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Response of Lettuce (*Lactuca sativa* L.) Plant to Bio-stimulants Under Various Irrigation Regimes in Reclaimed Sandy Soils

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The study conducted at private farm in reclaimed sandy soils in the western district of, El- Minia Governorate, Egypt during winter of two successive seasons of 2020/2021, 2021/2022. To investigate the influences of different irrigation regimes, bio-stimulants and their interaction on lettuce growth, yield and chemical composition, A split-plot design was used, the main plot was irrigation treatments (100% of crop evapotranspiration, 80% ETc and 60% ETc), while the bio-stimulants (control, potassium humate (K-H), biofertilizers (EM) and mixture from K-H plus EM) were assigned in sub-plot. The results showed that in most aspects there was a significant affect between 100% or 80% ETc compared to 60% ETc but insignificant between 100% ETc and 80% ETc in both seasons. Moreover, soil application of the bio-stimulants reduced the negative impact of water deficit compared to control. Combined K-H and EM caused significant increase in all estimated parameters for plant growth and yield. In addition, increase mineral contents, protein and carbohydrate contents in lettuce leaves. In opposite, it causing significant decrease by (23.20, .18.38 and 18.18, 14.33%) for nitrate and proline through both seasons respectively, compared with control. Furthermore, the highest irrigation water use efficiency was recorded with decreasing the required amount of water (60% ETc). Integration both bio-stimulants caused significant increase in irrigation water use efficiency by 58.60 %in the first season and were 59.14% in the second season. Available N, P and K in soil significantly increase with application of mixture of bio-stimulants followed by sole application of K-H.

Keywords: Sandy soil, Potassium humate, EM, Drip irrigation system, Lettuce plant.

1. <u>Introduction</u>

Lettuce (*Lactuca sativa* L.) is grown all over the world and is considered a winter cash crop for Egyptian farmers. It is rich with vitamins C and E, polyphenols, luthein and fibers **(Chen *et al.*, 2019).** Lettuce plant cultivated since 4500 BC in the Mediterranean area. Lettuce plant contains high cellulose content, so it facilitates digestion. Moreover, lettuce contains lactocin and lactucopicrin which improves sleep. In the case of leafy vegetables, a high concentration of nitrates causes health problems. This is a particularly serious issue in lettuce, which can accumulate a lot of NO_3 **(Cruz *et al.*, 2012).** Toxicity of nitrate, when it is transformed to nitrite, as the probability of formation of toxic compounds increases **(Aboud and Abd-Alrahman, 2021).** Water stress is one of the most devastating environmental problems threatens many countries around the world, among them is Egypt. Researchers face many challenges under increasing population and changing climate conditions are likely to increase water scarcity, which will lead to further decline in crop productivity. Irrigation scheduling might be used to improve plant quality by decreasing excessive vigor and increasing water use efficiency **(Abdel-Fattah *et al.*, 2020).** Moreover, Egypt has limited agricultural land, associated with the lack of irrigation water and rapid population growth **(Okasha *et al.*, 2022).** Sandy soils widely in arid and semi-arid regions in Egypt's east and west deserts. Sandy soils faced a lot of challenges for agricultural production as nutrient deficiencies, low water holding capacity, excessive drainage, susceptibility to wind erosion on sandy dunes, low irrigation water retention, high

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evaporation, low soil organic matter content and low fertility **(Hoa *et al.*, 2010).** In general, sandy soil lacks plant nutrients and the nutrients provided to it are lost due to irrigation water. Plants under water stress can avoid the harmful of drought through several ways as stomata closure, osmotic adjustments, leaf rolling, reductions and thus decreases in cellular expansion, and alterations of different essential physiological and biochemical processes that effect on plant vitality **(Farouk and Ramadan, 2012).** In this respect, anti-transpirants are substances that able to increase leaf resistance to water vapor loss, consequently improving plant water use and increasing biomass or yield **(Zahran *et al.*, 2020).** Bio-stimulants have been described as non-nutritional products that may reduce fertilizer use and increase yield and resistance to water and temperature stress **(Poincelot, 1993).** Bio-stimulants whether substance or microorganism applied to plants to improve nutrition efficiency, abiotic stress tolerance and crop quality **(Patrick, 2015).** Bio-stimulants such as EM, which has role in restoration of healthy ecosystem in both soil and water by using some major genera of microorganisms, which are found in nature: *Rhodopseudomonas*, *Lactobacillus* and *Saccharomyces* **(Shalan, 2014).** Biofertilizers application enhanced growth, yield and ripening of pea plant under drought stress conditions **(Itelima *et al.*, 2018).** One of these bio-stimulants is humic acid that improves soil aggregation, and stimulate microbial diversity **(Chen *et al.*, 2006 and Kocira *et al.*, 2018),** its essential role in carbon and nitrogen cycling, and stability of soil structure **(El-Naqma, 2020).** Humic acid combined with potassium led to rapidly absorbed and incorporated potassium into plants whether via soil addition or foliar application methods **(Abd El-Aal *et al.*, 2010).** Potassium humate is a promising natural resource to improve growth, yield and nutritional state. It is a natural material that can improve soil physical and chemical properties and nutrient dynamics **(Mahdi *et al.*, 2021 and Mohammed *et al.*, 2021).** It can be used as an organic potassium fertilizer to supply the plants with high levels of soluble potassium in a readily available form. As, K-H contains carboxyl, phenolic hydroxyl, and other functional groups that can reduce the loss of ammonium nitrogen, enhance sugar, starch and protein synthesis. Application of bio-stimulants improved onion plant stress tolerance to water deficit irrigation **(Hefzy *et al.*, 2020).**

The goal of this study is evaluation the effect of bio-stimulants whether K-H and/or EM under different irrigation regime to improve lettuce growth and yield with high quality under different irrigation regimes to reduce the negative effect of water stress in reclaimed sandy soils.

2. <u>Materials and Methods</u>

2.1. Experimental site

A field experiment was conducted at private farm in reclaimed sandy soils in the western district of EL-Minia city, El- Minia Governorate, Egypt during winter of two successive seasons of 2020/2021 and 2021/2022. To test the impact of humate potassium (K-H), biofertitatizers (EM; Effective microorganisms) and mixture K-H and EM under three irrigation regimes on vegetative growth, yield parameters and chemical constituents of lettuce plant as well as available macronutrient contents of sandy soil after harvesting. The soil physical and chemical analyses of the experimental site are presented in Table (1). Physical parameters were determined according to the methods of **Haluschak (2006),** while chemical was according to **Reeuwijk (2002).** The experiment includes 12 treatments was arranged in randomized complete block design in a split-plot with three replications. The experimental plot area was 21 m^2. The treatments were arranged as followed:

1. Main plots (irrigation regime with three treatments).

A- Irrigation with 100% of (ETc)

B- Irrigation with 80% (ETc)

C- Irrigation with 60% (ETc)

2- Sub-plots (included four treatments).

1. Control.
2. Potassium Humate (K-H) applied at 20.00 $Lfed^{-1}$
3. EM applied at (5L fed^{-1})
4. Mixture of Potassium Humate (K-H) and EM

2.2. Experimental details:

2.2.1. Organic fertilizers:

Compost fertilizers (commercial compost namely Nile compost (plant residues). Compost was added at the rate of 10 ton fed^{-1} was broadcasted and thoroughly mixed with soil surface layer (0 - 25cm) during plots preparation with all treatments. Chemical analysis of the compost used presented in Table (2).

2.2.2. Chemical fertilizers:

Granular (22.50 kg P_2O_5) fed^{-1} (150 kg super calcium phosphate, 15.5% P_2O_5) was broadcasted and thoroughly mixed with soil surface layer (0 - 25cm) during plots preparation; 60 kg nitrogen/fed (180 kg ammonium nitrate, 33.5% N); in six equal doses with irrigation water (first doses with transplant) and 24 kg

K_2O fed^{-1} (50 kg potassium sulfate, 48% K_2O) in four equal doses with irrigation water (first doses with three doses of nitrogen fertilizers). Potassium humate was applied with the irrigation water through the Fertigation after two weeks from transplanting at rates of 20.00 L fed^{-1} and repeated twice after two weeks interval according to treatments. The chemical analyses of used K-H were shown in Table (3).

2.2.3. Biofertilizers:

EM is a commercial bio-stimulant, contains various selected strains of "effective microorganisms" (photosynthetic bacteria, lactic acid bacteria, yeast, actinomycetes and various fungi) EM were kindly obtained from Agriculture Faculty, Minya university. EM application were performed by addition of 5 L fed^{-1} of Effective microorganisms. According to treatment, the first dose was added after two weeks from transplanting date and repeated twice after two weeks interval.

2.2.4. Irrigation treatments:

CROPWAT model was used to calculate reference evapotranspiration according to Penman Monteith. Crop evapotranspiration (ETc) was calculated according to **(Allen *et al*., 1998).**

$ET_C = ET_O \times K_c$

Where:-

ET_C = Crop evapotranspiration.

ET_O = Reference evapotranspiration.

K_c = Crop coefficient (from FAO paper 56)

The amounts of actual irrigation water applied under each irrigation treatment were determined using the following equation: **James (1988).**

$$\text{I. Ra} = \frac{\text{ETc} + \text{Lf}}{\text{ER}}$$

Where:

I.Ra = total actual irrigation water applied mm/ interval.

Etc = Crop evapotranspiration using CROPWAT model (8)

Lf = leaching factor 10 %.

Er = irrigation system efficiency.

2.2.5. Water use efficiency (WUE)

The irrigation water use efficiency (IWUE, kg m^{-3}) values were calculated as follows:

$$\text{IWUE} = \frac{Marketable\ yield\ (Kg/fed.)}{2\ arrigation\ water\ Applied\ (m\ 3\ /fed)}$$

2.3. Plant material and growth conditions:

Seeds of head lettuce (Big-Bell) were germinated in a tray filled with peat and vermiculite mixture (1:1) and incubated for three days at (10/8/2020 and 12/8/2021). After germination, the trays were placed in the greenhouse for six weeks. Thereafter, seedlings with uniformly size were transplanted under drip irrigation system during second week of September in the two growing seasons, 2020/2021 and 2021/2022. Seedlings planted in one side of drip irrigation lateral line of 0.5 m lateral lines which has drippers at 0.3m distance.

2.4. Data collection and measurements

2.4.1. Growth and yield parameters:

After 70 days from transplanting five plants were collected from each replicate as a representative sample to measure plant fresh weight (g), head length (cm), head diameter (cm), head fresh weight (g), root fresh weight (g) and total yield (ton/fed.).

2.4.2. Chemical analysis:

Determination of leaf mineral contents (N, P and K %)**:** Fresh samples of 100 g of leaves were oven dried at 65 ^{o}C for 48 h. The dry matter was finely ground and wet digested with sulphuric acid - perchloric acid mixture (1:1). Total nitrogen content by using the modified Micro-kjeldahl apparatus was employed for total N-determination as described by **Jones *et al.* (1991).** Total phosphorus was determined spectrophotomitrically by **Peters *et al.* (2003).** Total potassium was estimated flame photometerically by **Peters *et al.* (2003).** Crude protein was calculated by multiplying the total percentage of nitrogen by the factor of 5.75**.** Determination of total carbohydrate**:** Carbohydrates were estimated in the leaves according to anthrone method **(Shumaila and Safdar, 2009).** Proline estimation: proline was determined according to **(Marin *et al*., 2010).** Nitrate determination: by **Cheng and Tsang (1998).**

Soil samples were collected after plant harvest at 0-15 cm depth. The collected samples were air dried, crushed, and sieved through a 2 mm sieve and prepared for chemical determinations of available concentrations of N, P and K in soil according to the methods that described by according to **Reeuwijk (2002).`**

2.5. Statistical Analysis

Data were analyzed with statistical analysis software; CoState (2005). All multiple comparisons were first subjected to analysis of variance (ANOVA). Comparisons among means were made using Duncan's multiple range test at a P level of 0.05.

Table 1. Physical and chemical properties of representative soil samples from the field experimental site through two successive seasons of 2020/2021- 2021/2022.

Soil properties	(1st season) 2020/2021	(2nd season) 2021/2022
I- Physical properties particle size distribution		
sand %	89.5	89.1
silt %	7.6	7.8
clay %	2.9	3.1
Texture grade	Sandy	Sandy
(Field capacity) (%)	8.19	8.22
(Max Water hold capacity) (%)	19.5	19.8
(Wilting point) (%)	2.94	2.92
Available water (%)	5.25	5.30
Saturation percent	31.00	32.00
Bulk density (g cm^{-3})	1.81	1.86
II – Chemical properties		
pH (1:2.5, Soil: water)	8.54	8.37
$EC.dSm^{-1}$ (1:5, Soil: Water)	0.49	0.46
Organic Carbon (g kg^{-1})	1.00	1.10
Cation Exchange capacity ($cmol_c$ kg $^{-1}$)	4.5	4.8
$CaCO_3$ (g kg^{-1})	92.3	92.00
Available nitrogen (mg kg^{-1})	16	17
Available Phosphorus (mg kg^{-1})	4.49	4.41
Available Potassium (mg kg^{-1})	152	158
Organic matter (g kg^{-1})	1.72	1.76

Table 2. Physical and chemical properties of the used compost.

Properties	2020/2021	2021/ 2022	Properties	2020/2021	2021/ 2022
Organic matter (%)	27.25	27.75	K (%)	1.11	1.21
Carbon (%)	15.6	15.75	Fe (ppm)	979.4	818.6
Total N (%)	0.83	0.94	Zn (ppm)	271.1	269.2
C/N ratio	18.80	16.76	Mn (ppm)	227.3	237.5
Humidity (%)	8.11	7.99	pH 1:10	7.45	7.21
P (%)	0.26	0.29	E. C. (dSm^{-1})	1.08	1.06

Table 3. Some characteristics of K- Humate.

properties	Humic acid	Fulvic acid	K	P	Fe	Zn	Mn	Mg
Values (%)	10	1	2.5	1	1	0.5	0.5	2

Table 4. Average monthly meteorological data of Minia weather station during the two growth seasons of 2020/2021 and 2021/2022.

Parameter Month	2020/2021				2021/2022			
	Temperature (°C)		Relative Humidity %	Wind speed (km/h)	Temperature (°C)		Relative Humidity %	Wind speed (km/h)
	Max	Min			Max	Min		
November	24.3	9.6	50.1	15.1	23.1	9.1	49.2	14.2
December	21.5	8.7	54.6	14.2	20.2	8.2	55.2	16.2
January	15.1	5.1	52.5	15.6	13.5	4.2	51.8	13.4
February	17.8	6.2	41.3	14.2	15.6	6.1	50.2	18.6

3. Results

Vegetative and yield attributes

The data in Table (5) concerned with the effects of various irrigation regime and bio-stimulants whether potassium humate (K-H) and/or biofertilizers (EM) on plant fresh weight (g). Plant fresh weight was significantly increased when plants were irrigated with 100% or 80% ETc compared to 60% ETc. but insignificant between 100% ETc and 80% ETc in the first season. Combined K-H and EM caused significant increase in plant fresh weight by (29.88, 51.56%) respectively in the 1st and 2nd season. Interaction between irrigation regime and bio-stimulants gave the best result of fresh weight (901.43 and 956.50 g plant^{-1}) due to irrigation with 100% ETc combined bio-stimulants, in both seasons, respectively. in the same trend, head length and head diameter there was a significant affect between 100% or 80% ETc compared to 60% ETc but insignificant between 100% ETc and 80% ETc in both seasons. Integration K-H and EM caused significant increase for head length and head diameter compared with control by (14.88, 11.01 and 28.14, 19.56%) respectively through both seasons. but there was no significant difference among both bio-stimulants in second seasons. Regarding the interaction between irrigation regimes and bio-stimulants treatments, highest values for head length (12.94 and 12.83 cm) and for head diameter (12.67 and 12.80 cm) was recorded by irrigation with 100% ETc combined both bio-stimulants, in 1st and 2nd seasons, respectively. Furthermore, plots irrigated with 100% and 80% ETc gave significant increase in weight of fresh head and root in comparison with 60% ETc. but insignificant between 100% ETc and 80% ETc in both seasons. Combining K humate and EM caused significant increase by (39.28, 44.44 and 26.65, 56.79%) for head fresh weight and root fresh weight compared with control. There was significant difference among bio-stimulants in both seasons for head fresh weight. Also, in 2nd season for root fresh weight. While, there were no significant in 1st season among them. The interaction effect between irrigation with 100% ETc with both bio-stimulants was superior for head and root fresh weight (888.00, 877.00 and 41.12, 41.89 g plant^{-1}) in 1st and 2nd season respectively Table (6).

Plants irrigated with 100% and 80% ETc gave significant increase in total yield comparing with these irrigated with 60% ETc. but insignificant among 100% ETc and 80% ETc in both seasons. Integration K-H and EM caused significant increase by (37.21, 46.16%) for total yield compared with control plants through 1st and 2nd seasons respectively. There was no significant difference among bio-stimulants in both seasons. The interaction effect between irrigation with 100% ETc and both bio-stimulants gave best total yield of lettuce (24.20 and 21.73 ton fed^{-1}) in 1st and 2nd season respectively Table (6).

Table 5. Effect of bio-stimulants (potassium humate and/or EM) on fresh weight, head length and head diameter of lettuce plant under irrigation regime treatments.

Treatments	Plant fresh weight (g)							
	1st season				2nd season			
	Irrigation							
	60%	80%	100%	Mean	60%	80%	100%	Mean
NPK (100%chemical)	416.33g	750.73d	776.88c	647.98D	433.50j	620.33h	700.00f	584.61D
Potassium humate	571.37f	790.2c	796.43c	719.27C	560.75i	802.00c	791.17c	717.97C
Biofertilizers (EM)	638.3e	848.57b	839.9 b	775.59B	655.22g	772.50d	847.56b	758.43B
Mix (EM+K)	734.53d	888.93a	901.43 a	841.63A	747.45e	954.00a	956.50a	886.09A
mean	590.13C	819.56A	828.66A		599.23C	787.21.B	823.891A	
LSD 5%	A. 10.68	B. 12.34	AB. 21.37		A. 5.59	B. 6.99	AB. 12.10	
	Head length (cm)							
NPK (100%chemical)	9.55f	11.27cd	11.23cd	10.68D	9.73f	11.50bcd	11.75bc	10.99C
Potassium humate	10.37e	11.50c	11.62c	11.15C	10.50ef	12.16ab	12.20ab	11.61B
Biofertilizers (EM)	10.46E	12.48b	12.53ab	11.82B	10.75de	12.08ab	12.16ab	11.66B
Mix (EM+K)	11.00d	12.88ab	12.94a	12.27A	11.12cde	12.63a	12.83a	12.20A
mean	10.34B	12.03A	12.08A		10.53B	12.09A	12.24A	
LSD 5%	A. 0.207	B. 0.239	AB. 0.415		A. 0.40	B. 0.46	AB. 0.80	
	Head diameter (cm)							
NPK (100%chemical)	8.67e	9.48d	9.77d	9.31D	8.50f	10.85cd	10.70d	10.02C
Potassium humate	9.37f	11.48b	11.35b	10.73C	9.00ef	11.35bc	11.63b	10.66B
Biofertilizers (EM)	10.23c	11.53b	11.44b	11.07B	9.45e	11.45b	11.50b	10.80B
Mix (EM+K)	10.62c	12.51a	12.67a	11.93A	10.50d	12.65a	12.80a	11.98A
mean	9.72B	11.25A	11.31A		9.36B	11.56A	11.66A	
LSD 5%	A. 0.206	B. 0.24	AB. 0.41		A. 0.30	B. 0.35	AB. 0.60	

Table 6. Effect of bio-stimulants (potassium humate and/or EM) on head fresh weight, root fresh weight and total yield of lettuce plant under irrigation regime treatments.

Treatments	Head fresh weight (g)							
	1st season				2nd season			
	Irrigation							
	60%	**80%**	**100%**	**Mean**	**60%**	**80%**	**100%**	**Mean**
NPK (100%chemical)	356.67h	711.00e	724.33d	597.33D	470.00h	602.00g	622.00f	564.67D
Potassium humate	548.00g	817.00bc	809.67c	724.89C	595.50g	714.50d	718.50cd	676.17C
Biofertilizers (EM)	615.67f	823.33b	816.67bc	751.89B	613.50f	739.50b	727.00c	693.00B
Mix (EM + K)	731.00d	877.00a	888.00a	832.00A	700.00e	870.00a	877.00a	815.66A
mean	562.83B	807.08A	809.67A		594.75B	731.50A	736.13A	
LSD 5%	**A. 5.64**	**B. 6.52**		**AB. 11.29**	**A. 4.91**	**B. 5.67**	**9.83**	**AB.**
	Root fresh weight							
NPK (100%chemical)	16.97h	34.81e	36.33d	29.37C	18.50e	25.70d	27.13cd	23.77D
Potassium humate	27.27g	39.14b	38.27bc	34.35B	27.50cd	36.50b	36.63b	33.54B
Biofertilizers (EM)	27.87g	37.96c	37.21cd	34.89B	25.00d	35.66b	34.56b	31.74C
Mix (EM + K)	30.13f	40.37a	41.12a	37.20A	29.16c	40.75a	41.89a	37.27A
mean	25.56B	38.07A	38.23A		25.04B	34.65A	35.05A	
LSD 5%	**A. 0.53**	**B. 0.61**		**AB. 1.06**	**A. 1.27**	**B.**	**2.10**	**AB. 2.55**
	Total yield (ton fed^{-1})							
NPK (100%chemical)	10.033h	19.45f	19.70cd	16.39C	10.50f	15.75d	16.00d	14.08C
Potassium humate	17.00	22.00e	22.07b	20.24B	14.07e	17.15c	17.22c	16.15B
Biofertilizers (EM)	16.75e	22.45d	22.27b	20.48B	14.67e	17.23c	17.34c	16.41B
Mix (EM + K)	19.50c	23.77b	24.20a	22.49A	18.50b	21.50a	21.73a	20.58A
Mean	15.73B	21.92A	22.06A		14.44B	17.91A	18.07A	
LSD 5%	**A. 0.35**	**B. 0.41**		**AB .0.75**	**A. 0.37**	**B.**	**0.43 0.75**	**AB.**

Chemical constituents

Table (7) indicated the effects of bio-stimulants under various irrigation regimes for mineral contents in lettuce leaves. Nitrogen content was significantly increased when plants were irrigated with 100% and 80% ETc compared to 60% ETc. but insignificant between 100% ETc and 80% ETc in both season. Combined K-H and EM caused significant increase in nitrogen content comparing with control by (22.58 , 13.01%) respectively in the 1st and 2nd season . Highest nitrogen content (3.93 and 3.96%) by irrigation with 100% ETc combined bio-stimulants, in both seasons, respectively. In the same way, potassium and phosphorous gave the same trend. Integration K-H and EM caused significant increase for phosphorous by (39.13, 35.01%) and for potassium by (30.65, 27.83%) respectively through both seasons. Regarding the interaction between irrigation regimes and bio-stimulants treatments, highest values for phosphorous content (0.37 and 0.39 %) and for potassium content (3.50 and 3.67%) was recorded by irrigation with 100% ETc combined both bio-stimulants, in 1st and 2nd seasons, respectively

Plots irrigated with 100% and 80% ETc gave significant increase for crude protein and total carbohydrate compared with 60% ETc but insignificant between 100% ETc and 80% ETc in both seasons. Combining K-H and EM caused significant increase by (22.58, 13.17 and 39.50, 29.22%) for protein and total carbohydrate through both seasons compared with control. There was significant difference among bio-stimulants in both seasons. The interaction effect between irrigation with 100% ETc with both bio-stimulants was superior for crude protein (22.60 and 22.77%) by same way for carbohydrate (6.41 and 6.57%) through 1st and 2nd seasons respectively Table (8)

On other hand, irrigated with 100% and 80% ETc gave significant decrease comparing with 60% ETc. for nitrate and **proline** but insignificant effect between 100% ETc and 80% ETc in both seasons Table (8). Combining bio-stimulants caused significant decrease by (23.20, 18.83. and 18.18, 14.33%) for nitrate and **proline** through both seasons compared with control. There were significant differences among bio-stimulants in both seasons. But there were no significant effects between control and application of

EM for **proline** content in second seasons. The interaction effect between irrigation with 100% ETc with both bio-stimulants gave lowest nitrate content (214.66 and 220.56 mg kg^{-1}), and (2.17 and 2.32 µmol g^{-1}) for **proline** through 1st and 2nd seasons respectively.

Table 7. Effect of bio-stimulants (potassium humate and/or EM) on nitrogen, phosphorous and potassium contents of lettuce leaves under irrigation regime treatments.

Treatments	Nitrogen %							
	1st season				2nd season			
	Irrigation							
	60%	80%	100%	Mean	60%	80%	100%	Mean
NPK (100%chemical)	2.86g	3.27e	3.17f	3.10D	2.90h	3.54e	3.69d	3.38D
Potassium humate	3.17f	3.50c	3.41d	3.36C	3.21g	3.77bc	3.71cd	3.56C
Biofertilizers (EM)	3.36de	3.67b	3.69b	3.57B	3.33f	3.83b	3.80b	3.65B
Mix (EM + K)	3.60b	3.88a	3.93a	3.80A	3.58e	3.92a	3.96a	3.82A
mean	3.25B	3.57A	3.56A		3.25B	3.77A	3.79A	
LSD 5%	A.0.046	B. 0.053	AB.0.092		A. 0.032	B . 0.038	AB. 0.065	
	Phosphorus %							
NPK (100%chemical)	0.17i	0.25f	0.27e	0.23D	0.19	0.29	0.29	0.257D
Potassium humate	0.22g	0.31c	0.30cd	0.27B	0.24	0.34	0.34	0.306B
Biofertilizers (EM)	0.20h	0.29d	0.27e	0.25C	0.21	0.30	0.32	0.278C
Mix (EM + K)	0.25f	0.35b	0.37a	0.32A	0.27	0.38	0.39	0.347A
mean	0.21B	0.30A	0.30A		0.23C	0.33B	0.34A	
LSD 5%	A. 0.010	B. 0.011	AB. 0.020		A. 0.007	B. 0.008	AB. 0.014	
	Potassium %							
NPK (100%chemical)	2.17h	2.95ef	3.09e	2.74D	2.23f	3.27d	3.24d	2.91D
Potassium humate	2.49g	3.43cd	3.39d	3.10C	2.55e	3.68c	3.57c	3.27C
Biofertilizers (EM)	2.76f	3.67bc	3.59bcd	3.34B	2.70e	3.73c	3.77bc	3.40B
Mix (EM + K)	3.00ef	3.81ab	3.94a	3.58A	3.07d	3.98ab	4.11a	3.72A
Mean	2.61B	3.46A	3.50A		2.64B	3.66A	3.67A	
LSD 5%	A. 0.122	B. 0.141		AB. 0.244	A. 0.115	B. 0.132	AB. 0.229	

Table 8. Effect of bio-stimulants (potassium humate and/or EM) on protein, carbohydrate, nitrate and proline contents of lettuce leaves under irrigation regime treatments.

Treatments	Protein%							
	1st season				2nd season			
	Iirrigation							
	60%	80%	100%	Mean	60%	80%	100%	Mean
NPK (100%chemical)	16.48g	18.80e	18.23f	17.84D	16.68h	20.35e	21.22d	19.42D
Potassium humate	18.22f	20.13c	19.59d	19.31C	18.46g	21.68bc	21.32cd	20.49C
Biofertilizers (EM)	19.32de	21.10b	21.22b	20.55B	19.15f	22.02b	21.85b	21.00B
Mix (EM + K)	20.70bc	22.31a	22.60a	21.87A	20.58e	22.54a	22.77a	21.97A
Mean	18.68B	20.58A	20.41A		18.72 B	21.65A	21.79A	
LSD 5%	A. 0.267	B. 0.308	AB. 0.533		A. 0.188	B. 0.217 AB.0.375		
	Carbohydrate%							
NPK (100%chemical)	3.23i	4.36ef	4.56e	4.05D	4.07h	4.85de	4.93d	4.62D
Potassium humate	3.89g	5.84bc	5.98b	5.24B	4.64f	5.94b	5.88b	5.49B
Biofertilizers (EM)	3.49h	5.53d	5.62cd	4.88C	4.32g	5.58c	5.63c	5.17C
Mix (EM + K)	4.17f	6.37a	6.41a	5.65A	4.70ef	6.63a	6.57a	5.97A
mean	3.70B	5.53A	5.64A		4.43B	5.75A	5.75A	

LSD 5%	**A. 0.117**	**B. 0.136**		**AB. 0.235**	**A. 0.097**	**B. 0.112**		**AB. 0.194**
				Nitrate mg kg^{-1}				
NPK (100%chemical)	319.16a	300.00cd.	303.5bc	307.56A	297.00a	289.50b	288b	291.50A
Potassium humate	297.81d	253.42g	249.50g	266.91C	274.34c	247.42f	244.59f	255.45C
Biofertilizers (EM)	305.57b	271.59f	268.75f	281.97B	291.75b	265.16e	270.44c-e	275.79B
Mix (EM + K)	281.43e	212.50h	214.66h	236.20D	266.24	223.12g	220.56g	236.62D
mean	300.99A	259.38B	259.10B		282.33A	256.30B	255.89B	
LSD 5%	**A. 2.52**	**B. 2.91**		**AB. 5.04**	**A. 2.19**	**B. 2.53**		**AB. 4.38**
				Proline µmol g^{-1}.				
NPK(100%chemical)	3.73a	2.97d	2.88d	3.19A	3.66a	2.79c	2.75c	3.07A
Potassium humate	3.49c	2.53f	2.50f	2.84 C	3.38b	2.56d	2.50d	2.81B
Biofertilizers (EM)	3.60b	2.66e	2.68e	2.98 B	3.58a	2.72c	2.75c	3.02A
Mix (EM + K)	3.44c	2.21g	2.17g	2.61 D	3.27b	2.30e	2.32e	2.63C
mean	3.57A	2.59B	2.55B		3.71A	2.60B	2.58B	
LSD 5%	**A. 0.0471**	**B. 0.054**		**AB. 0.094**	**A. 0.060**	**B. 0.069**		**AB. 0.119**

Irrigation water efficiency

The trends of irrigation water use efficiency are negatively correlated with the total amount of irrigation water. Irrigation water use efficiency is defined as marketable yield per unit of irrigation water applied of growing plants. The effects of irrigation regime and bio-stimulants whether K-H and/or EM on the irrigation water use efficiency are represented in Table (9). Irrigation water use efficiency was significantly decreased when plants were irrigated with 100% and 80% ETc compared to 60% ETc but insignificant between 100% ETc and 80% ETc in the first and second seasons. Integration both bio-stimulants caused significant increase in irrigation water use efficiency by 58.64 %in the first season and were 59.14% in the second season. Interaction between irrigation regime and bio-stimulants gave the best result of IWUE (19.68 and 20.23 Kg fed^{-1}) due to application of K-H and EM under irrigation with 60% ETc, in the first and second seasons,respectively.

Table 9. Effect of bio-stimulants (potassium humate and/or EM) on Irrigation water efficiency Kg / m^3 of lettuce plant under irrigation regime treatments.

Treatments	**Irrigation water efficiency Kg/ m^3**							
	1st season				**2nd season**			
	Irrigation							
	60%	**80%**	**100%**	**mean**	**60%**	**80%**	**100%**	**mean**
NPK(100%chemical)	9.00g	13.14d	10.65f	10.93C	9.42g	10.64f	8.65h	9.57C
Potassium humate	15.25c	14.87c	11.93e	14.02B	12.62d	11.59e	9.31g	11.17B
Biofertilizers (EM)	15.02c	15.10c	12.04e	14.08B	13.16c	11.64e	9.37g	11.39B
Mix (EM + K)	17.49a	16.06b	13.08d	15.54A	16.60a	14.53b	11.75e	14.29A
Mean	14.11A	14.81B	11.92C		12.95A	12.10B	9.77C	
LSD 5%	**A. 0.26**	**B. 0.30**	**AB. 0.51**		**A. 0.76**	**B. 0.87**	**AB. 52**	

Available macronutrients content

The impact of K-M and EM on available N, P and K for the investigated soil under water stress is represented in Fig.1. The results indicated that plots irrigated with 100% or 80% ETc gave significant effect comparing with 60% ETc. but insignificant between 100% ETc and 80% ETc in both seasons. Combining K-H and EM caused significant increase by (9.23, 6.79%) for nitrogen and (13.97, 12.75%) for phosphorous and (43.45, 41.43%) for potassium compared with control through both seasons. There were significant differences among bio-stimulants in both seasons. Where, Application of K-H gave significant increase in available NPK in soil comparing with EM. The interaction effect between irrigation with 80% ETc with both bio-stimulants gave highest available nitrogen in first season (172.50 mg kg^{-1}) but under 100% ETc gave (175.83 mg kg^{-1}) for nitrogen in second season, (7.41 and 7.53 mg kg^{-1}) for phosphorous through two seasons respectively, (188.00 mg kg^{-1}) for potassium through first season while (189.50 mg kg^{-1}) under 80%ETc for available potassium through second season**.**

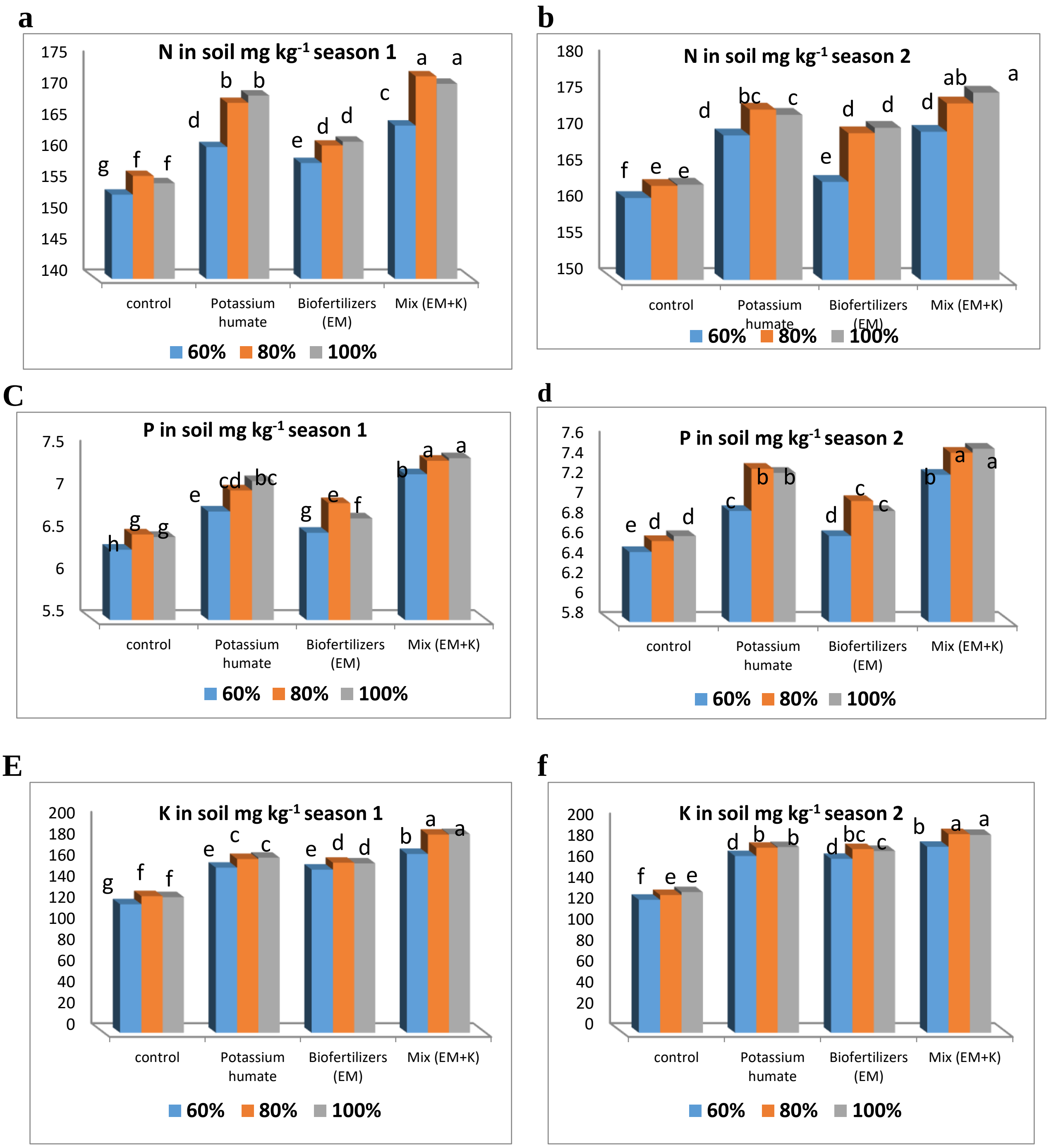

Fig. 1. Effect of bio-stimulants (potassium humate and/or EM) on available N, P and K in soil mg kg^{-1} after lettuce crop harvested under irrigation regime treatments through two studied seasons.

4. Discussion

Lettuce is low-calorie plant, has loosely bunched leaves and is used mainly for salads **(Hamerschmidt *et al.*, 2013).** It is rich with vitamins C and E, polyphenols, luthein and fibers **(Chen *et al.* 2019).** Previous reports concluded that water deficit cause yield reduction by decreasing the growth of crop and biomass (**El Shahawy *et al.* 2020 and Zahran *et al.* 2020**). Combining of bio-stimulants (K-H and EM) under various irrigation regimes on lettuce growth and yield, indicated that application of bio-stimulants under less water (80% of ETc) leads to increase the studied parameters.

Vitality of lettuce plants may be attributed to application of bio-stimulants. Where, potassium humate effects, humic compounds used as a soil conditioner to improve root and whole plant growth under normal or stress conditions (**Rady *et al*., 2016),** prevents crop transpiration, increases the soil water content, and slows down the rate of soil water consumption **(Abou-Elseoud and Abdel-Megeed 2012).** Potassium humate contains many carboxylic and phenolic groups, provides good conditions for chemical reactions, biological activity, increases buffering of pH, accelerates the transfer of nutrients to plants **(Amjad *et al.,* 2014)** and enhancing protein and carbohydrate **(Amruthesh *et al.*, 2003).** Potassium plays an important role in reducing abiotic stress on plants **(Waraich *et al*., 2011).** Bio-stimulants improve plant performance, enhance plant growth and productivity, interact with several processes involved in plant responses to stress, and increase the accumulation of antioxidant compounds that allow decrease in plant stress sensitivity **(Bulgari *et al.,* 2019).** The use of microbial bio-stimulants recommended for creating ecologically friendly technologies (**Gaveliene *et al*., 2021**), supporting plants exposed to abiotic stresses **(Santoyo *et al*., 2021),** improving plant development under water stress conditions (**Sangiorgio *et al*., 2020**). Furthermore, production of protective osmolytes or phytohormones, secretion of volatile organic compounds **(Fadiji, *et al*., 2022)** and synthesize IAA, which stimulates plant growth and root branching of the plant under drought stress (**Ouyang *et al.,* 2017**). These results agreed with **Refai *et al.* (2018)** who revealed that interaction irrigation (80% of ETc) plus adding bio-stimulants had a positive effect on growth and yield of cauliflower. Same results gave by **Amer *et al.* (2020)** on sugar beet and cotton. Likewise**, Abou Basha *et al*. (2021)** who found that application of potassium humate combined with biofertilizer gradually increased growth, yield and yield quality of maize under water stress conditions.

Besides, the role of organic acids in potassium humate, Application of K-H increases permeability of plant cell membranes and improved nutrient uptake **(Verlinden *et al.* 2009)** and facilitating transport of nutrients, especially N, P, K which led to Increasing plant efficiency to absorb and accumulate these elements in the leaves **(Hashem, 2014)**. Applied K-H directly or indirectly, affect the nutrient status of faba bean plants **(Mahdi *et al*., 2021)**. Likewise, the humic substances react with the cell membrane structures and interact as a carrier of nutrients **(Garca *et al*., 2016).** The increases in the protein content might be attributed to the high content of leaves from the mineral elements that give efficiently to the protein **(El-Zehery, 2019).** Where, nitrogen is directly entered in the synthesis of amino acids, which are the essential compounds for protein synthesis **(Barak, 1999).** Phosphorous enters inside the synthesis of DNA and RNA, which directly influences protein synthesis and enhancing of crop yield and quality **(Scalenghe *et al.,* 2012).** Furthermore, potassium effects the enzyme activation and carbohydrate metabolism, as well as potassium enhancement the efficiency of nitrogen uptake and consequently increases in protein synthesis **(Elhakim *et al.* 2016).**

Interestingly, the nitrate content in leaf of lettuce treated with bio-stimulants make it safely marketable. As the ability of bio- stimulants to avoid nitrate accumulation by regulation of nitrate reductase, which responsible for higher assimilation of nitrates into amino acids **(Tsouvaltzis *et al*., 2014).** These results agreed with **(Soliman and Hamed , 2019)** who found that application of bio-stimulants reduced the nitrate content in spinach leaves. Proline is a source of carbon and nitrogen for quick recovery and growth in stress. Proline is the main component of osmotic adjustment and this mitigates oxidative damage, stabilizes cell membranes **(Matysik *et al.,* 2002).** Under drought stress, a higher accumulation of proline **(Yi *et al.* 2016 and Abid *et al.,* 2018),** may be attributed to bio-stimulants that applied when the stress occurs or during stress conditions. Beside bio-stimulants involved in the activation and biosynthesis of bioactive compounds, these products are able to counteract environmental stress such as water deficit **(Pokluda *et al*., 2016).**

Referring to IWUE are in general agreement with those reported by **Khan *et al.* (2017).** Where, lower irrigation treatments induce higher values of IWUE. This was confirmed by **Zahran *et al*. (2020)** who concluded that the highest the values IWUE for potato were higher under irrigation with 60 % ETc, than drip-irrigated with higher water amounts (100% ETc) in both seasons. values of IWUE for summer squash were obtained under the lowest irrigation conditions. In the same direction, **El-Gindy *et al.* (2009)** showed that lower water amounts (60% of ETc) recorded higher IWUE than drip-irrigated summer squash with higher water amounts (80% ETc). In contrast, **Cantore *et al.* (2014)** reported that IWUE was not influenced by the applied supplementary irrigation. **Badawy *et al.* (2019)** reported that, irrigation water use efficiency (IWUE) values for potato increased with application bio-stimulants that enhancement of water stored in the effective root zone and these observations indicated that addition bio-stimulants mitigated the harmful effect of water stress, because the trudged cells of the stomata closed most of time, so transpiration rate decreased, however there is no need for more water to be absorbed by plant roots which in turn reduce the amount of absorbed water **(Zein El-abdeen *et al.,* 2018)**.

The significant increase in available nutrients content of the soil after harvesting of the crop may be attributed to K-H that can be used to enhance the physio-biochemical properties of soils, because its containing most elements that improve soil fertility and increase nutrients availability **(Mahdi, *et al*., 2021),** good chelating properties, which reduce loss of nutrients due to leaching and run off **(Hassan *et al*., 2017),** Increase the activity microorganism to mobile the unavailable forms of nutrient elements and cation exchange capacity **(Natsheh and Mousa, 2014)** and production of exo-polysaccharides alongside the enrichment of nutrients and soil organic matter **(Fadiji *et al*., 2022**). Its ability to interact with metal ions and soil minerals which forming complexes of varying properties, especially phosphorus (**Filip and Bielek, 2002)** and increasing chemical stability. Likewise, Microorganisms has essential role in solubilize phosphor, digestion of organic materials, soil aggregation **(Sarabia *et al*., 2018)**. Similar results were reported by **Awwad *et al*. (2015)** concluded that irrigation by 100% with application of K-H improves some soil propertie

5. Conclusion

It could be concluded that irrigation with 20 % water-saving from required amount of irrigation water accompanied with bio-stimulants (K-H and EM) alleviate the negative effect toward water deficit irrigation and gave more efficient for growth, yield and chemical constituents of lettuce plants grown under reclaimed sandy soil conditions. Furthermore, improved available nutrients content of the soil after harvesting of crop

6. Conflicts of interest

The authors declare that they have no competing interests.

7. Formatting of funding sources

No funding

9. References

Abd El-Aal H. A.; Abo El-Fadl, N. I. and Moussa, S. A. M. (2010). Effect of mineral and organic potassium fertilization on sweet potato crop grown in the newly reclaimed land. Alex. Sci. Exch. J., 31: 266-278.

Abdel-Fattah, I. M.; Attia, E. A. and El-Banna, G. M. (2020). Irrigation scheduling and its impacts on freesia-water productivity, vegetative and flowering parameters under greenhouse cultivation. Env. Biodiv. Soil Security, **4**, 59-71.

Abid, M.; Ali, Sh.; Zahoor , R. *et al.* (2018). Physiological and biochemical changes during drought and recovery periods at tillering and jointing stages in wheat (*Triticum aestivum* L.) Scientific Reports 8:4615, 1-15 | DOI:10.1038/s41598-018-21441-7.

Abou Basha, D.M.; Hellal, F. and El Sayed,S. (2021). The combined effect of potassium humate and bio-fertilizers on maize productivity and quality under water stress conditions. Sci. Arch. 2 (3), 162-170.

Aboud, F. S. and Abd-Alrahman, H. A. (2021). Effect of nitrogen mineral fertilizer, vermicompost and biofertilizer on lettuce yield and quality. Middle East J. of Agric. Res. 10 (2): 2706-7955 DOI: 10.36632/mejar/2021.10.2.44.

Abou-Elseoud I. I. and Abdel-Megeed, A. (2012). Impact of rock materials and biofertilizations on P and K availability for maize (*Zea maize*) under calcareous soil conditions. Saudi J. Bio. Sci., 19, 55–63.

Allen, G .R.; Pereira, L .S.; Raes, D. and Smith, M. (1998). Crop evapotranspiration, guidelines for competing crop water requirements. FAO. Irrigation and drainages paper 56. Rome, Italy.

Amer, M M.; Gazia, E. A. W.; Aboelsoud, H. M. and Rashed, S. H. (2020). Management of Irrigation water and organic matter application contribution in improving some soil properties and yields-water productivity of sugar beet and cotton in salt affected soil. Env. Biodiv. Soil Security, **4**, 7-18.

Amjad, M.; Javaid, A. and Anwar-ul-Haq, M. (2014). Soil and foliar application of potassium enhances fruit yield and quality of tomato under salinity. Turk. J. Bio., 38(2):208-218.

Amruthesh, K. N.; Raj, S. N.; Kiran, B.; Shetty, H. S. and Reddy, M. S. (2003). Growth promotion by plant growth promoting Rhizobacteria in some economically important crop plants. In: 6th int. PGPR Workshop, 5-10 October, Calicut, India, 97-103.

Awwad, M. S.; El-Hedek, K. S.;. Bayoumi, M.A.and. Eid, T. A. (2015). Effect of potassium humate appliction and irrigation water levels on maize yield, crop water productivity and some soil properties. Soil Sci. and Agric. Eng., Mansoura Univ., 6 (4): 461 – 482.

Badawy, A. S.; Zahran, M. M. A. and Hefzy,M. M. (2019). Effect of irrigation level and bio-stimulants on productivity and quality of garlic (*Allium sativum* l) in calcareous soils. Fayoum J. Agric. Res. & Dev., (1)33, 90-101.

Barak, (1999). Essential elements for plant's growth published by Nature publishers. 1- 5.

Bulgari R.; Franzoni G. and Ferrante A. (2019). Biostimulants application in horticultural crops under abiotic stress conditions. Agronomy 9(6); 306; 30 p. DOI: 10.3390/agronomy9060306.

Cantore, V.; Wassar, F.;Yamaç, S. S.; Sellami, M. H.; Albrizio, R.; Stellacci, A. M. and Todorovic, M. (2014). Yield and water use efciency of early potato grown under diferent irrigation regimes. Int.J. Plant Prod., 8: 409–428.

Chen, J. X. and Wang, X. F. (2006). Plant physiology experimental guide. Higher Education Press, Beijing, pp 24–25: 55–56.

Chen, Z.; Han,Y.; Ning,K.; *et al* (2019). Assessing the performance of different irrigation systems on lettuce (*Lactuca sativa* L.) in the greenhouse. PLoS ONE 14(2): 1-18 e0209329. DOI: https://doi.org/10.1371/journal.pone.0209329.

Cheng, C. F. and Tsang, C. W. (1998). Simultaneous determination of nitrite, nitrate and ascorbic acid in canned vegetable juices by reverse-phase ion-interaction HPLC. Food Additives and Contaminants, 15 (7), 753-758. http://dx.doi. org/10.1080/02652039809374706.

CoStat (2005). CoHor tsoftware,version 6.3. 798 Lighthouse Ave. PMB 320 Monterey, CA, 93940, USA.

Cruz, R.; Baptista, P.; Cunha,S.; Pereira,J. A. and Casal, S. (2012). Carotenoids of lettuce (*Lactuca sativa* L.) grown on soil enriched with spent coffee grounds.

Molecules 17: 1535-1547. DOI: https://doi:10.3390 /molecules17021535.

El Shahawy, H. A. A.; El-Sherbieny, A. E.; Sheha, A. A. and Habashy, N. R. (2020). Impact of irrigation water regimes and anti-transpirations with hydro-gel on nutritional status of peanut grown in sandy soil. Zagazig J. Agric. Res., 47 (4). 963-974.

El-Gindy, A. G. M., El-Banna, E. S., El-Adl, M. A., and Metwally, M. F. (2009). Effect of fertilization and irrigation water levels on summer squash yield under drip irrigation. Misr J. of Agri.Engi., 26, 94-106

Elhakim, S.A.Z.; El-Mesirry, D.S. and Yousry, M.M. (2016). Impact of potassium fertilization rates and *Bacillus circulans* on the growth, yield and color of processed potato (*Solanum tuberosum* L.) tubers chips. Alex. Sci. Exch. J., 37(4): 594-605.

El-Naqma, K. (2020). The role of humate substances in controlling synergism and antagonism of nutrients uptake by potato plants. Env. Biodiv. Soil Security,**4**, 149-165.

El-Zehery, T. M. (2019). Incorporated use impact of organic, bio and mineral fertilizers on potato (*Solanum tuberosum*. L) productivity and quality. J. of Soil Sci. and Agric. Engin., Mansoura Univ., 10 (12): 857-865. https://doi: 10.21608/jssae.2019.79681.

Fadiji, A. E.; Babalola, O. O. Santoyo, G. and Perazzolli, M. (2022). The Potential Role of Microbial Biostimulants in the Amelioration of Climate Change-Associated Abiotic Stresses on Crops Frontiers in Microb. 12 | Article 829099 1-17.

Farouk, S. and Ramadan, A. A. (2012). Improving growth and yield of cowpea by foliar application of chitosan under water stress. Egypt. J. Biol., 14: 14-26.

Filip, Z. and Bielek, P. (2002). Susceptibility of humic acids from soils with various some bio-fertilizers to reduce nitrogen rates on cucumber (*Cucumis sativus* L.) in relation to vegetative growth, yield and chemical composition. J. Plant Prod. Mansoura Univ., 1: 1041-1051.

Garca, A. C.; Santos, L. A.; de Souza, L. G. A.; Tavares, O. C. H.; Zonta, E.; Gomes, E. T. M. (2016). Vermicompost humic acids modulate the accumulation and metabolism of ROS in rice plants. J. Plant Physiol., 192, 56–63.

Gaveliene, v.; Šocik, B.; Jankovska-Bortkeviˇc, E. and Jurkoniene, S. (2021). Plant microbial biostimulants as a promising tool to enhance the productivity and quality of carrot root crops. Microorganisms 9, 1850. https://doi.org/10.3390/microorganisms9091850 1-16.

Haluschak, P. (2006). Laboratory methods of soil analysis. Canada-Manitoba Soil Survey. April.

Hamerschmidt, I.; Leonardecz, A.; Gheller, J. A. *et al.* *(*2013). Manual técnico de olericultura. Curitiba: Emater, 266.

Hashem, H. A. (2014). The effect of spraying with organic liquid fertilizer (LiQ Humus) and licorice extract on the growth of local orange seedlings. The Tech. J., 27 (3**).**

Hassan; W. Z.; El-Farghal, W. M; Khalil, F. A. F. and EL-Etr, W. M. (2017). Effect of some soil amendments and irrigation treatments on wheat crop productivity in middle Egypt. J. .Soil Sci. and Agric. Eng., Mansoura Univ., 8 (10): 553 – 563.

Hefzy, M.; Mostafa, H. H. A. and Zahran, M. M. A. A. (2020). Enhancement of onion (*Allium cepa* L.) drought tolerance in calcareous soils by using bio-stimulants. Env. Biodiv. Soil Security Vol. 4 pp. 239-251

Hoa, H. T. T.; Cong, P. T.; Tam, H. M.; Chen, W. and Bell, R. (2010). Sandy soils in South Central Coastal Vietnam: Their origin, constraints and Management. In: 19th World Congress of Soil Science, Soil Solutions for a Changing World, 1–6 August 2010, Brisbane, Australia.

Itelima, J. U.; Bang, W. J. and Onyimba, I.A. (2018). A review: biofertilizer; a key player in enhancing soil fertility and crop productivity. J Micro. Biol. Biot. Rep. 2:22–28.

James, L. G. (1988). Principles of farm irrigation systems design. Washington State University. 543pp.

Jones, J.; Wolf, B. J. B. and Mills, H. A. (1991). A practical sampling, preparation, analysis, and interpretative guide. Plant analysis handbook, Micro-Macro Publishing, Athens.

Khan, A.; Khan, R. U.; Khan, Z.; Khan, F. H. A. and Shah, S. U. (2017). Effect of humic substances alone and in combination with micronutrients on potato yield and nutrients status, Int. J. Agron. Agri. 11(5): 24-29.

Kocira, S.; Szparaga, A.; Kocira, A.; *et al*. (2018). Modelingbiometric traits, yield and nutritional and antioxidant properties of seeds of three soybean cultivars through the application of biostimulant containing seaweed and amino acids. Front. Plant Sci. 9:1-18. doi:10.3389/fpls.2018.00388.

Mahdi,A.H.A.; Badawy, Sh. A.; Abdel Latef, A. A. H.; *et al*., (2021). Integrated Effects of potassium humate and planting density on growth, physiological Traits and yield of *Vicia faba* L. Grown in newly reclaimed soil. Agronomy. 11(461) 1-12. https://doi.org/10.3390/agronomy11030461.

Marin, J. A.; Andreu, P.; Carrasco, A. and Arbeloa, A. (2010). Determination of proline concentration, an abiotic stress marker, in root exudates of excised root cultures of fruit tree rootstocks under salt stress. Revue Des Régions Arides – Numéro Spécial – 24.

Matysik, J. B.; Bhal, B. and Mohanty, P. (2002). Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. Curr. Sci. 82 (5), 525–532.

Mohammed, M. H.; Abd-Alrahman, H. A.; Abdel-Kader, H. H. and Aboud, F. S. (2021). Effect of potassium humate and levels of potassium fertilization on growth, yield and nutritional status of tomato plants. J. Hort. Sci. & Ornam. Plants. 13 (2): 124-133, DOI: 10.5829/idosi.jhsop.2021.124.133.

Natsheh, B. and Mousa, S. (2014). Effect of organic and inorganic fertilizers application on soil and Cucumber (*Cucumis Sativa* L.) plant productivity. Int. J. Agric. For., 4: 166-170.

Okasha, A. M.; Eldib,E .M.; Elmetwalli, A. H. *et al*. (2022). Maximization of water productivity and yield of two iceberg lettuce cultivars in hydroponic farming system using magnetically treated saline water. Agriculture. 12 (101) 1-18. https://doi.org/10.3390/agriculture12010101.

Ouyang, L.; Pei, H., and Xu, Z. (2017). Low nitrogen stress stimulating the indole- 3-acetic acid biosynthesis of Serratia sp. ZM is vital for the survival of the bacterium and its plant growth-promoting characteristic. Arch.

Microbiol. 199, 425–432. doi: 10.1007/s00203-016-1312-7.

Patrick, D. J. (2015). Plant biostimulants: definition, concept, main categories and regulation. Sci. Hort., 196, 3-14.

Peters, I. S.; Combs,B.; Hoskins, I.; Iarman, I.; Koverwatson ,M. and Wolf, N. (2003). Recommended methods of manure analysis. University of Wisconsin, cooperative extension published, Madison.

Poincelot, R. P. (1993). The use of a commercial organic biostimulant for bedding plant production. J. Sust. Agric., 3 (2): 99-110.

Pokluda, R.; Sekara, A.; Jezdinský, A.; Kalisz, A.; Neugebauerová, J.; Grabowska, A. (2016). The physiological status and stress biomarker concentration of *Coriandrum sativum* L. plants subjected to chilling are modified by biostimulant application. Biol. Agric. Hortic., 32, 258–268.

Rady, M. M.; Abd El-Mageed, T. A., Abdurrahman, H. A. and Mahdi, A. H. (2016). Humic acid application improves field performance of cotton (*gossypium barbadense* L.) under saline conditions. J. Animal & Plant Sci., 26(2): 487–493.

Reeuwijk, L. P. (2002). Procedures for soil analysis. Inter. Soil Ref. and Info. Center. Food and Agric. Organization of the United Nations.

Refai, E. F. S.; Hassan H. A. M.; Hefzy ,M. M. and Zahran, M. M. A (2018). Influence of irrigation water quantity and biofertilizer on cauliflower productivity in sandy calcareous soil. Fayoum J. Agric. Res. & Dev., 2(32) 119-134.

Sangiorgio, D.; Cellini, A.; Donati, I.; Pastore, C.; Onofrietti, C. and Spinelli, F. (2020). Facing climate change: application of microbial biostimulants to mitigate stress in horticultural crops. Agronomy 10:794. doi: 10.3390/ agronomy10060794.

Santoyo, G.; Gamalero, E. and Glick, B. R. (2021). Mycorrhizal-bacterial amelioration of plant abiotic and biotic stress. Front. Sustain. Food Syst. 5:139. doi: 10.3389/fsufs.2021.672881.

Sarabia, M., Cazares, S., González-Rodríguez, A., Mora, F., Carreón-Abud, Y., and Larsen, J. (2018). Plant growth promotion traits of rhizosphere yeasts and their response to soil characteristics and crop cycle in maize agroecosystems. Rhizosphere 6, 67–73. doi: 10.1016/j.rhisph.2018.04.002.

Scalenghe, R.; Edwards, A.C. and Barberis, E. (2012). Agricultural soil under a continental temperatureclimate susceptible to episodic reducing conditions and increased leaching of phosphorus. J. Env. Manag. (97):141-147.

Shalan, A. M. (2014). Effect of bio-stimulant and soil amendment on vegetative growth, yield and fruit quality of Pyrus communis cv. 'le conte' pear trees. J. Plant Production, Mansoura Univ., 5(12): 1973-1987.

Shumaila, G. and Safdar, M. (2009) Proximate composition and mineral analysis of cinnamon. Pakistan J. Nut., 8 (9), 1456-1460. http:// dx.doi.org/10.3923/pjn.2009.1456-1460.

Soliman, M. A. E. and Hamed, L. M. M. (2019). Application of bio-stimulants in comparison with organic and mineral n fertilizers for growth promotion of spinach and common bean grown on sandy soil. J. Soil Sci. and Agric. Eng., Mansoura Univ., Vol. 10(1): 79 – 85.

Tsouvaltzis, P.; Koukounaras, A. and Siomos, S. A. (2014). Application of amino acids improves lettuce crop uniformity and inhibits nitrate accumulation induced by the supplemental inorganic nitrogen fertilization. Int. J. Agric. Biol., 16, 951-955.

Verlinden, G., Pycke, B.; Mertens, J.; *et al.* (2009). Application of humic substances results in consistent increases in crop yield and nutrient uptake. J. of Plant Nut., 32: 1407-1426.

Waraich, E. A.; Ahmad, R.; Saifullah, A.; Ehsanullah, M. Y. (2011). Role of mineral nutrition in alleviation of drought stress in plants. Aust. J. Crop Sci., 5(6): 764-777.

Yi, X. P. *et al.* (2016) Rapid recovery of photosynthetic rate following soil water defcit and re-watering in cotton plants (*Gossypium herbaceum* L.) is related to the stability of the photosystems. J. Plant Physiol., 193, 23–34.

Zahran, M. A. M; Mohamed, H. and Mostafa, H. H. A. (2020). Enhancing the productivity of potato crop under drought stress by using some biological treatments. Inter. J. Environ. 9(2):83-183. DOI: 10.36632/ije/2020.9.2.6.

Zein El-abdeen, H. A.; Khalil, F. A. F. and El-Etr, W. M. T. (2018). Effect of calcium humate, nitrogen fertilizer rates and irrigation regime on soil chemical properties and faba bean productivity. J.Soil Sci. & Agric. Eng., Mansoura Univ., 9 (11): 575–586, 2018.