

## Impact of Some Bio-Stimulants on Growth, Yield and Quality of Head Lettuce (cv. Big bell)

Waleed A. El-Saady<sup>1</sup> and Genesis F. Omar<sup>2</sup>

<sup>1</sup>Vegetable and Floriculture Dept., Fac. of Agric., Mansoura Univ., Mansoura, Egypt.

<sup>2</sup>Horticulture Dept., Fac. Agric., Suez Canal Univ., Ismailia, Egypt.

Received: 19 Oct. 2017/ Accepted: 17 Dec. 2017/ Publication Date: 30 Dec. 2017

### ABSTRACT

The purpose of this investigation was to assess the response of growth, chemical composition and physical and chemical qualities of head lettuce plants yield to effective microorganisms (EM) and moringa leaves extract (MLE) as a foliar application. During the consecutive winter seasons of 2015 and 2016 at the Experimental Station, Faculty of Agriculture, Mansoura University, Dakahlia Governorate, Egypt, two field experiments had been carried out. Three EM levels (0, 5 and 10 cm<sup>3</sup>/l) and four different MLE application numbers (0, 1, 2 and 3 times) and their interactions were applied as a foliar spraying to head lettuce cv. Big bell. The experiment was conducted in a strip-plot design with three replicates. The vertical plots were assigned to EM foliar application levels, whereas the horizontal plots were allocated for foliar spraying numbers with MLE. The results indicated that the highest values of vegetative growth characters (plant height, foliage weight/plant, leaves fresh weight/plant, leaves number/plant and leaf area/plant); chemical composition of outer leaves (chlorophyll a, b, carotenoids contents and total nitrogen, phosphorus and potassium percentages), heads yield and its components (head weight, edible head weight, diameter and compactness and total heads yield/fed.) and edible head chemical quality (vitamin C, TSS and dry matter percentage of outer and inner leaves), and lowest values of edible head nitrate content were obtained from head lettuce plants sprayed with EM at 5 cm<sup>3</sup>/l in both seasons. The same trend of the forecited obtained results was achieved with lettuce plants received three times foliar application of MLE. The interaction treatment between 5 cm<sup>3</sup>/l of EM and foliar spraying of MLE three or two times, respectively gave the maximum records for all studied parameters, with the lowest nitrate (NO<sub>3</sub>) content in edible head lettuce in two seasons. Regarding the mentioned results, it could be recommended that foliar application of head lettuce plants twice with EM at 5 cm<sup>3</sup>/l combined with three times sprayings of MLE are promised for enhancement of head lettuce (cv. Big bell) productivity and quality under Dakahlia Governorate conditions.

**Keywords:** Head lettuce (Big bell cultivar), Effective microorganisms (EM), Moringa leaf extract (MLE), growth, yield, quality.

### Introduction

Head lettuce (*Lactuca sativa* var. *capitata* L.) belongs to family Compositae (Asteraceae) and considered a main salad crop, which contains good amounts of minerals, many vitamins and rich in water. It is one of the most popular among leafy crops and salad vegetables in most countries of the world grown in cool season (Rubatzky and Tamaguchi, 1997). In Egypt, lettuce is an important vegetable crop for both farmers and consumers. For farmers, it is amongst fast cash crop in the winter season. For consumers, lettuce is eaten fresh and salad, where it is consider as a nutritious and aperitif vegetable (El-Bassyouni, 2016).

The utilization of biostimulants and fertilizers to increase plant productivity nowadays is a regular farming procedure. Biostimulants contain various substances and microorganisms that improve plant growth (Calvo *et al.*, 2014). The impacts of diverse biostimulants on plant growth, yield and quality of lettuce have been studied by some investigators till now (Amanda *et al.*, 2009; Lucini *et al.*, 2015; Shahein *et al.*, 2015; Dudas *et al.*, 2016 and Abdel Nabi *et al.*, 2017). Some of the most beneficial and efficient biostimulants that are used either a foliar or a soil amendment for vegetables are effective microorganisms (EM) and moringa leaf extract (MLE).

**Corresponding Author:** Waleed, A. El-Saady, Vegetable and Floriculture Dept., Fac. of Agric., Mansoura Univ., Mansoura, Egypt. E-mail: elsady\_2003@mans.edu.eg

EM is a commercial bio-stimulant or soil amendment, produced by EM Research Organization (EMRO) and developed by Professor Teruo Higa, Ryukyu Univ., Okinawa, Japan (Higa, 1991). EM comprises of more than 60 beneficial and naturally occurring strains of effective microorganisms including yeasts, actinomycetes, lactic acid bacteria, photosynthetic bacteria and different other microorganisms types (Woodward, 2003). Therefore, it must be effective to enhance soil health, quality and its productivity and in turn to improve the growth, yield and quality of different crops (Higa and Wididana, 1991 and Kengo and Hui-lian, 2000) through their effects on stimulation and activation of plant root system growth, improving availability of nutrients and increasing nutrient-water uptake processes by plants, which in turn leads to increase plant growth and productivity (Muthaura *et al.*, 2010).

Although various *Moringa oleifera* plant part extracts are known to possess diverse biological and medicinal activity on animals and human, little is known scientifically about its potential effect as a growth improvement in major vegetable crops because very few published literature are available that clearly explain the effects of moringa leaf extract in this regard. Moringa leaves are potential source of vitamins (A, C and E), iron, calcium, riboflavin,  $\beta$ -carotene, phenolics (Nambiar *et al.*, 2005) and considered as a powerful natural antioxidants (Njoku and Adikwu, 1997). Nowadays, moringa plant has attained enormous attention because of having naturally-occurring cytokinin (zeatin), antioxidants and macro and micro nutrients in its leaves (Abdalla and El-Khoshiban, 2012 and Abdalla, 2013) which in turn act as natural plant growth enhancer. Thus, MLE is low-cost and friendly for environment.

Therefore, the present study aimed to evaluate response of growth, yield and quality of head lettuce to foliar spraying with different levels of EM and number of moringa leaf extract application times as well as their interactions under the environmental conditions of Mansoura, Dakahlia Governorate, Egypt.

## Materials and Methods

Two field experiments were carried out during the two winter seasons of 2015 and 2016 under clay loamy soil conditions using furrow irrigation system at the Experimental Station, Faculty of Agriculture, Mansoura University, Mansoura Governorate, Egypt to study the response of growth and crop performance of head lettuce cv. Big bell to exogenous foliar application with EM, moringa leaf extract and their interactions. The experiments were carried out in a strip-plot design with three replicates. The vertical plots were assigned to EM foliar application levels, whereas the horizontal plots were allocated for foliar spraying numbers with MLE. Physical and chemical analysis of the experimental soil was listed in (Table 1).

**Table 1:** Soil physical and chemical characters during the both seasons of 2016 and 2017

Seasons	Silt %	Clay %	Sand %	Soil texture	pH	E.C (dsm <sup>-1</sup> )	OM %	CaCO <sub>3</sub> %	N ppm	P ppm	K ppm
2015	41.3	37.2	21.5	Clay loamy	8.12	1.73	1.93	3.07	53.9	6.6	289
2016	41.4	37.5	21.1	Clay loamy	8.19	1.79	2.01	3.01	52.3	6.7	278

OM: Organic matter

Head lettuce transplants (cv. Big bell 40 days old) were transplanted on 20<sup>th</sup> and 21<sup>th</sup> of October during both seasons at distance of 35 cm a part on two sides of ridge (30000 plant per fed.). The plot area was 12. 0 m<sup>2</sup> which consisted of five ridges of 0.8 m wide and 3.0 m long.

EM solution was obtained from Ministry of Agriculture. Three foliar application treatments of EM, namely 0 (as control), 5 and 10 cm<sup>3</sup> EM/l were sprayed twice in the same day of the first and second irrigations.

To prepare a moringa leaf extract (MLE), a quantity of 20 g of green tender moringa leaves was obtained from growing moringa shrubs at the Experimental Farm of Agronomy Dept., Faculty of Agric., Mansoura Univ., Egypt. This amount was mixed with 675 ml of 80 % ethanol as described by Makker and Becker (1996). The mixture was ground and stirred utilizing a homogenizer to aid maximizes the extract amount. The solution was then filtered by wringing the solution employing a heavy pored cloth. The resultant solution was re-filtered by using No. 2 Whatman filter paper. Using method as reported by Fuglie (2000), the obtained extract was diluted with distilled water at a

proportion of 1:32 (v/v) and thereafter sprayed directly onto plants. The remaining of prepared extract was stored within refrigerator and only taken out when needed for use.

Prepared MLE was used as foliar sprays at mentioned rate three times; the first and second sprays were done after three days of the 1<sup>st</sup> and 2<sup>nd</sup> irrigation time, while the third spray was conducted in the same day of the 3<sup>rd</sup> irrigation time. Control treatment was sprayed with tap water only.

Plants in all treatments were fertilized with mineral NPK fertilizers at a full recommended dose; 41 kg N/fed., 31 kg P<sub>2</sub>O<sub>5</sub>/fed. and 37.5 kg K<sub>2</sub>O/fed. as a form of ammonium sulphate (20.5 % N), single calcium superphosphate (12.5 % P<sub>2</sub>O<sub>5</sub>) and potassium sulfate (50 % K<sub>2</sub>O), respectively. Farmyard manure at 20 m<sup>3</sup>/fed. and full single calcium superphosphate dose were added during soil preparation. Potassium sulphate rate was applied at two equal portions, before transplanting, and before the first irrigation time. Ammonium sulphate fertilizer was added in two equal rates, before the first and second irrigation time. The process of irrigation was carried out at three times by 3 weeks intervals of transplanting date. All other agricultural procedures were executed according to the Ministry of Agriculture and Land Reclamation recommendations.

#### **Measurements:**

Five plants were randomly taken from each plot after 75 days from the transplanting date to determine the following parameters for the two seasons:

#### **Vegetative growth characters:**

Plant height, foliage fresh weight/plant (outer and inner stem plus outer and inner leaves), leaves fresh weight /plant, leaves number/plant and leaf area/plant were measured.

#### **Chemical composition of outer leaves:**

Chlorophyll a, chlorophyll b and carotenoids content and N, P and K percentages were analyzed according to AOAC (1990).

#### **Heads yield and its physical and chemical qualities:**

Head fresh weight /plant (inner and outer leaves plus inner stem), edible head fresh weight /plant (inner stem plus firm and compacted inner leaves) and edible head diameter were recorded. Edible head compactness rate was computed according to (Riad *et al.*, 2009) as shown in the following equation:

$$\text{Compactness rate} = \frac{\text{Edible head volume } (0.75 \times 3.14 \times \text{radius}^3)}{\text{Edible head weight}}$$

Also, total heads yield /fed. and outer and inner leaf dry matter percentage were determined. Vitamin C, TSS and nitrate content were estimated according to AOAC (1990).

#### **Statistical analysis:**

All data were statistically analyzed using the analysis of variance according to Snedecor and Cochran (1989). Least significant difference (LSD) at the probability of 5 % was used due to the procedure reported by (Gomez and Gomez, 1984).

### **Results and Discussion**

#### **1- Vegetative growth characters:**

Data presented in Table (2) show that plant height, foliage fresh weight per plant, leaves no. per plant, leaves fresh weight per plant and leaves area per plant of head lettuce were significantly increased with all EM levels compared to control (untreated plants) during both seasons. Mostly, the highest records of above parameters were obtained with EM at 5 cm<sup>3</sup>/l, but no significant differences between 5 cm<sup>3</sup>/l and 10 cm<sup>3</sup>/l EM were observed in both seasons. Similar positive effects of EM application on plant growth were noticed in case of cucumber, where EM influenced significantly on the number of leaves and the total surface area of leaves (Abdelaziz and Pokluda, 2009). Likewise,

Chantal *et al.* (2010) reported that EM increased cabbage leaf area and improved photosynthesis. As well as Doklega and Abd El-Hady (2017) on broccoli stated that the highest records of vegetative growth parameters were obtained from plants treated with EM.

Concerning the foliar application no. of MLE, data shown in the same Table (2) mainly indicate that aforementioned parameters were also significantly increased with all MLE foliar application times as compared with control (untreated plants) during two seasons, without significant differences among the three MLE foliar application numbers in the first season particularly for plant height, leaves no. and leaves fresh weight parameters. The highest values in this respect were achieved with head lettuce plants sprayed three times with MLE. Whereas, the lowest values were recorded with control treatment. Similar results were stated by Culver *et al.* (2012) on tomato, Rady and Mohamed (2015) on common bean and Hassan and Abd El-Samee (2015) on roselle plant.

**Table 2:** Effect of foliar applications with EM and MLE on vegetative growth characters of head lettuce during 1<sup>st</sup> (2015) and 2<sup>nd</sup> (2016) seasons.

Characters	Plant height (cm)		Foliage FW* (g/ plant)		Leaves No. /plant		Leaves FW* (g /plant)		Leaves area (cm <sup>2</sup> /plant)		
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season							
<b>Foliar application with EM (cm<sup>3</sup>/l)</b>											
Zero	17.2	16.5	926	887	31.0	29.6	842	807	5148	4931	
5	20.2	19.7	1087	1058	36.4	35.2	992	966	6042	5883	
10	20.1	19.5	1082	1049	36.2	35.0	988	958	6016	5837	
LSD 5%	1.0	0.9	54	52	1.8	1.1	50	49	300	295	
<b>Foliar applications No. of MLE</b>											
Zero	17.3	16.6	932	893	31.2	29.7	847	812	5184	4967	
One	18.9	18.2	1015	978	34.0	32.7	926	892	5644	5439	
Two	19.8	19.3	1067	1036	35.6	34.5	974	946	5934	5761	
Three	20.7	20.2	1111	1084	37.3	36.2	1016	991	6180	6034	
LSD 5%	0.9	0.8	51	46	1.7	1.4	48	38	288	229	
<b>Interaction between EM foliar application levels and MLE foliar applications No.</b>											
Zero	Zero	16.1	15.6	870	829	29.3	27.5	788	751	4837	4610
	One	16.6	15.9	894	855	30.0	28.6	815	779	4972	4755
	Two	17.1	16.4	922	884	30.8	29.5	840	805	5127	4910
	Three	18.9	18.2	1017	980	34.1	32.8	927	893	5657	5448
5	Zero	17.7	17.0	954	916	31.8	30.5	868	834	5306	5094
	One	19.5	18.9	1050	1014	35.3	33.8	958	924	5841	5637
	Two	21.6	21.2	1165	1141	39.0	38.0	1066	1045	6476	6347
	Three	21.9	21.6	1177	1161	39.5	38.8	1076	1061	6546	6455
10	Zero	18.1	17.4	972	935	32.5	31.1	885	850	5408	5197
	One	20.5	19.8	1100	1065	36.8	35.6	1004	972	6120	5924
	Two	20.7	20.1	1115	1084	37.1	36.1	1018	989	6199	6026
	Three	21.2	20.7	1139	1113	38.3	37.1	1043	1019	6335	6200
LSD 5%	1.6	1.5	89	80	3.0	2.5	83	67	500	397	

\*FW: Fresh weight

The several interactions between the two studied factors, *i.e.*, foliar application with EM and foliar spraying times with MLE on forecited vegetative growth characters of head lettuce plants during 2015 and 2016 seasons were tabulated in Table 2. Whereat, the highest values in this concern were obtained from foliar application of EM at 5 cm<sup>3</sup>/l accompanied with the three or two times foliar application of MLE followed by the treatment of EM at 10 cm<sup>3</sup>/l combined with the same numbers of MLE application compared with the other individual applications of them or control treatment (without both EM and MLE foliar application). On the other hand, the lowest values for these above mentioned parameters were obtained by control treatment in the two studied seasons.

The enhancement of head lettuce plant growth parameters by using EM may be due to increasing beneficial microorganism's populations such as yeasts, actinomycetes, lactic acid and photosynthetic bacteria in the soil which results in improvement of vegetative growth by increasing the rate of organic matter mineralization that lead to an increase in plant-available nutrients and suppression of soil born pathogens.

The positive effects of these treatments (EM level, MLE spraying number and their interactions) may be ascribed to the important physiological role of EM in vegetable plants (Daly and

Stewart, 1999). Also, moringa leaf extracts is a rich source of amino acids, potassium, calcium, iron, vitamin E, ascorbates, phenolic compounds and growth regulating hormones like zeatin (Makkar and Becker, 1996 and Nagar *et al.*, 2006) that reflected on an increase in growth characters of head lettuce plants.

## 2- Chemical composition of outer leaves:

Data of both seasons in Table (3) indicate that, in most cases, chlorophyll a and b as well as total carotenoids contents and total nitrogen, phosphorus and potassium percentages were significantly increased with two EM levels (5 and 10 cm<sup>3</sup>/l) under study compared with untreated plants (control) in both seasons. The highest values in this connection were obtained by the treatment of 5 cm<sup>3</sup>/l level which followed by 10 cm<sup>3</sup>/l level without significant differences between them. Many investigators concluded that EM application had an important physiological and biochemical functions on structure of photosynthetic pigments, metabolism of nitrogen, phosphorus and potassium. These results are in general agreement with those found by Kleiber *et al.* (2013) on lettuce plant.

**Table 3:** Effect of foliar applications with EM and MLE on pigments, N, P and K in outer leaves of head lettuce during 1<sup>st</sup> (2015) and 2<sup>nd</sup> (2016) seasons.

Characters	Chl. A (mg/100g FW*)		Chl. b (mg/100g W*)		Total carotenoids (mg/100g FW*)		N (%)		P (%)		K (%)		
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	
<b>Foliar application with EM (cm<sup>3</sup>/l)</b>													
Zero	55.4	53.0	26.9	25.8	18.8	18.0	3.63	3.48	0.424	0.402	3.93	3.77	
5	65.0	63.3	31.6	30.8	22.1	21.5	4.27	4.15	0.498	0.485	4.62	4.49	
10	64.7	62.7	31.5	30.5	22.0	21.4	4.24	4.11	0.496	0.481	4.60	4.46	
LSD 5%	3.2	2.3	1.5	1.2	1.1	0.8	0.21	0.18	0.024	0.024	0.23	0.21	
<b>Foliar applications No. of MLE</b>													
Zero	55.7	53.4	27.1	26.0	19.0	18.2	3.65	3.50	0.427	0.409	3.96	3.80	
One	60.7	58.5	29.5	28.4	20.7	19.9	3.98	3.84	0.465	0.448	4.31	4.16	
Two	63.8	62.0	31.0	30.1	21.7	21.1	4.19	4.07	0.489	0.475	4.54	4.40	
Three	66.5	64.9	32.3	31.5	22.6	22.1	4.36	4.25	0.510	0.491	4.72	4.61	
LSD 5%	3.1	2.6	1.5	1.2	1.0	0.9	0.20	0.16	0.023	0.021	0.22	0.19	
<b>Interaction between EM foliar application levels and MLE foliar applications No.</b>													
Zero	Zero	52.0	49.6	25.3	24.1	17.7	16.9	3.41	3.25	0.399	0.380	3.70	3.52
	One	53.5	51.1	26.0	24.9	18.2	17.4	3.51	3.35	0.410	0.392	3.80	3.63
	Two	55.1	52.9	26.8	25.7	18.8	18.0	3.62	3.47	0.422	0.405	3.92	3.75
	Three	60.8	58.6	29.6	28.5	20.7	19.9	3.99	3.84	0.466	0.432	4.32	4.17
5	Zero	57.1	54.8	27.7	26.6	19.4	18.6	3.74	3.59	0.437	0.420	4.06	3.89
	One	62.8	60.6	30.5	29.5	21.4	20.6	4.12	3.98	0.481	0.464	4.47	4.31
	Two	69.7	68.3	33.9	33.2	23.7	23.2	4.57	4.48	0.534	0.523	4.95	4.85
	Three	70.4	69.4	34.2	33.8	24.0	23.6	4.62	4.55	0.540	0.532	5.01	4.93
10	Zero	58.2	55.9	28.3	27.2	19.8	19.0	3.81	3.66	0.446	0.428	4.14	3.97
	One	65.8	63.7	32.0	31.0	22.4	21.7	4.32	4.18	0.504	0.489	4.68	4.53
	Two	66.7	64.8	32.4	31.5	22.7	22.1	4.37	4.25	0.511	0.497	4.74	4.61
	Three	68.1	66.6	33.1	32.4	23.2	22.7	4.47	4.37	0.522	0.510	4.84	4.73
LSD 5%	5.3	4.6	2.6	2.2	1.8	1.5	0.35	0.28	0.041	0.036	0.38	0.34	

\*FW: Fresh weight

It is quite clear from the data shown in Table 3 that, chlorophyll a, chlorophyll b and carotenoids contents, total nitrogen, phosphorus and potassium percentages of the outer leaves of lettuce were significantly affected by all MLE foliar application frequencies as compared with control treatment in both seasons. The best treatment in this respect was foliar spraying with moringa leaf extract three times followed by the treatment of two times application in the first and second seasons, respectively. The increase in nitrogen and potassium percentages may be attributed to the fact that moringa leaf extract is a rich source of amino acids, potassium, calcium, iron, vitamin E, ascorbates, phenolic compounds and growth regulating hormones like zeatin (Nagar *et al.*, 2006 and Hamad *et al.*, 2017).

Results under discussion in Table 3 indicate that, the highest values of chemical composition of lettuce outer leaves (the photosynthetic pigments and NPK percentages) were recorded with lettuce plants receiving EM at 5 or 10 cm<sup>3</sup>/l level interacted with foliar spraying with MLE three or two times, respectively. This trend was true in both seasons. On the contrary, the general control treatment (no foliar spraying with EM and MLE) registered the lowest values for the forecited parameters in two seasons, consecutively. These results are in harmony with those reported by Abdallah *et al.* (2016) who found that fennel plants inoculated with arbuscular mycorrhizal (AM) fungi combined with aqueous extract foliar spray of moringa leaves gave the highest herb contents of nitrogen, phosphorus and potassium.

### 3- Heads yield and its physical qualities:

Data in Table 4 show that, the level of EM at 5 and 10 cm<sup>3</sup>/l recorded the highest values of head fresh weight/plant, edible head fresh weight/plant, edible head diameter, edible head compactness and total heads yield/fed. compared with non-applied plants (control) during both seasons. Likewise, the abovementioned characters were showed gradually significant increase with increasing EM levels from zero to 5cm<sup>3</sup>/l then it was decreased in the first and second seasons. Kleiber *et al.* (2014) on tomato reported that application of EM had positive significant effect when applied either as seed inoculation or combined seed inoculation + spraying of plants on enhance of total and commercial yields. Also, similar results had been obtained by Xu *et al.* (2001) on tomato, Dawa, *et al.* (2013) on pea and Doklega and Abd El-Hady (2017) on broccoli.

**Table 4:** Effect of foliar applications with EM and MLE on heads yield and its physical quality of head lettuce during 1<sup>st</sup> (2015) and 2<sup>nd</sup> (2016) seasons.

Characters	Head FW*		Edible head FW*		Edible head diameter		Edible head compactness		Total heads yield		
	(g/plant)		(g/plant)		(cm)				(ton./ fed.)		
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	
<b>Foliar application with EM (cm<sup>3</sup>/l)</b>											
Zero	870	833	606	581	17.5	16.7	2.63	2.41	26.10	25.00	
5	1021	994	712	693	20.5	20.0	3.64	3.46	30.64	29.83	
10	1016	986	708	687	20.4	19.8	3.60	3.35	30.50	29.58	
LSD 5%	50	37	35	27	1.0	0.8	0.35	0.27	1.52	1.11	
<b>Foliar applications No. of MLE</b>											
Zero	876	839	610	585	17.6	16.9	2.66	2.44	26.28	25.18	
One	954	919	665	640	19.2	18.5	3.18	2.95	28.62	27.57	
two	1003	974	699	679	20.2	19.6	3.52	3.33	30.09	29.22	
Three	1044	1019	728	710	21.0	20.4	3.80	3.58	31.33	30.58	
LSD 5%	48	41	34	28	0.9	0.8	0.32	0.26	1.46	1.23	
<b>Interaction between EM foliar application levels and MLE foliar applications No.</b>											
Zero	Zero	817	779	569	543	16.4	15.7	2.32	2.10	24.52	23.37
	One	840	803	585	560	16.9	16.2	2.46	2.24	25.21	24.10
	Two	866	830	604	579	17.4	16.7	2.61	2.39	25.99	24.92
	Three	956	920	666	641	19.2	18.5	3.16	2.93	28.68	27.62
5	Zero	896	861	625	600	18.0	17.3	2.79	2.56	26.90	25.82
	One	987	952	688	664	19.8	19.2	3.39	3.15	29.61	28.58
	Two	1094	1072	763	747	22.0	21.6	4.17	4.00	32.84	32.18
	Three	1106	1090	771	760	22.2	22.0	4.24	4.11	33.19	32.73
10	Zero	914	878	637	612	18.4	17.7	2.89	2.67	27.42	26.35
	One	1034	1001	721	698	20.8	20.0	3.71	3.47	31.03	30.03
	Two	1047	1018	730	710	21.1	20.5	3.81	3.59	31.43	30.55
	Three	1070	1046	746	729	21.5	20.8	4.00	3.70	32.12	31.38
LSD 5%	84	71	58	49	1.7	1.4	0.56	0.46	2.53	2.13	

\*FW: Fresh weight

The data given in Table 4 suggest that, foliar spraying of moringa leaves extract two or three times significantly increased lettuce heads yield and its physical quality compared with control in both experiment seasons. The maximum mean values of head fresh weight/plant, edible head fresh weight/plant, edible head diameter, edible head compactness and total heads yield /fed. under study have been recorded with applying moringa extract two or three times in the two tested seasons. These

results are in agreement with those reported by Hassan and Abd El-Samee (2015) on roselle, Rady and Mohamed (2015) on *Phaseolus vulgaris* and Matthew (2016) on pepper.

Lettuce heads yield and its physical quality that are mentioned above were significantly affected by EM levels combined with MLE foliar spraying numbers. In most cases, the interaction between different EM levels and numbers of application with moringa leaves extract gave higher yield components values compared with control treatment. The highest values in this connection were obtained with plants treated with EM at 5 cm<sup>3</sup>/l + foliar spray with MLE three times in both seasons (Table 4). However, Daly and Stewart (1999) found that the application of a mixture of EM and molasses in growing of onion plant contributed to yielding of this vegetable crop which increased by 29%. In case of pea plant the increase in yields amounted to 31%, while in sweet corn it was 23%. Also, Hamad *et al.* (2017) on dill found that foliar spraying of aloe and moringa leaves extracts significantly increased yield components compared with control.

#### **4- Edible heads chemical quality:**

The effects of EM levels shown in Table (5). Vitamin C, TSS, and dry matter percent of both outer and inner leaves of lettuce plant were significantly affected by the effective microorganisms in both seasons. Using EM levels at 5 or 10 cm<sup>3</sup>/l led to significant increases in this regard compared with control, with no significant differences between two EM levels were observed in both seasons. Whereas, edible head NO<sub>3</sub> content (mg/kg expressed on the dry weight basis) was significantly decreased by EM application in the first and second seasons compared with control treatment. Since, the content of nitrate was lower in EM treated plants than in control ones. Furthermore, Ncube and Calistus (2012) stated that if EM were found to reduce nitrate content also in cucumber, pumpkin and squash fruits, this would be a very desirable and important effect on their quality. Also, Olle and Williams (2015) reported that EM improves the growth and reduces the nitrate content of cucumber, pumpkin and squash transplants. Likewise, Shahein *et al.* (2015) pointed out that the lowest nitrate content in lettuce leaves was achieved when Big bell cultivar treated with humic substances extracted from biogas manure as soil application. Likewise, Dudas *et al.* (2016) stated that vitamin C and dry matter were increased when head lettuce plants were treated with Bio-algee S-90 as biostimulant, moreover they obtained the lowest nitrate content in lettuce leaves after treatment of plants either Bio-algee S-90 or megagreen.

Data recorded in Table 5 pointed out that, all moringa leaf extract foliar treatments significantly increased vitamin C, TSS in inner lettuce leaves, and dry matter percent of both outer and inner leaves of head lettuce plant compared with control in both seasons. The best treatment in this regard was the foliar spraying with moringa leaf extract three times followed by the treatment of two times application in the first and second seasons. While, edible head NO<sub>3</sub> mg/kg as dry weight significantly decreased by all foliar treatments with moringa leaf extract in the first and second seasons compared with control one. Abdelnaser *et al.* (2017) indicated that all moringa leaf extract concentrations had a positive effect on biochemical, yield and yield related traits as well as yield quality of snap bean compared with control. In conclusion, the leaf extract of moringa provides a good source of phytohormones that have a positive role to stimulate growth and productivity of snap bean plants.

The different interaction effects between EM foliar application levels and MLE foliar applications numbers gave the best values for Vit. C, TSS and nitrate content of edible head as well as dry matter of outer and inner leaves of lettuce plant as compared with control treatment (without foliar application with both EM and MLE) in both seasons as presented in Table (5). The interaction treatment which including a foliar application with EM at 5 cm<sup>3</sup>/l level + three or two times spraying with MLE recorded the highest values of aforementioned chemical quality parameters and in the same time it achieved significant values for nitrate (NO<sub>3</sub>) content (less nitrate content) in edible head lettuce during the two seasons. On the contrary, the control treatment registered the lowest values for Vit. C, TSS and outer and inner leaves dry matter, whereas it recorded the highest nitrate content of edible head lettuce in both seasons. These results are in harmony with the obtained results by Kleiber *et al.* (2013) on lettuce plant and reported that the application of EM had a positive influence on total dry weight, percentage of dry matter and chemical composition of leaves. Also, Abou El-Nour and Ewais (2017) found that moringa leaves extract increased pepper fruit yield quality as well as fruit chemical contents such as ascorbic acid.

**Table 5:** Effect of foliar applications with EM and MLE on edible head Vit. C, TSS, nitrate and dry matter of outer and inner leaves of head lettuce during 1<sup>st</sup> (2015) and 2<sup>nd</sup> (2016) seasons.

Characters	Vit. C (mg/100g FW*)		TSS (%)		Edible head NO <sub>3</sub> (mg/kg DW**)		Dry matter (%)				
							Outer leaves		Inner leaves		
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	
<b>Foliar application with EM (cm<sup>3</sup>/l)</b>											
Zero	3.04	2.91	2.85	2.73	126.9	121.6	4.41	4.22	2.47	2.36	
5	3.57	3.47	3.34	3.26	109.6	106.5	5.18	5.04	2.89	2.82	
10	3.55	3.44	3.33	3.24	113.0	109.5	5.15	5.00	2.88	2.79	
LSD 5%	0.18	0.15	0.16	0.18	0.9	0.9	0.25	0.14	0.14	0.08	
<b>Foliar applications No. of MLE</b>											
Zero	3.06	2.93	2.87	2.74	126.4	121.1	4.44	4.25	2.48	2.37	
One	3.33	3.21	3.12	3.02	120.2	115.7	4.84	4.66	2.70	2.60	
Two	3.50	3.40	3.28	3.19	112.2	108.8	5.08	4.94	2.84	2.76	
Three	3.65	3.56	3.42	3.35	107.3	104.6	5.29	5.17	2.96	2.89	
LSD 5%	0.17	0.14	0.15	0.14	0.8	0.6	0.24	0.21	0.13	0.11	
<b>Interaction between EM foliar application levels and MLE foliar applications No.</b>											
Zero	Zero	2.85	2.72	2.68	2.53	134.2	127.9	4.14	3.95	2.32	2.19
	One	2.94	2.81	2.75	2.65	128.1	122.5	4.26	4.07	2.38	2.28
	Two	3.03	2.90	2.84	2.72	125.8	120.6	4.39	4.21	2.46	2.35
	Three	3.34	3.22	3.13	3.02	119.8	115.3	4.85	4.67	2.71	2.61
5	Zero	3.13	3.01	2.94	2.82	123.0	118.0	4.55	4.36	2.54	2.44
	One	3.45	3.33	3.23	3.12	119.1	114.9	5.00	4.83	2.80	2.70
	Two	3.83	3.75	3.59	3.51	100.2	98.2	5.55	5.44	3.10	3.04
10	Three	3.86	3.81	3.63	3.58	96.5	95.1	5.61	5.53	3.14	3.09
	Zero	3.19	3.07	3.00	2.88	122.1	117.3	4.63	4.45	2.59	2.49
	One	3.61	3.50	3.39	3.28	113.5	109.8	5.24	5.08	2.93	2.84
LSD 5%	Two	3.66	3.56	3.43	3.34	110.9	107.7	5.31	5.16	2.97	2.89
	Three	3.74	3.65	3.51	3.46	105.9	13.4	5.43	5.30	3.04	2.97
		0.29	0.24	0.27	0.25	1.4	1.8	0.42	0.37	0.24	0.20

\*FW: Fresh weight

\*\*DW: Dry weight

Finally, the obtained results in this regard may be explained on the basis of adding EM supplies the soil with diverse beneficial and effective microorganisms populations which mainly aids to rapid decomposition and mineralization of farmyard manure added to the experimental soil and releasing essential plant growth promoters and nutrients in the root environment that lead to considerable increase in nutrient availability and uptake them which might have an important role in enhancement of different plant metabolism processes resulting in high quality of head lettuce edible portion. Also, both EM and MLE may be acting simultaneously in order to improve absorbance of essential major elements like potassium which enhances the production of protein via stimulating the formation of energy-rich ATP compounds; reduction of NO<sub>3</sub> to NH<sub>2</sub> and the providing of assimilates for synthesis of amino acids and in turn protein. Hence, good abundant supply of potassium together with balanced levels of nitrogen, phosphorus, magnesium and other nutrients favours the rapid conversion of inorganic-N into protein and consequently decreasing NO<sub>3</sub>-N content in edible plant parts which in turn would be safe for human health.

## Conclusion

Regarding the forecited results, it could be concluded that using effective microorganisms at 5 cm<sup>3</sup>/l level + foliar spraying with moringa leaf extract three times had a positive significant effect on vegetative growth characters, yield quality and leaves chemical composition with less nitrate content of head lettuce plants cv. Big bell under Dakahlia Governorate conditions.

## References

Abdalla, M.M., 2013. The potential of *Moringa oleifera* extract as a bio-stimulant in enhancing the growth, bio-chemical and hormonal contents in rocket (*Eruca vesicaria* subsp. *sativa*) plants. Int. J. Plant Physiol. Biochem., 5(3): 42-49.

- Abdallah, Sonia A.S., H.M.S. Hassan and M.A.I. Mansour, 2016. Effect of mycorrhiza inoculation and foliar spray of some plants extracts on fennel growth and productivity. *Zagazig J. Agric. Res.*, 43 (2): 395-406.
- Abdelaziz, M.E. and R. Pokluda, 2009. Response of cucumbers grown on two substrates in an open soilless system to inoculation with microorganisms. *Acta Hort.*, 819: 157–164.
- Abdel Nabi, H., K. Dawa, E.I. El-Gamily and Y.F. Imryed, 2017. Effect of magnetic water, foliar application with nano material and nitrogen levels on productivity and quality of head lettuce. *Int. J. Adv. Res. Biol. Sci.*, 4 (5): 171-181.
- Abdelnaser, A.E., E.A. Mohamed, H.F. Maswada and T.D. Xuan, 2017. Enhancing growth, yield, biochemical, and hormonal contents of snap bean (*Phaseolus vulgaris* L.) sprayed with moringa leaf extract. *Archives of Agronomy and Soil Sci.*, 63 (5): 687-699.
- Abou El-Nour, H.H. and N.A. Ewais, 2017. Effect of *Moringa oleifera* leaf extract (MLE) on pepper seed germination, seedlings improvement, growth, fruit yield and its quality. *Middle East J. Agric. Res.*, 6 (2): 448-463.
- Amanda, A., M. Valagussa, A. Piaggese and A. Ferrante, 2009. Effect of biostimulants on quality of baby leaf lettuce grown under plastic tunnel. *Acta Horticulturae*, 807, 407–412.
- AOAC, 1990. Official methods of analysis. 15<sup>th</sup> ed., Association of Official Analytical Chemists, International, Arlington, Virginia, USA.
- Calvo, P., L. Nelson and J. Kloepper, 2014. Agricultural uses of plant biostimulants. *Plant Soil*, 383, 3–41.
- Chantal, K., S. Xiaohou, W. Welmu and B. Ong'or, 2010. Effects of effective microorganisms on yield and quality of vegetable comparatively to nitrogen and phosphorus fertilizers. *Pakistan J. of Nutrition*, 9 (11): 1039-1042.
- Culver, M., T. Fanuelli and A.Z. Chiteka, 2012. Effect of moringa extract on growth and yield of tomato. *Greener J. Agric. Sci.* 2 (5): 207-211.
- Daly, M.J. and D.P.C. Stewart, 1999. Influence of effective microorganisms (EM) on vegetable production and carbon mineralization- a preliminary investigation. *J. of Sustain. Agric.*, 14 (2/3): 15–25.
- Dawa, K.K., A.H. Amer and M.M. Helmy, 2013. Effect of magnetite, humic acid and biofertilizer as well as N, P and K levels application on growth and yield of pea (*Pisum sativum* L). *J. Plant Production, Mansoura Univ.*, 4 (4): 641 – 654.
- Doklega, S.M and M.A. Abd El-Hady, 2017. Impact of organic, mineral and bio-fertilization on broccoli. *J. Plant Production, Mansoura Univ.*, 8 (9): 945 – 951.
- Dudas, S., I. Sola. B. Sladonja, R. Erhatic, D. Ban and D. Poljuha, 2016. The effect of biostimulants and fertilizer on "low input" lettuce production. *Acta Bot. Croat.*, DOI: 10.1515/botcro, 1-12.
- El-Bassyouni, M.S.S., 2016. Effect of different nitrogen sources and doses on lettuce production. *Middle East J. Agric. Res.*, 5 (4): 647-654.
- Fuglie L. J., 2000. New uses of moringa studied in Nicaragua: ECHO's Technical Network Site-networking global hunger solutions. ECHO, Nicaragua.
- Gomez, K. A. and A. A. Gomez, 1984. *Statistical Procedures for Agricultural Research*. John Wiley & Sons Inc., New York, 2nd ed., 68 p.
- Hamad, E.H.A., M.S.S. El-Basuony and M.A.I. Abdelkader, 2017. Enhancing dill (*Anethum graveolens* L.) growth and yield by NPK fertilization and some plant extracts. *Inter. J. Agric. and Econ. Development*, 5 (2): 57-78.
- Hassan, H.M. and M.A. Abd El-Samee, 2015. Growth, yield and nutritional value of *Hibiscus sabdariffa* L. as influenced by licorice and moringa aqueous extracts under North Sinai conditions. *Zagazig J. Agric. Res.*, 42 (5): 1069-1079.
- Higa, T., 1991. Effective microorganisms: A biotechnology for mankind. P.7-14. In J. F. Parr, S. B. Hornick and C. E. Whitman, ed., *Proceedings of the First International Conference on Kyusei Nature Farming*. U. S. Dept. Agric., Washington. D. C., USA.
- Higa, T. and A.N. Wididana, 1991. Changes in the soil microflora induced by effective microorganisms. P, 153-162. In J. F. Parr, S. B. Hornick and C.E. Whitman, ed., *Proceedings of the First International Conference on Kyusei Nature Farming*. U. S. Dept. Agric., Washington. D. C., USA.

- Kengo, Y. and X. Hui-lian, 2000. Properties and applications of an organic fertilizer inoculated with effective microorganisms. *J. Crop Production*, 3 (1): 255-268.
- Kleiber, T., J. Starzyk and M. Bosiacki, 2013. Effect of nutrient solution, effective microorganisms (EM-A), and assimilation illumination of plants on the induction of the growth of lettuce (*Lactuca sativa* L.) in hydroponic cultivation. *Acta Agrobotanica*, 66 (1): 27–38.
- Kleiber, T., J. Starzyk, R. Górski, K. Sobieralski, M. Siwulski, A. Rempulska and A. Sobiak, 2014. The studies on applying of effective microorganisms (em) and crf on nutrient contents in leaves and yielding of tomato. *Acta Sci. Pol., Hortorum Cultus*, 13 (1): 79-90.
- Lucini, L., Y. Roupael, M. Cardarelli, R. Canaguier, P. Kumar and G. Colla, 2015. The effect of a plant-derived biostimulant on metabolic profiling and crop performance of lettuce grown under saline conditions. *Scientia Horticulturae*, 182, 124-133
- Makkar, H. P. and K. Becker, 1996. Nutritional value and antinutritional components of whole and ethanol extracted *Moringa oleifera* leaves. *Animal Feed Sci. and Technol.*, 63: 211-288.
- Matthew, A., 2016. Moringa leaf extract on the growth and yield of pepper (*Capsicum annum* L.). *J. Agric. Biol. Sci.*, 11 (3): 107-109.
- Muthaura, C., D.M. Musyimi, J.A. Ogur and S.V. Okello, 2010. Effective microorganisms and their influence on growth and yield of pigweed (*amaranthus dubians*). *ARPJ. Agric. Biol. Sci.*, 5 (1): 17-22.
- Nagar, P.K., R.I. Iyer and P.K. Sircar, 2006. Cytokinins in developing fruits of *Moringa pterigosperma* Gaertn. *Physiol. Plant.*, 55: 45-50.
- Nambiar, V.S., R. Mehta and M. Daniel, 2005. Polyphenol content of three Indian green leafy vegetables. *J. Food Sci. Technol.*, 42 (6): 312- 315.
- Ncube, L. and B. Calistus, 2012. Effects of the integrated use of effective microorganisms, compost and mineral fertilizer on greenhouse grown tomato. *African J. Plant Sci.*, 6: 120-124.
- Njoku, O.U. and M.U. Adikwu, 1997. Investigation on some physico-chemical antioxidant and toxicological properties of *Moringa oleifera* seed oil. *Acta Pharmaceutica Zagreb*, 47 (4): 287-290.
- Olle, M. and I. Williams, 2015. The influence of effective microorganisms on the growth and nitrate content of vegetable transplants. *J. Adv. Agric. Techno.*, 2 (1): 25-28.
- Rady, M.M. and G.F. Mohamed, 2015. Modulation of salt stress effects on the growth, physio-chemical attributes and yields of *Phaseolus vulgaris* L. plants by the combined application of salicylic acid and *Moringa oleifera* leaf extract. *Scientia Hort.* 193:105–113.
- Riad, G., A. Ghoname, A. Ahmed, M. Abd El- Baky and A. Hegazi, 2009. Cabbage nutritional quality as influenced by planting density and nitrogen fertilization. *Fruit, Vegetable and Cereal Sci. and Bio.*, 3 (1): 68-74.
- Rubatzky, V.E. and M. Tamaguchi, 1997. *World vegetables, principles, production and nutritive values*. 2<sup>nd</sup> ed., Chapman and Hall International Thomson Publishing. New York, U.S.A. pp. 843.
- Shahein, M.M., M.M. Afifi and A.M. Algharib, 2015. Study the effects of humic substances on growth, chemical constituents, yield and quality of two lettuce cultivars (cv.s. dark green and big bell). *J. Mater. Environ. Sci.*, 6 (2): 473-486.
- Snedecor, G.W. and W.G. Cochran, 1989. *Statistical methods*. 8<sup>th</sup> ed. Iowa State Univ. Press, Ames Iowa, USA.
- Woodward, D., 2003. *Soil and sustainability: Effective microorganisms as regenerative systems in earth healing*. MSc. Dissertation. Brighton. Web [http:// www. Livingsoil.co.uk/learning/soilsustain.html](http://www.livingsoil.co.uk/learning/soilsustain.html).
- Xu, H. L., R. Wang and M.A. Mridha, 2001. Effects of organic fertilizers and microbial inoculants on leaf photosynthesis and fruit yield and quality of tomato plants. *J. Crop Production*, 3: 173-182.