⁻¹ as ammonium nitrate + biofertilizer (N4) recorded the highest increases in the total yield per hectare, the average weight and height of the heads as well as vitamin C and TSS. In the second order, comes N3 and N5 treatments which recorded significant increases in the head diameter during both seasons of study (Table 5).

Table 5. Yield, yield components and quality traits of head broccoli as affected by the application of N-fertilizer sources and alga cell extract either solely or in combinations

Nitrogen sources (N)	Foliar spray rate (F)							
	First season				Second season			
	F0	F1	F2	Mean	F0	F1	F2	Mean
Total yield of heads (ton ha ⁻¹)								
N1	12.847 j	14.923 fg	15.288 ef	14.352 E	14.297 h	15.022 f	15.401 f	14.906 D
N2	13.438 i	15.559 de	16.020 d	15.005 D	14.491 h	15.528 ef	15.672 def	15.230 C
N3	14.467 gh	16.714 c	17.770 b	16.318 B	14.494 gh	15.766 de	15.835 d	15.365 C
N4	14.964 fg	17.138 c	19.039 a	17.047 A	14.863 f	17.155 c	18.722 a	16.913 A
N5	13.894 hi	15.902 d	15.900 d	15.233 C	14.784 fg	15.859 d	17.930 b	16.190 B
Mean	13.92 C	16.05 B	16.802 A		14.585 C	15.866 B	16.711 A	
Curd weight (g)								
N1	577.5 f	637.7 cdef	632.2 cdef	615.8 D	585.1 g	642.6 de	632.2 defg	620.0 C
N2	586.2 ef	645.4 cde	649.6 cd	627.1 CD	586.2 fg	635.3 defg	666.7 d	629.4 C
N3	629.3 cdef	666.7 c	677.9 c	658.0 B	600.8 efg	649.6 de	653.2 de	634.5C
N4	688.8 c	836.5 b	909.9 a	811.7 A	676.5 cd	727.0 bc	909.9 a	771.1 A
N5	600.8 def	657.6 cd	653.2 cd	637.2 C	640.2 def	657.6 d	758.2 b	685.3 B
Mean	616.5 B	688.7 A	704.6 A		617.8 C	662.4 A	724.0 A	
Curd diameter (cm)								
N1	24.56 f	25.32 def	25.74 bcd	25.21 D	24.59 i	25.50 efg	25.74 def	25.28 C
N2	24.65 f	25.60 cde	25.65 cde	25.30 D	24.65 hi	25.42 efg	26.42 cd	25.50 C
N3	25.34 def	26.42 bc	26.57 b	26.11 B	25.41 efgh	26.09 cde	27.28 b	26.26 B
N4	25.55 de	27.60 a	28.28 a	27.14 A	25.22 fg	26.60 bc	28.28 a	26.70 A
N5	24.87 ef	26.09 bcd	25.67 cde	25.54 C	24.87 ghi	25.65 def	25.67 def	25.40 C
Mean	24.99 B	26.21 A	26.38 A		24.95 C	25.85 A	26.68 A	
Vitamin C (mg/100g FW)								
N1	88.94 f	97.36 def	102.90 cd	96.40 D	89.79 gh	100.30 def	102.90 cde	97.66 C
N2	89.18 f	101.40 cde	100.80 cde	97.13 D	89.18 h	98.61 efg	110.40 bc	99.40 C
N3	96.44 def	110.40 bc	113.30 b	106.70 B	91.76 fgh	100.80 def	110.00 bcd	100.90BC
N4	99.55 de	118.80 b	136.10 a	118.20 A	95.82 efgh	113.30 b	136.10 a	115.10 A
N5	91.76 ef	103.30 cd	110.00 bc	101.70 C	97.99 efgh	103.30 cde	115.00 b	105.40 B
Mean	93.17 C	106.25 B	112.62 A		92.91 C	103.26 B	114.88 A	
Total soluble solids (TSS %)								
N1	5.64 i	6.76 efg	6.78 defg	6.39 D	5.77 i	6.90 defg	6.78 efgh	6.48 D
N2	5.87 hi	6.98 def	7.21 cde	6.69 C	5.87 i	6.62 fgh	7.55 bcd	6.68 CD
N3	6.45 fgh	7.55 bcd	8.07 ab	7.36 B	6.14 hi	7.21 cdef	7.13 cdef	6.83 C
N4	6.72 efg	7.79 bc	8.66 a	7.72 A	6.37 ghi	8.05 ab	8.66 a	7.69 A
N5	6.14 ghi	7.31 cde	7.13 cdef	6.86 C	6.83 efg	7.31 cde	7.62 bc	7.25 B
Mean	6.16 B	7.28 A	7.57 A		6.20 C	7.22 B	7.55 A	

See footnote Table 4.

On the other hand, the treatment 216 kg N ha⁻¹ as compost manure (N1) recorded the least values in the outcome yield, average head weight and TSS during both seasons. It is worthy to mention that this treatment recorded comparable results with N2 in the head diameter and vitamin c.

Foliar application with algal extract improved significant yield properties and heads quality as compared with plants sprayed with distilled water. Moreover, increasing the dose of application from 2.5 mL L⁻¹ (F1) to 5 mL L⁻¹ (F2) resulted in further significant increases in yield, vitamin C and TSS were recorded for both seasons,

while recorded comparable effect on average weigh and diameter of the heads.

The highest increases in yield and quality of heads were attained by the treatment N4F2, which recorded significant increases in the outcome yield, average head weight, head diameter, TSS and vitamin c during both seasons of study. N3F2 and N5F2 came second and improved significantly total yield per hectare. Likewise, N4F1 came in the third rank and recorded comparable and insignificant increases with N3F2 and N5F2 in head quality (TSS and vitamin c).

Total phenol and antioxidants content in broccoli heads as affected by the application of N-fertilizer sources and alga cell extract either solely or in combinations

Application of nitrogen fertilizers either in the organic form solely or combined with mineral fertilizers (+/- biofertilizers, i.e. N1, N2, N3 and N4) increased significantly the total phenol and antioxidants content in broccoli heads as compared with the application of 100% mineral nitrogen fertilizer (N5). In this concern, there were

no significant variations in the total phenol and antioxidant activity content in heads among these four sources of N-fertilizers. In this concern, the highest increases in the total phenols and antioxidants in broccoli heads were recorded for the application of “108 kg N ha⁻¹ as compost manure + 108 kg N ha⁻¹ as ammonium nitrate” with or without biofertilizer. Likewise, spraying plants with alga cells extract (2.5 or 5 mL L⁻¹) recorded further significant increases in the investigated heads quality, i.e. total phenol and antioxidants as compared to those received distilled water (Table 6).

It is worthy to mention that the interaction between the soil application of organic fertilizer treatments (N1, N2, N3 and N4) and the foliar application of alga cell extracts (0, 2.5 and 5 mL L⁻¹) (F0, F1 and F2) recorded the highest significant increases in the total phenol and antioxidant contents in heads during the two studied seasons. Such increases exceeded the corresponding ones attained for the application of 100% mineral-N and sprayed with or without alga cell extracts (N5F0, N5F1 and N5F2).

Table 6. Total phenol and antioxidants content in broccoli heads as affected by the application of N-fertilizer sources and alga cell extract either solely or in combinations

Nitrogen sources (N)	Foliar spray rate (F)							
	First season				Second season			
	F0	F1	F2	Mean	F0	F1	F2	Mean
Total phenol mg/g ww								
N1	0.557 bc	0.592 abc	0.645 abc	0.598 AB	0.545 bc	0.601 abc	0.629 abc	0.592 AB
N2	0.595 abc	0.625 abc	0.672 abc	0.631 AB	0.582 abc	0.634 abc	0.659 abc	0.625 AB
N3	0.696 abc	0.709 abc	0.744 ab	0.716 A	0.685 abc	0.720 ab	0.721 ab	0.709 A
N4	0.727 ab	0.735 ab	0.765 a	0.743 A	0.706 ab	0.746 a	0.753 a	0.735 A
N5	0.507 d	0.522 c	0.557 bc	0.529 B	0.532 bc	0.543 bc	0.492 c	0.522 B
Mean	0.616 B	0.637 AB	0.677 A		0.610 B	0.649 AB	0.651 A	
Antioxidant activity (micro mol/g ww)								
N1	0.399 cde	0.431 bcde	0.477 abcde	0.435 BC	0.389 bcd	0.438 abcd	0.466 abcd	0.431 BC
N2	0.483 abcde	0.490 abcde	0.497 abcde	0.490 AB	0.476 abcd	0.481 abcd	0.493 abcd	0.483 AB
N3	0.517 abcd	0.537 abc	0.580 ab	0.544 AB	0.501 abc	0.548 ab	0.561 a	0.537 AB
N4	0.551 abc	0.572 ab	0.617 a	0.580 A	0.532 abc	0.584 a	0.600 a	0.572 A
N5	0.337 e	0.360 de	0.400 cde	0.365 C	0.324 d	0.368 cd	0.388 bcd	0.360 C
Mean	0.457 B	0.478 AB	0.514 A		0.444 B	0.484 AB	0.502 A	

See footnote Table 4.

N, P and K contents in broccoli as affected by application of N-fertilizer sources and alga cell extract either solely or in combinations

Results shown in Table 7 reveal that the foliar application of alga cell extract (F1 and F2) increased significantly concentrations of N and K within the leaves and heads of broccoli plants during the two successive years of study i.e. 2016/2017 and 2017/2018 as compared with the control treatments (distilled water, F0). The effect of alga cell extract was also pronounced on increasing P contents in broccoli shoots during the two successive years of study, yet this effect seemed to be significant on P content in heads of broccoli only in the first growing season. The treatment that received 50% organic-N+50% inorganic-N + biofertilizer (N4) resulted in further significant increases in concentrations of the investigated nutrients within the heads and shoots of broccoli plants than other nitrogen fertilizer sources. Results also reveal that concentrations of N, P and K increased significantly in both shoots and heads of broccoli owing to the dual application of N4 and F2

(N4F2) as compared to the reference treatment (N5F0). Likewise, the treatment N4F1 improved significantly the nutrient contents within shoots and heads of broccoli. Coming second after N4F2, its insignificant effect was detected on N and P contents in shoots of broccoli in both seasons.

Soil enzyme activity

Significant increases occurred in the activity of dehydrogenase enzyme when soil treated with either compost or biofertilizer as compared to the mineral (inorganic) N-fertilizer (Table 8). In this concern, the highest activity of DH enzyme was attained due to the application of N4 (50 % mineral-N + 50 % organic-N + biofertilizer) which recorded the highest increases in the vegetative growth and total yield as shown in Tables 4 and 5. On the other hand, the activity of DH enzyme increased progressively after broccoli transplanting and reached the highest values after 30 days from transplanting; afterwards, significant reductions occurred.

Table 7. The nutritional status of broccoli as affected by application of N-fertilizer sources and alga cell extract either solely or in combinations

Nitrogen sources (N)	Foliar spray rate (F)															
	Shoot							Heads (flowering buds)								
	First season			Second season				First season			Second season					
	F0	F1	F2	Mean	F0	F1	F2	Mean	F0	F1	F2	Mean	F0	F1	F2	Mean
	N (g kg ⁻¹)															
N1	3.04 h	3.40 efg	3.48 ef	3.31 D	3.24 g	3.65 cd	3.55cde	3.48 D	3.37 g	3.68 ef	3.73 e	3.59 D	3.52 gh	3.69 ef	3.95 c	3.72 C
N2	3.20 gh	3.60 cde	3.58cdef	3.46 C	3.24 g	3.51cdef	3.87 ab	3.54 C	3.42 g	3.77de	3.82cde	3.67 C	3.69 ef	3.74def	3.65 fg	3.69 C
N3	3.32 fg	3.81 bc	3.97 ab	3.70 B	3.50def	3.65cd	3.69bcd	3.61B	3.65ef	3.96bcd	4.14ab	3.92 B	3.83cde	3.97bc	3.83cde	3.88A
N4	3.51def	3.88ab	4.13a	3.84A	3.44efg	3.94a	3.94a	3.77A	3.68ef	4.02bc	4.32a	4.01A	3.67fg	3.76def	4.29a	3.91A
N5	3.17gh	3.76bcd	3.53def	3.49C	3.33fg	3.71bc	3.56cde	3.53C	3.5 fg	3.77de	3.81cde	3.69 C	3.47h	3.85cd	4.12b	3.81B
Mean	3.25 B	3.69 A	3.74 A		3.35 B	3.69 A	3.72 A		3.52 C	3.84 B	3.96 A		3.64B	3.80AB	3.97A	
	P (g kg ⁻¹)															
N1	0.243f	0.333bcd	0.330bcd	0.302D	0.255g	0.323bcd	0.310cdf	0.296C	0.243h	0.313def	0.333de	0.296E	0.32def	0.378bc	0.28fg	0.325D
N2	0.278ef	0.340bcd	0.335bcd	0.318C	0.270fg	0.303cdf	0.343abc	0.305C	0.260gh	0.335cde	0.353cd	0.316D	0.33def	0.305efg	0.32def	0.318D
N3	0.310de	0.360bc	0.360bc	0.343B	0.295defg	0.329abcde	0.368ab	0.331AB	0.300efg	0.380bc	0.415b	0.365B	0.39bc	0.343cde	0.36cd	0.363B
N4	0.320cde	0.368ab	0.413a	0.367A	0.288efg	0.369a	0.370a	0.342A	0.315def	0.400b	0.470a	0.395A	0.35cde	0.310efg	0.47a	0.375A
N5	0.343bcd	0.298de	0.333bcd	0.325C	0.310cdf	0.320cde	0.340abcd	0.323B	0.275fgh	0.353cd	0.353cd	0.327C	0.36cd	0.410b	0.27g	0.346C
Mean	0.299B	0.340A	0.354A		0.284B	0.329A	0.346A		0.279C	0.356B	0.385A		0.35A	0.349A	0.34A	
	K (g kg ⁻¹)															
N1	2.43 f	2.52 ef	2.57 de	2.51 D	2.37 h	2.52cdef	2.50 def	2.46 C	1.70 i	1.90 efg	1.91 efg	1.84 D	1.90fghi	2.01 cd	1.84 ij	1.92CD
N2	2.48 ef	2.57 de	2.62 cd	2.56 C	2.40 gh	2.45efgh	2.59 bcd	2.48 C	1.77 hi	1.96 cdef	1.98 bcde	1.90C	1.95defg	1.88hi	1.90fghi	1.91 D
N3	2.50 ef	2.63 cd	2.76 ab	2.63 B	2.43 fgh	2.54 cde	2.59 bcd	2.52 B	1.87 fgh	2.02 bcd	2.08 a	1.99B	2.02bc	1.95defg	1.93efgh	1.96AB
N4	2.51 ef	2.68 bc	2.84 a	2.68 A	2.44 fgh	2.65 b	2.80 a	2.63 A	1.90 efg	2.03 bcd	2.13 a	2.02A	1.96cdef	1.89ghi	2.13a	1.99A
N5	2.50 ef	2.55 de	2.64 cd	2.56 C	2.47 efg	2.50 def	2.60 bc	2.52 B	1.83 gh	1.97 cdef	1.92 defg	1.91C	1.99cde	2.08ab	1.80j	1.95BC
Mean	2.48 C	2.59 B	2.69 A		2.42 C	2.53 B	2.61 A		1.81 B	1.98B	2.01 A		1.96C	1.96B	1.92A	

See footnote Table 4.

Table 8. Effect of mineral, organo- and bio-fertilizer combinations on dehydrogenase activity.

Treatments	Soil dehydrogenase (µg TPF g ⁻¹ dw h ⁻¹)							
	First season				Second season			
	7 days	30 days	60 days	Mean	7 days	30 days	60 days	Mean
N1	19.7 ef	31.8 bcd	25.7 cdef	25.7 B	20.4 cd	33.1 b	27.1 bcd	26.9 BC
N2	21.4 def	38.8 ab	29.5 bcdef	29.9 AB	21.6 cd	45.8 a	30.4 bcd	32.6 AB
N3	20.2 ef	32.4 bc	26.4 cdef	26.3 B	20.8 cd	33.7 b	29.4 bcd	28.0 BC
N4	21.4 def	47.2 a	30.3 bcde	33.0 A	21.7 cd	53.4 a	30.9 bc	35.3 A
N5	19.3 f	29.6 bcdef	24.1 cdef	24.3 B	19.5 d	31.1 bc	23.4 bcd	24.7 C
Mean	20.4 B	35.9 A	27.2 AB		20.8 B	39.4 A	28.2 AB	

For more details about treatments: N1 to N5 see footnote Table 4

Values followed by the same letters are not significantly different by Duncan's test at 0.05 level

Discussion

Effect of the different N-sources on broccoli growth parameters and yield components

Managing N-inputs is an important factor in broccoli production (Schellenberg *et al.*, 2009; Luna *et al.*, 2018) especially in light textured soils (Abd-Alrahman *et al.*, 2016). In such soils, the traditional mineral N-fertilizers are still in use for plant production; however, the extensive use of these fertilizers is associated with significant losses of N from soil through volatilization or nitrate leaching (Peng *et al.*, 2015; Kadyampakeni *et al.*, 2018). This probably possesses ecological hazards. Thus, organic fertilizers are recommended in this research to substitute the traditional inorganic N-fertilizers. These organic forms are slow release fertilizers (Farid *et al.*, 2014) which can, therefore, minimize N-losses from soil. Furthermore, introducing free living N-fixing bacteria can partially substitute mineral N-fertilizers (Abbas *et al.*, 2011). To what extent can these two sources of N partially substitute the full dose on mineral N-fertilizers was a matter of concern in this study which was conducted in an arid sandy loam soil cultivated with broccoli. Our results highlighted the positive effect of the combinations between the investigated three N-sources i.e. mineral fertilizers + compost + biofertilizers on NPK uptake by plants, the growth and yield components of broccoli. In this concern, larger heads have a high marketable value than the smaller ones (Takahashi *et al.*, 2018). The effect of this

combination exceeded the sole effect of the traditional inorganic fertilization (reference treatment). Likewise, amending the soil with “organic+mineral” N fertilizer improved significantly the growth parameters and yield components of broccoli plants when compared with the mineral N-fertilizer (the reference treatment); however, to a lower extent when compared with the treatment “inorganic N-fertilizers +compost +biofertilizers”. On the other hand, the investigated treatments stimulated the activity of DH enzyme. The stimulatory effect of organic fertilizer might have been directly exerted through its effect on the growth and proliferation of the bacteria, thereby creating a favourable habitat for better survival of the inoculated bacteria (Kumar *et al.* 2009). This result probably reflects the positive effect of the amended organic matter on protecting and maintaining soil enzymes in their active forms (Mandal *et al.*, 2007). Increasing the activity of DH resulted from the concurrent increases in root exudates during plant growth (Dakora and Phillips, 2002; Hugoni *et al.*, 2018) beside of the increases that occurred in the available nutrients upon organic matter decomposition (Farid *et al.*, 2014 and 2018). After 30 days from transplanting, the activity of dehydrogenase decreased.

This might be attributed to the reductions that occurred in plant exudates while shifting from the vegetative to the reproductive growth (Hupe *et al.*, 2018), beside of the reductions that occurred in the easily oxidized organic matter in soil (Abdelhafez *et al.*, 2018). Moreover,

intracellular enzyme activities are short-lived because they are degraded by proteases (Burns and Dick, 2002). This might in turn reduce the activity of soil biota and consequently the DH activity.

Effect of the foliar spray of alga extract on broccoli growth parameters and yield components

Spraying plants with the alga extract improved significantly the growth parameters, yield and yield components of broccoli plants grown on a sandy loam soil. Moreover, this extract increased significantly NPK contents within the grown plants. Our results agree with those of Abd El-Mawgoud *et al.* (2010) and Abo Sedera *et al.* (2010) on strawberry, Shehata *et al.* (2011) on celery, Shalaby and El-Ramady (2014) on garlic, Anitha *et al.* (2016) on tomato and Abd El-Aleem *et al.* (2017) on fennel. Such increases take place probably because the alga extract is rich in nutrients, and plant growth regulators e.g. auxins and cytokinins (Zhang & Ervin, 2004 and Papenfus *et al.*, 2013). Moreover, it contains cytokines which affects positively the activity of photosynthesis and this might, in turn, improve plant growth characteristics (Kim & Chojnacka, 2015).

The combination between different N-sources and foliar spray of alga extract on broccoli growth parameters and yield components

Our results support the hypothesis of the study indicating that the algal extracts improved significantly the growth parameters and yield components of broccoli plants grown on a sandy loam soil. Probably, these extracts increased the utilization of soil nutrients by plants through inducing soil biota which decomposed the organic matter and therefore enriched soils with nutrients. On the other hand, the application of the organic amendments acts as slow release fertilizers while decreased the leachability of soil nutrients from the sandy loam soil. The combination between the organic and inorganic N sources in presence of N-fixing bacteria and the alga extract resulted in further N, P and K uptake by plants, consequently their contents in shoots increased. This might in turn increase the total head yield of broccoli exceeding those attained due to the application of the total nitrogen as a mineral form.

CONCLUSION

We recommend soil application of 108 kg N ha⁻¹ as compost manure + 108 kg N ha⁻¹ as ammonium nitrate + biofertilizer (N4) in presence of alga cell extract sprayed at a rate of 5 ml L⁻¹ (F2) to improve the growth parameters, yield and head quality of broccoli as compared with the organic farming or even the traditional inorganic fertilizers on a sandy loam soil.

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مصادر النيتروجين ومستخلص الطحالب كمرشحين لتحسين نمو ومحصول وسمات الجودة في نباتات البروكلي

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ساهمت زيادة وعي المستهلك في تأسيس قضايا تتعلق بسلامة الأغذية والمخاوف البيئية من خلال إدخال معاملات عضوية وحيوية كبديل للتسميد الكيميائي التقليدي. ومع ذلك، بدأ أن كفاءة هذه الأسمدة منخفضة نسبياً بالمقارنة مع الأسمدة المعدنية التقليدية. تفترض هذه الدراسة أن مستخلص الطحالب من *Chlorella sp.* قد يحفز نمو النباتات. ولدراسة هذا الافتراض، أجريت دراسة حقلية لموسمين متتاليين (٢٠١٦/٢٠١٧ و ٢٠١٥/٢٠١٦) باستخدام مصادر مختلفة للنيتروجين مثل السماد العضوي (الكمبوست)، والسماد الحيوي (*Azotobacter chroococcum* و *Azospirillum lipoferum*) والأسمدة المعدنية (نترات الأمونيوم) مع أو بدون مستخلص طحالب *Chlorella sp.* لدراسة كفاءتها لتحسين قياسات النمو والمحصول ومكوناته من نباتات البروكلي المنزوعة في تربة رملية طبيعية. أظهرت النتائج أنه قد تحقق أعلى زيادات من امتصاص النيتروجين والفوسفور والبوتاسيوم لمعاملات "الكمبوست + نترات الأمونيوم + الأسمدة الحيوية". كما أدت هذه المعاملات إلى زيادات مترامنة في ارتفاع النبات، عدد الأوراق للنبات، مساحة الورقة؛ الوزن الطازج، كلوروفيل أ و ب، الوزن والقطر الرؤوس، وفيتامين ج، المواد الصلبة الذائبة، محتوى المركبات الفينولية الكلية ومضادات الأكسدة في رؤوس البروكلي ومحصول الرؤوس الناتجة. علاوة على ذلك، أدى الجمع بين مصادر النيتروجين المختلفة والرش الورقي بمستخلص الطحالب إلى زيادات أخرى كبيرة في امتصاص النيتروجين والفوسفور والبوتاسيوم من النباتات وهذا بدوره حسن بشكل كبير من معاملات النمو المذكورة أعلاه ومكونات المحصول. ووفقاً لذلك، تدعم نتائجنا الفرضية التي تشير إلى أن الرش الورقي بمستخلص الطحالب قد حسن بشكل ملحوظ من قياسات النمو ومكونات المحصول لنباتات البروكلي المنزوعة في تربة رملية طبيعية.

الكلمات المفتاحية: البروكلي، مصادر النيتروجين، السماد العضوي، الأسمدة الحيوية، *Azospirillum*، *Azotobacter*، مستخلص الطحالب، *Chlorella sp.*، النمو، محصول الرؤوس