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EFFECT OF NITROGEN AND BIOFERTILIZATION, SEAWEED EXTRACT AND THIAMINE ON GROWTH, YIELD AND ESSENTIAL OIL OF PARSLEY PLANT

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ABSTRACT: A field experiment was carried out to study the effect of reducing the recommended dose of mineral N fertilizer on parsley (*Petroselinum sativum*, Hoffm.) plants. To achieve this aim, some bio-stimulants commercial product (Minia azotein) which contain N fixing bacteria, seaweeds extract at 100 and 200 ppm and thiamine (Vit. B1) at 100 and 200 ppm, separately or in combinations were used. Results revealed that mineral N at 50 to 75% + Minia azotein significantly stimulated the vegetative growth traits, yield components, essential oil content, photosynthetic pigments and N % relatively to control plants (recommended dose of mineral N). Using mineral N fertilizer at 25% and Minia azotein significantly reduced the above-mentioned characters compared to check treatment. All treatments of seaweeds extract and/or thiamine (Vit. B1) significantly augmented the vegetative growth parameters, yield components, essential oil photosynthetic pigments and N % compared with control plants. The most effective treatment was seaweeds extract and Vit. B1 both at 200 ppm. The best results with regard to the herb dry and, fruit weight and essential oil productivity were obtained as a result of using N fertilizer at 50% plus Minia azotein in combinations with seaweeds extract + Vit. B1 each at 200 ppm.

Key words: *Petroselinum sativum*, bio-stimulants, N fixing bacteria, seaweeds extract, thiamine.

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INTRODUCTION

Parsley (*Petroselinum sativum*, Hoffm.) plants belong to the family Apiace (Umbelliferae) is an aromatic bright green, annual or biennial herb (Simon, 1999). Leafy parsley is recognized as a spice native to the Mediterranean countries. It is widely used as fresh, dry or frozen herb to enhance the flavor of different types of foods. Parsley seeds have been used as antiseptic treatment, inflammation, kidney stones and also as carminative (Moazedi *et al.*, 2007 and Behtash *et al.,* 2008). Parsley plants have been used as a medicinal plant for diverse medicinal purposes in traditional and folklore medicine of many countries (Blumenthal *et al.,* 2000). Parsley seeds produce high amount of essential oil (EO) with monoterpenes as a main constituents. This EO could be used in the food industry and as a perfume fragrance (Diaz-Maroto *et al.,* 2002).

Nitrogen is an essential element for all living organisms as it is involved in biosynthesis of many molecules such as proteins, enzymes and nucleic acids. However, a small ratio of it is present in the soil, yet a tiny portion of soil-N is available to the plants (Mengel *et al.*, 2001). A plant bio-stimulant is a substance or microorganism such as seaweed and thiamine (vitamin B1), that could encourage natural developments of plants and promoting their nutrient use efficiency (Torre *et al*., 2013). Biofertilizers are one of these bio-stimulants which afford an

economically cheap and ecologically safe fertilizer moreover, they are vital for sustainable agriculture by reducing the doses of chemical fertilizers (Chatterjee *et al.*, 2017). Minia azotein (MA) (N-fixing biofertilizer) as a bio-stimulant has been applied to reduce mineral N fertilizer of different herbs (Abdallah *et al.,* 2012) and (Shehata, 2019) on parsley; (Abdou *et al*., 2020) on fennel and (El-Sayed *et al.,* 2020) on dill.

Seaweeds (SE) are significant marine renewable materials that used as a replacement of chemical fertilizer due to their content of many macronutrients, micronutrients and growth regulators (Khan *et al.*, 2009). Seaweeds extract has been used to improve the growth and productivity of many medicinal plants (Ramya *et al.,* 2011 on guar; Aqeel *et al.,* 2014 on *Nigella sativa*, Hassan, 2015 on *Anethum graveolens*; Mahmoud, 2016 on *Calendula officinalis* and Veeranan *et al.,* 2018 on *Ocimum sanctum*).

Vitamin B1 (thiamine) acts as cofactor and activator for many plant metabolic activities especially enzymes which involve in the synthesis of pentose phosphate pathway, amino acids, tricarboxylic acid cycle (Colinas *et al.*, 2015 and Subki *et al.*, 2018). Vit. B1 has been used as a bio-stimulants by many investigators on different medicinal crops (Hendawy and Ezz El-Din, 2010 on fennel; Botros, 2013 on caraway; Abdou *et al*., 2019 on cumin and Abdelkader *et al.,* 2018 on black cumin).

Therefore, this study was conducted to assess the effects of replacing some mineral N fertilizer requirements by MA, SE and Vit. B1 on the vegetative growth, fruit yield and EO and productivity of parsley plant.

MATERIALS AND METHODS

This investigation was carried out during the two successive seasons; 2016/2017 and 2017/2018 at the nursery and laboratory of ornamental plants, Fac. of Agric., Minia Univ., Egypt. Seeds of parsley were obtained from Nursery of Floriculture, Fac. of Agric., Minia Univ. and sown on November 6th in clay loamy soil. The physical and chemical analysis of the experimental soil is listed in Table (a).

A complete randomized block design following the split plot arrangement was executed. The main plots included 4 treatments of mineral N which were N as a unique source or in combination with the biofertilizer Minia azotein (MA) whereas, the sub plots contained 7 treatments of SE and/or Vit. B1. Minia azotein which is a commercial biofertilizer contains free living N-fixing bacteria was obtained from Center of Biofertilizers, Dept. of Genetics, Fac. of Agric. Minia Univ. The components of seaweed extract which obtained from Lab. Chem, Cairo, Egypt is shown in Table (b).

The main plot was 3 × 4.8 m with 60 cm distance between the rows and 30 cm between the hills within the row. So, each plot contained 7 rows and 70 hills (10 hills/row). Plants were thinned twice, the 1st after 3 weeks from plating date and the 2nd after 2 weeks from the 1st one, finally each hill contained 2 plants. Overall there were 38 thousand plants/fed.

The main plots (A) treatments were control (recommended dose (100%) of

Table a. Physical and chemical analysis of the experimental soil.

Soil characters	Value	Soil characters		Value
Soil type	Clayey loam	Avail. P (%)		15.40
Sand (%)	28.59	Exch. K (mg/100g)		2.45
Silt (%)	30.29	Exch. Ca (mg/100g)		31.43
Clay	41.12	Exch. Na (mg/100g)		2.46
Organic Matter (%)	1.65		Fe	8.39
$CaCO_3$ (%)	2.10	DTPA	Cu	2.04
pH (1:2.5)	7.79	Ext. (ppm)	Zn	2.81
EC (mmhos/cm)	1.06		Mn	8.19
Total N (%)	0.08			

Table b. The chemical properties of the seaweeds extract.

Characters	Values	Character	Values
Moisture (%)	6.0	S (%)	3-9
Organic matter (%)	45-65	Ca (%)	0.2-1.5
Inorganic matter (%)	45-65	B ppm	20-100
Protein (%)	6-8	Mo ppm	1-5
Carbohydrate (%)	35-50	Fe ppm	50-200
Alginic acid (%)	10-20	Cu ppm	1.0-6.0
Mannitol	4-7	Mn ppm	5-12
Total N (%)	1.0-1.5	Zn ppm	10-100
K (%)	1.0-1.2	Cytokines (%)	0.02
Mg (%)	0.5-0.9	IAA (%)	0.03
P (%)	0.02-0.09	ABA (%)	0.01

mineral N fertilizer as a unique source of N) and N fertilizer at 25, 50 and 75% of the recommended dose plus MA. The amounts of mineral N fertilizer for 25, 50, 75 and 100% were 75, 150, 225 and 300 kg/fed, respectively of ammonium sulphate (20.6% N). The N fertilizer was divided into two batches, added with 3 weeks interval, starting on 20th December. A volume of 50 ml/hill of the suspension culture of MA contains 1×10^7 cell was added twice, to the soil beside the plant at the same times of N fertilization. While, the sub plots (B) treatments were untreated plants (tap water), SE at 100 or 200 ppm, vit B1 at 100 or 200 ppm and SE + Vit. B1 each one at 100 or 200 ppm. Parsley plants were foliar sprayed thrice with 3 weeks interval till run off starting on 19th December. In addition to those plants were sprayed after the 1st, 2nd and 3rd cuts of the herb at one month intervals, starting on 15th Feb. Therefore, the experiment involved 28 treatments each consisted of one row and replicated thrice.

All parsley plants received phosphorus at 200 kg/fed of calcium superphosphate (15.5% P_2O_5) and potassium fertilizers 50 kg/fed of potassium sulphate (48% K_2O). Organic farm yard manure at 20 m^3/fed and P fertilizer were added during preparing of the soil for cultivation, while K fertilizer was added on 20th December in both growing seasons. All other agriculture practices were carried out as usual in both seasons.

At harvesting time, half number of the plants was harvested to obtain the EO of herb (the herb was collected four times at one month intervals starting 5th February however, the other plants were harvested once at end of the season (end of June) to obtain the essential oil (EO) from mature fruits. EO % of herb and fruits were determined according to British Pharmacopoeia (1963). Samples of fresh leaves were taken after 3 weeks from the last treatment to estimate the contents of the photosynthetic pigments (chlorophyll a, b, and carotenoids, mg/g f.w.) according to Fadl and Sri El-Deen (1978). At the end of growing season the percentage of N in the dry leaves was determined according to Wilde *et al.* (1985). The obtained data were tabulated and subjected to statistical analysis according to Mead *et al.* (1993) using MSTAT-C (1986).

RESULTS

1. Herb fresh and dry weights:

Data presented in Table (1) show that factor A treatments except N at 25% + MA caused a significant increase in herb fresh and dry weights/fed as compared to the control treatment in the two seasons. The heaviest fresh and dry weights of herb/fed were obtained due to N at 75% + MA treatment followed by N at 50% + MA (with no significant difference between them, in both seasons). The increments in herb dry weight/fed as a result of the previous treatments were 21.64 and 16.86% in the 1st season and 23.01 and 15.34% in the 2nd one, respectively over the control plants. Regarding the effect of factor B treatments, it was obvious that all tested treatment significantly augmented the fresh and dry

Table 1. Effect of nitrogen, biofertilization, seaweeds extract and vitamin B1 on fresh weight of herb/plant and /fed of parsley plants in two growing seasons.

Foliar spray treatments (ppm) (B)	Fertilization treatments (A)				Mean (B)	Fertilization treatments (A)				Mean (B)
	100% N (Control)	25% N + MA	50% N + MA	75% N + MA		100% N (Control)	25% N + MA	50% N + MA	75% N + MA	
	Fresh weight of herb/plant (g)					Fresh weight of herb/fed (ton)				
First season (2016/2017)										
Tap water	102	90	112	116	105	3.96	3.49	4.36	4.53	4.08
SE (100)	139	131	159	165	148	5.40	5.11	6.18	6.41	5.77
SE (200)	156	139	176	182	163	6.06	5.42	6.87	7.06	6.35
Vit. B1 (100)	114	100	126	131	118	4.42	3.88	4.90	5.11	4.58
Vit. B1 (200)	123	110	142	153	132	4.79	4.39	5.54	5.96	5.15
SE (100) + Vit. B1 (100)	171	159	192	197	180	6.66	6.18	7.48	7.66	6.99
SE (200) + Vit. B1 (200)	186	172	209	214	196	7.23	6.72	8.13	8.34	7.60
Mean (A)	141	59	160	166		5.50	5.01	6.21	6.44	
L.S.D. at 5%		A: 18	B: 8	AB: 16			A: 0.69	B: 0.32	AB: 0.63	
Second season (2017/2018)										
Tap water	109	93	116	122	110	4.23	3.63	4.53	4.75	4.28
SE (100)	147	139	168	175	157	5.73	5.40	6.55	6.82	6.12
SE (200)	166	147	187	195	174	6.46	5.72	7.27	7.57	6.76
Vit. B1 (100)	120	106	135	141	126	4.67	4.13	5.26	5.47	4.88
Vit. B1 (200)	131	118	150.	157	139	5.12	4.58	5.84	6.09	5.41
SE (100) + Vit. B1 (100)	181	169	203	208	190	7.05	6.59	7.88	8.08	7.40
SE (200) + Vit. B1 (200)	194	179	218	225	204	7.54	6.95	8.48	8.74	7.92
Mean (A)	150	136	168	175		5.83	5.29	6.54	6.79	
L.S.D. at 5%		A: 16	B: 7.3	AB: 15			A: 0.63	B: 0.28	AB: 0.57	

Control: 100% of recommended dose of mineral N fertilization, MA: Minia azotein, SE: Seaweed extract and Vit. B1: Thiamine

weights of parsley herb/fed compared with the check treatment (Table, 1). The best treatment which produced the heaviest fresh and dry weights was the dual treatment of seaweeds extract + Vit. B1 (each 200 ppm). This treatment increased the herb dry weight/fed. by 99.86 and 89.48% over that of the control plants in the two seasons, respectively.

The interaction between factor A and B was significant in the two growing seasons as shown in Table (1). Overall, the most effective treatments which produced the heaviest herb fresh and dry weights/fed in both seasons was N fertilizer at 75% or 50% + MA in combination with seaweeds extract+ Vit. B1 each at 200 ppm.

2. Fruit yield:

Parsley fruit yield was augmented due to fertilization treatments (except N fertilizer at 25% + MA) in comparison with control plants in both seasons. The augmentation of the yield was only significant for N fertilizer at 75% + MA, which augmented fruit yield/fed by 9.91% in 1st season and 10.21% in the 2nd one over the control plants (Table, 2). A significant increment of fruit yield was detected due to all treatments of factor B comparing with tap water treatment in both seasons as shown in Table (2). The highest value of fruits yield was obtained due to SE + Vit. B1 each at 200 ppm. This treatment enhanced fruit yield/fed by 77.79 and 75.75% over the tap water treated plants in the both seasons, respectively.

The interaction between the two investigated factors was significant in the two growing seasons (Table, 2). The best results of fruit yield were obtained from the treatment of N fertilizer at 50% + MA in combination with SE + Vit. B1 each at 100 ppm in the 1st season and in combination with SE +Vit. B1 both at 200 ppm in the 2nd one.

3. Essential % and yield/fed of parsley herb:

Data recorded in Table (3) revealed that EO % and yield/fed of parsley herb were significantly increased, in most cases due to N fertilizer + MA treatments (except N at 25% + MA) compared with control treatment in both seasons. The highest percentage and yield/fed were achieved due to supplying plants with N at 75% + MA followed by N at 50% + MA (with no significant differences between themselves) in the two seasons. These treatments augmented the EO yield by 25.65 and 19.65% in the 1st season and by 26.42 and 16.93% in the 2nd one, respectively over those of control plants.

The obtained results (Table, 3) suggested that spraying parsley plants with SE and/or Vit. B1 at all tested concentrations resulted in a significant increment of EO % and oil yield/fed as compared to tap water treatment in the two season. The highest values in this concern were obtained from spraying the plants with SE + Vit. B1 each one at 200 ppm. The increment percentages of the EO yield/fed were 120.14 and 118.26% over that of control plants in the two seasons, respectively.

There was a significant interaction between the two investigated factors on EO % and oil yield/fed of parsley herb for both seasons. The highest values were obtained with the treatment of N fertilizer at 75% or 50% + MA in combination with seaweeds extract + Vit. B1 each at 200 or 100 ppm for the two growing seasons, respectively.

4. Essential % and yield/fed of fruits:

Data in Table (4) reveal that fertilizing parsley plants with all doses of N fertilizer (except 25%) + MA significantly promoted the EO % and yield/fed compared to the control plants in the two experimental seasons. The most effective treatments were mineral N at 75% + MA for EO% and N at 75% followed by 50% each plus MA (with no significant differences between themselves) for EO yield/fed in both seasons. These two treatments increased the EO yield of fruits/fed over those of control treatment by 18.88 and 12.48% in the 1st season and by 18.38 and 11.13% in the 2nd one, respectively.

Table 2. Effect of nitrogen, biofertilization, seaweeds extract and vitamin B1 on fruit yield/plant and /fed of parsley plants in two growing seasons.

Foliar spray treatments (ppm) (B)	Fertilization treatments (A)				Mean (B)	Fertilization treatments (A)				Mean (B)
	100% N (Control)	25% N + MA	50% N + MA	75% N + MA		100% N (Control)	25% N + MA	50% N + MA	75% N + MA	
	Fruit yield / plant (g)					Fruit yield/fed (ton)				
First season (2016/2017)										
Tap water	28.4	23.9	30.2	31.9	23.60	1.11	1.31	1.17	1.24	1.11
SE (100)	41.7	38.5	45.4	45.7	42.83	1.62	1.50	1.77	1.78	1.67
SE (200)	43.7	40.9	48.1	48 .3	45.29	1.70	1.59	1.87	1.88	1.76
Vit. B1 (100)	34.1	29.9	36.2	38.5	34.67	1.33	1.16	1.41	1.50	1.35
Vit. B1 (200)	38.3	35.1	40.7	41.6	38.92	1.49	1.37	1.58	1.62	1.51
SE (100) + Vit. B1 (100)	46.6	43.9	50.2	51.8	48.13	1.81	1.71	1.95	2.01	1.87
SE (200) + Vit. B1 (200)	50.5	46.8	52.4	53.6	50.84	1.97	1.82	2.04	2.08	1.98
Mean (A)	40.5	37.0	43.3	44.5		1.57	1.44	1.68	1.73	
L.S.D. at 5%		A: 3.8	B: 2.4	AB: 4.9			A: 1.48	B: 9.40	AB: 1.89	
Second season (2017/2018)										
Tap water	28.5	24.9	32.5	33.3	29.8	1.11	1.69	1.26	1.29	1.16
SE (100)	42.1	40.6	43.9	46.1	42.2	1.64	1.58	1.71	1.79	1.68
SE (200)	44.8	42.7	45.8	49.2	45.6	1.74	1.66	1.78	1.91	1.77
Vit. B1 (100)	34.2	31.3	39.2	39.9	36.1	1.33	1.22	1.52	1.55	1.41
Vit. B1 (200)	39.1	37.0	40.5	43.2	39.9	1.52	1.44	1.58	1.68	1.56
SE (100) + Vit. B1 (100)	48.6	47.3	49.9	52.2	49.5	1.89	1.84	1.94	2.03	1.93
SE (200) + Vit. B1 (200)	51.6	49.1	53.9	54.8	52.34	2.01	1.91	2.10	2.13	2.04
Mean (A)	41.3	39.0	43.7	45.5		1.61	1.52	1.67	1.77	
L.S.D. at 5%		A: 3.6	B: 1.7	AB: 3.4			A: 1.38	B: 6.60	AB: 1.32	

Control: 100% of recommended dose of mineral N fertilization, MA: Minia azotein, SE: Seaweed extract and Vit. B1: Thiamine

Table 3. Effect of nitrogen, biofertilization, seaweeds extract and vitamin B1 on essential oil percentage of herb and on essential oil yield of herb of parsley plants in two growing seasons.

Foliar spray treatment (ppm) (B)	Fertilization treatments (A)				Mean (B)	Fertilization treatments (A)				Mean (B)
	100% N (Control)	25% N + MA	50% N + MA	75% N + MA		100% N (Control)	25% N + MA	50% N + MA	75% N + MA	
	Essential oil % of herb					Essential oil yield of herb (l/fed)				
First season (2016/2017)										
Tap water	1.67	1.62	1.70	1.71	1.68	14.7	11.9	16.9	17.8	15.3
SE (100)	1.79	1.72	1.84	1.87	1.81	21.2	17.1	26.7	27.4	23.4
SE (200)	1.83	1.77	1.88	1.91	1.85	24.3	20.1	29.7	31.1	26.4
Vit. B1 (100)	1.71	1.65	1.75	1.76	1.72	16.3	13.4	18.9	20.1	17.2
Vit. B1 (200)	1.76	1.68	1.80	1.81	1.76	18.3	14.8	22.3	24.4	19.9
SE (100) + Vit. B1 (100)	1.88	1.83	1.93	1.95	1.90	28.2	23.9	33.3	34.9	30.1
SE (200) + Vit. B1 (200)	1.93	1.87	1.97	1.98	1.94	31.6	26.9	37.6	38.9	33.8
Mean (A)	1.80	1.73	1.84	1.86		22.1	18.5	26.9	27.8	
L.S.D. at 5%		A: 0.03	B: 0.03	AB: 0.06			A: 3.6	B:1.6	AB: 3.1	
Second season (2017/2018)										
Tap water	1.72	1.63	1.74	1.77	1.72	16.3	12.5	18.0	20.0	16.7
SE (100)	1.85	1.77	1.89	1.92	1.86	23.5	19.8	28.1	30.7	25.5
SE (200)	1.91	1.81	1.94	1.97	1.91	27.7	21.9	32.4	35.3	29.3
Vit. B1 (100)	1.77	1.69	1.80	1.82	1.77	17.9	14.1	21.0	22.2	19.0
Vit. B1 (200)	1.80	1.73	1.83	1.86	1.81	20.3	16.2	24.0	26.3	21.7
SE (100) + Vit. B1 (100)	1.95	1.86	1.97	2.00	1.95	30.9	25.7	36.2	38.4	32.8
SE (200) + Vit. B1 (200)	1.99	1.90	2.00	2.02	1.98	34.5	28.4	40.3	42.6	36.4
Mean (A)	1.86	1.77	1.88	1.91		24.4	19.8	28.9	30.9	
L.S.D. at 5%		A: 0.03	B: 0.03	AB: 0.06			A: 2.9	B: 1.5	AB: 2.9	

Control: 100% of recommended dose of mineral N fertilization, MA: Minia azotein, SE: Seaweed extract and Vit. B1: Thiamine

Table 4. Effect of nitrogen, biofertilization, seaweeds extract and vitamin B1 on essential oil percentage of fruits and on essential oil yield of fruits/fed of parsley plants in two growing seasons.

Foliar spray treatments (ppm) (B)	Fertilization treatments (A)				Mean (B)	Fertilization treatments (A)				Mean (B)
	100% N (Control)	25% N + MA	50% N + MA	75% N + MA		100% N (Control)	25% N + MA	50% N + MA	75% N + MA	
	Essential oil % of fruits					Essential oil yield of fruits/fed (l/fed)				
First season (2016/2017)										
Tap water	2.80	2.68	2.85	2.93	2.82	31.0	24.9	33.3	36.4	31.4
SE (100)	3.04	3.00	3.24	3.32	3.15	49.3	44.7	57.3	59.0	52.6
SE (200)	3.06	3.03	3.28	3.36	3.18	51.8	48.2	61.4	63.0	56.1
Vit. B1 (100)	2.94	2.81	2.98	3.08	3.95	39.0	32.7	42.0	46.8	40.1
Vit. B1 (200)	3.02	2091	3.15	3.21	3.07	45.0	39.8	50.0	51.8	46.7
SE (100) + Vit. B1 (100)	3.12	3.07	3.31	3.44	3.24	56.6	52.5	64.5	69.2	60.7
SE (200) + Vit. B1 (200)	3.25	3.17	3.43	3.54	3.35	36.9	57.4	70.0	73.9	66.3
Mean (A)	3.03	2.95	3.18	3.27		48.1	42.9	54.1	57.2	
L.S.D. at 5%		A: 0.05	B: 0.04	AB: 0.08			A: 4.4	B: 2.7	AB: 5.4	
Second season (2017/2018)										
Tap water	2.85	2.70	2.90	2.94	2.85	31.6	26.0	36.7	38.2	33.1
SE (100)	3.14	3.02	3.37	3.41	3.24	51.5	47.7	57.7	61.2	54.5
SE (200)	3.16	3.06	3.40	3.47	3.27	55.0	50.9	60.5	66.5	58.2
Vit. B1 (100)	2.98	2.82	3.12	3.20	3.03	39.7	34.2	47.7	49.6	42.8
Vit. B1 (200)	3.12	2.92	3.24	3.30	3.15	47.4	42.1	51.1	55.5	49.0
SE (100) + Vit. B1 (100)	3.25	3.09	3.45	3.52	3.33	61.6	56.9	66.9	71.5	64.2
SE (200) + Vit. B1 (200)	3.35	3.20	3.49	3.59	3.41	67.4	61.2	73.1	76.6	64.2
Mean (A)	3.12	2.97	3.28	3.35		50.6	45.6	56.2	59.9	
L.S.D. at 5%		A: 0.05	B: 0.04	AB: 0.07			A: 4.6	B: 2.5	AB: 5.1	

Control: 100% of recommended dose of mineral N fertilization, MA: Minia azotein, SE: Seaweed extract and Vit. B1: Thiamine

Results in Table (4) show that SE and/or Vit. B1 treatments significantly augmented EO% and yield/fed compared to untreated plants in the two seasons. The dual treatment was significantly better than the single one, on the other hand, SE was more effective than Vit. B1. Overall, the highest EO % and yield/fed were obtained from plants treated with SE and Vit. B1 both at 200 ppm, such treatment increased the EO yield of fruits/fed by 111.18 and 109.89% over those of tap water treatment in both seasons, respectively.

There was a significant interaction between the two investigated factors in both seasons. The highest EO% of parsley fruit was achieved when the plants treated with N at 75% + MA in combination with SE + Vit. B1 both at 200 ppm. However, the highest EO yield/fed was produced due to supplying plants with N fertilizer at 75% + MA in combination with SE + Vit. B1 both at 200 or 100 ppm or N at 50% + MA combined with SE + Vit. B1 each at 200 ppm (Table, 4).

5. Photosynthetic pigments:

Data presented in Tables (5) and (6) suggested that application of the N fertilizer at different percentages (except 25%) + MA significantly increased the content of the three photosynthetic pigments namely, chlorophyll a, b and carotenoids in the fresh leaves of parsley plants compared to the control plants in the two experimental season. Supplying the plants with N fertilizer at 75% + MA gave the highest values of the three photosynthetic pigments. Also, the effect of SE and/or Vit. B1 application was significant in both seasons, where the photosynthetic pigments content were significantly augmented with the application of these treatments comparing with tap water treatment (Tables, 5 and 6). The dual treatment of SE + Vit. B1 each at 200 ppm was more effective than the other treatments as it produced the highest content of chlorophyll a, b and carotenoids in both growing seasons.

The interaction between (N + biofertilization) and (SE and/or Vit. B1) was significant in the two growing seasons. In this concern, the highest values of the three photosynthetic pigments were obtained in plants treated with N fertilizer at 75 or 50% + MA in combination with SE + Vit. B1 both at 200 ppm in both experimental seasons.

6. Nitrogen percentage:

It is evident from the data exhibited in Table (6) that N % in the dry leaves was enhanced in the parsley plants treated with N fertilizer at all percentages (except 25%) + MA as compared to the control plants in the two growing seasons. The highest N % was obtained due to applying mineral N fertilizer at 75% + MA in both seasons (Table, 6).

Also, all treatments of SE and/or Vit. B1 caused a significant increase in the N percentage in the dry leaves of parsley plants comparing with tap water treatment in both growing seasons. Treating plants with SE + Vit. B1 both at 200 ppm increased the content of N to the highest percentage in both seasons.

There was a significant interaction between (N and biofertilizetion) and (SE and/or Vit. B1) treatments in both seasons. The highest accumulation of N% was obtained due to treating parsley plants with N fertilizer at 75% or 50% each one plus MA combined with SE + Vit.B1 both at 200 ppm in the two growing seasons.

DISCUSSION

Parsley plants treated with the recommended dose of N had significantly higher herb dry as well as fruit yield (101.8 and 28.4 g/plant respectively) in the 1st season compared with those treated with 25% of the recommended dose of N even with MA (89.7 and 23.95 g/plant respectively) and similar results were estimated in the 2nd season. The vital role of N in plant growth is well documented N is an essential component in amino acids formation, cell division, photosynthetic, vitamins and carbohydrates production

Table 5. Effect of nitrogen, biofertilization, seaweeds extract and vitamin B1 on chlorophyll a and b content in the fresh leaves of parsley plants in two growing seasons.

Foliar spray treatment (ppm) (B)	Fertilization treatments (A)				Mean (B)	Fertilization treatments (A)				Mean (B)
	100% N (Control)	25% N + MA	50% N + MA	75% N + MA		100% N (Control)	25% N + MA	50% N + MA	75% N + MA	
	Chlorophyll a content (mg/g f.w.)					Chlorophyll b content (mg/g f.w.)				
First season (2016/2017)										
Tap water	2.164	2.109	2.199	2.217	2.172	0.765	0.758	0.775	0.786	0.771
SE (100)	2.431	2.365	2.492	2.522	2.453	0.846	0.812	0.864	0.873	0.849
SE (200)	2.486	2.419	2.547	2.579	2.508	0.858	0.832	0.881	0.884	0.864
Vit. B1 (100)	2.327	2.268	2.402	2.408	2.351	0.783	0.770	0.822	0.828	0.801
Vit. B1 (200)	2.382	2.321	2.455	2.470	2.407	0.796	0.787	0.836	0.838	0.814
SE (100) + Vit. B1 (100)	2.524	2.466	2.576	2.602	2.542	0.879	0.862	0.898	0.905	0.886
SE (200) + Vit. B1 (200)	22.55	.494	2.612	.635	2.574	0.895	0.881	0.916	0.922	0.904
Mean (A)	2.410	2.349	2.469	2.490		0.832	0.815	0.856	0.862	
L.S.D. at 5%		A: 0.013	B: 0.015	AB: 0.030			A: 0.004	B: 0.006	AB: 0.012	
Second season (2017/2018)										
Tap water	2.169	2.114	2.223	2.284	2.198	0.769	0.762	0.777	0.797	0.776
SE (100)	2.525	2.470	2.613	2.648	2.564	0.859	0.827	0.867	0.884	0.859
SE (200)	2.576	2.521	2.669	2.701	2.617	0.857	0.843	0.881	0.893	0.873
Vit. B1 (100)	2.342	2.286	2.439	2.486	2.388	0.789	0.773	0.828	0.838	0.807
Vit. B1 (200)	2.393	2.337	2.491	2.532	2.438	0.804	0.787	0.884	0.851	0.822
SE (100) + Vit. B1 (100)	2.693	2.634	2.713	2.720	2.690	0.897	0.874	0.907	0.912	0.898
SE (200) + Vit. B1 (200)	2.717	2.658	2.740	2.750	2.716	0.911	0.890	0.921	0.982	0.913
Mean (A)	2.488	2.431	2.555	2.589		0.843	0.822	0.861	0.872	
L.S.D. at 5%		A: 0.014	B: 0.013	AB: 0.026			A: 0.005	B: 0.005	AB: 0.010	

Control: 100% of recommended dose of mineral N fertilization, MA: Minia azotein, SE: Seaweed extract and Vit. B1: Thiamine

Table 6. Effect of mineral nitrogen, biofertilization, seaweeds extract and vitamin B1 on carotenoids content and on nitrogen percentage in the dry leaves of parsley plants in two growing seasons.

Foliar spray treatments (ppm) (B)	Fertilization treatments (A)				Mean (B)	Fertilization treatments (A)				Mean (B)
	100% N (Control)	25% N + MA	50% N + MA	75% N + MA		100% N (Control)	25% N + MA	50% N + MA	75% N + MA	
	Carotenoids content (mg/g f.w.)					Nitrogen % in the dry leaves				
First season (2016/2017)										
Tap water	0.856	0.836	0.873	0.883	0.862	1.32	1.24	1.42	1.48	1.36
SE (100)	0.914	0.898	0.936	0.938	0.322	1.52	1.44	1.65	1.69	1.57
SE (200)	0.928	0.916	0.952	0.956	0.938	1.58	1.49	1.73	1.77	1.65
Vit. B1 (100)	0.887	0.863	0.902	0.909	0.890	1.37	1.28	1.48	1.55	1.42
Vit. B1 (200)	0.899	0.880	0.919	0.920	0.905	1.45	1.35	1.55	1.62	1.49
SE (100) + Vit. B1 (100)	0.947	0.932	0.967	0.973	0.955	1.69	1.59	1.82	1.84	1.74
SE (200) + Vit. B1 (200)	0.961	0.947	0.983	0.990	0.970	1.75	1.66	1.89	1.91	1.80
Mean (A)	0.913	0.896	0.933	0.938		1.53	1.44	1.65	1.70	
L.S.D. at 5%		A: 0.005	B: 0.007	AB: 0.014			A: 0.047	B: 0.026	AB: 0.052	
Second season (2017/2018)										
Tap water	0.868	0.839	0.882	0.887	0.869	1.35	1.26	1.44	1.52	1.39
SE (100)	0.924	0.907	0.940	0.953	0.931	1.56	1.45	1.65	1.73	1.60
SE (200)	0.938	0.922	0.954	0.966	0.945	1.64	1.53	1.74	1.82	1.68
Vit. B1 (100)	0.896	0.879	0.911	0.926	0.903	1.41	1.31	1.50	1.59	1.46
Vit. B1 (200)	0.904	0.894	0.923	0.932	0.913	1.48	1.38	1.57	1.64	1.52
SE (100) + Vit. B1 (100)	0.949	0.932	0.968	0.977	0.957	1.72	1.61	1.827	1.87	1.75
SE (200) + Vit. B1 (200)	0.968	0.953	0.986	0.994	0.957	1.78	1.68	1.90	1.95	1.83
Mean (A)	0.921	0.9040	0.938	0.947		1.56	1.458	1.66	1.73	
L.S.D. at 5%		A: 0.006	B: 0.005	AB: 0.010			A: 0.043	B: 0.037	AB: 0.075	

Control: 100% of recommended dose of mineral N fertilization, MA: Minia azotein, SE: Seaweed extract and Vit. B1: Thiamine

(Mengel *et al.*, 2001). It is the most vital nutrient element for plant growth, development and application of adequate amount of N is compulsory for successful crop production particularly non-legume plants (Baset, 2015).

Results showed that MA (N-fixing bacteria) could partially supply the parsley plant by its N requirements. For example, plants inoculated with MA and received only 75% of the recommended dose of N had significantly higher herb dry and fruit weights than plants fertilized with the full dose of the recommended N. Moreover, the obtained results showed that the EO percentage in the herb as well as the fruits were increased due to MA inoculation in addition to 75% of N to 1.86 and 3.27% respectively in the 1st season whereas the control plants had 1.67 and 2.80% respectively. For most estimated traits plants treated with 50% N and MA had significantly higher or at least similar values like those treated with 100% of N in both seasons.

In this regard, Bhattacharjee and Day (2014) stated that biofertilizers colonize the rhizosphere and increase the amount or availability of primary nutrients and/or growth stimulus to the plant. The benefits of biofertilisers in the improvement of plant production and enhancing its quality are widely discussed. Biofertilizers play a significant role in improving soil fertility and structure due to adding organic matter to it (Son *et al.*, 2007). It has been reported that growth, yield and quality parameters of certain plants significantly improved when inoculated with N fixers (Youssef and Eissa, 2014). Moreover, it produced some photohormones such as indole acetic acid, gibberellins and cytokinins, as well as, antibacterial substances which is vital (Hauwka, 2000). Our results were similar to those reported by Abdallah *et al.* (2012) and Shehata (2019) on parsley; Abdou *et al.* (2020) on fennel and El-Sayed *et al.* (2020) on dill.

The recent study showed that SE as well as Vit. B1 significantly increased almost all assessed traits compared with that of tap water treated plant on both seasons. Moreover, the higher dose of any of them had better effect. These results are similar to those obtained by Ramya *et al.* (2011) on guar; Aqeel *et al.* (2014) on *Nigella sativa;* Hassan (2015) on *Anethum graveolens*; Mahmoud (2016) on *Calendula officinalis* and Veeranan *et al.* (2018) on *Ocimum sanctum*. Seaweed extract containing macronutrients, trace elements, organic substances like amino acids and plant growth regulators such as auxins, cytokinins and gibberllins, vitamins and fatty acids (Chapman and Chapman, 1980). They enhanced the crop yield by improving root growth and structures, which finally improved plant development included leaf development, fruit set and better ability to tolerate biotic and biotic stress (Calvo *et al.,* 2014 and Drobek *et al.,* 2019). Thiamin (Vit. B1) function as growth regulator or hormone precursor (Samiullah, 1988) and plays essential role as a cofactor for important metabolic activities (Colinas *et al.,* 2015). It plays an important role through increasing carbon assimilation and transfers it into storage sinks (Fitzpatrick and Chapman, 2020). Vit. B1 has been used as a bio-stimulants by (Hendawy and Ezz El-Din, 2012 on fennel; Botros, 2013 on caraway; Abdelkader *et al.,* 2018 on black cumin and Abdou *et al*., 2019 on cumin).

Results showed that N + MA, SE and Vit. B1 had significant effects on growth, yield and EO of parsley plants. All N fertilizer + MA treatments except N at 25% significantly improved almost all investigated traits over those of plants treated with the recommended dose of N. The herb dry and fruit weights were significantly increased following the application of N fertilizer at 75% or at 50% each one plus MA combined with SE + Vit. B1 both at 200 ppm in the two growing seasons. This combined treatment increased the herb dry weight to 214.5 g/plant whereas, control the plants which treated with tap water had

101.8 g/plant (1st season). Moreover, the above-mentioned treatment increased the fruit yield of parsley plants from 28.4 g/plant to 53.60 g/plant. Therefore, supplying parsley plants with the mineral N fertilizer at 50% as ammonium sulphate plus MA combined with seaweeds extract + Vit. B1 both at 200 ppm is suggested to maximize the herb, fruits and EO productivity and reducing mineral N fertilizer by 50%.

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تأثير التسميد النيتروجينى والحيوي ومستخلص الأعشاب البحرية والثيامين على النمو والمحصول والزيت الطيار لنبات البقدونس

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أجريت هذه التجربة الحقلية لدراسة تاثير خفض الجرعة الموصي بها من السماد النيتروجينى إلى ٢٥، ٥٠ و٧٥٪ على نباتات البقدونس باستخدام بعض المنشطات الحيوية كبديل، فاستعملت البكتريا المثبتة للنيتروجين (المستحضر التجاري المعروف بمنيا ازوتين)، ومستخلص الأعشاب البحرية بتركيز ١٠٠ و٢٠٠ جزء في المليون، والثيامين (فيتامين ب١) بتركيز ١٠٠ و٢٠٠ جزء في المليون في صورة منفرده او متحدين معاً. أظهرت النتائج ان استخدام السماد النيتروجينى بنسبة من ٥٠ او ٧٥٪ + منيا ازوتين كان فعالاً في تنشيط صفات النمو الخضري، وزيادة مكونات المحصول، والزيت الطيار، ومحتوى صبغات التمثيل الضوئى، والنسبة المئوية للنيتروجين مقارنة بالكنترول (الجرعة الموصي بها من التسميد النيتروجين). وقد سبب استخدام السماد بنسبه ٢٥٪ + منيا ازوتين انخفاضاً في الصفات المذكورة سابقاً مقارنة بمعاملة الكنترول. أدت كل معاملات مستخلص الأعشاب البحرية و/أو فيتامين ب١ إلى زيادة صفات النمو الخضري، ومكونات المحصول، والزيت الطيار، ومحتوى صبغات التمثيل الضوئى، والنسبة المئوية للنيتروجين بصورة معنوية مقارنة بالنباتات الغير معاملة. وكانت المعاملة الأكثر فعالية هي مستخلص الأعشاب البحرية + فيتامين ب١ كلاهما بتركيز ٢٠٠ جزء في المليون. وتم الحصول على أفضل النتائج فيما يتعلق بإنتاجية العشب، والثمار، والزيت الطيار لنباتات البقدونس بإستخدام السماد النيتروجينى بنسبة ٥٠٪ + منيا ازوتين مقترناً مع مستخلص الاعشاب البحرية + فيتامين ب١ كلاهما بتركيز ٢٠٠ جزء في المليون.