THE ACTIVITY OF ANTIOXIDANTS ENZYMES AND NPK CONTENTS AS AFFECTED BY WATER QAULITY, KINETIN, BIO AND ORGANIC FERTILIZATION IN LETTUCE (*Lactuca sativa* L.)

D. K. A. Altaey* **Z. Z. Majid***
Assistant Prof. **Researcher**
Dept. of horticulture, college of Agriculture, AL-Qasim Green University
duraidaltaey@gmail.com **zahraamajid2017@gmail.com**

ABSTRACT
A field experiment was conducted using split – split block design to study the effect of irrigation water quality ,organic composts ,kinetin and Bio- health application on N.P.K contents and anti-oxidant enzymes activity in lettuce, The experiment included three factors ,quality of irrigation water (W) at two levels (1.2, 6.1 dS m^{-1}), kind of organic composts Plam fronds and Rice wastes(20) ton /hectare , kinetin application (50 $mg.L^{-1}$) and bio-health fertilizers (5 kg/ hectare). The results indicated that elevation of water salinity levels led to elevation in activity of Peroxidase POX , Catalase CAT, Malondialdehyde MDA, Superoxide dismutase SOD enzymes and proline contents in leaves, while it decrease leaves contents of (N.P.K), Organic composts , kinetin and bio-health applications alleviated from the negative effects of saline water by increasing the (NPK) contents in leaves ,reduced POX ,CAT, MDA,SOD and proline , That possible to mitigation the negative affect of salt stress in lettuce the application of plant growth regulators , bio fertilizers and decomposed organic matter.

Key words: salt stress, lettuce, kinetin, organic composts, bio-heath.
Part of M.Sc. thesis of second author

فعالية الانزيمات المضادة للأكسدة ومحتوى NPK وتأثرها بنوعية مياه الري والمعاملة بالـ Kinetin والتسميد الحيوي والعضوي لنبات الخس *Lactuca sativa* L.

دريد كامل عباس الطائي زهراء زهير ماجد
أستاذ مساعد باحث
قسم البستنة – كلية الزراعة – جامعة القاسم الخضراء
zahraamajid2017@gmail.com duraidaltaey@gmail.com

المستخلص
تم تنفيذ تجربة عامليه حقليه وفق نظام الالواح المنشقة بثلاث عوامل، العامل الرئيسي نوعية مياه الري بمستويين (1.2و 6.1 ديسيسمنز/ م)، العامل الثاني هو التسميد العضوي بثلاث مستويات (السيطرة، كمبوست مخلفات سعف النخيل، كمبوست مخلفات الرز) كل منها 20 طن / هكتار، العامل الثالث بثلاث مستويات يمثل الرش بالـ Kinetin بـ(50) ملغم /لتر والتسميد الحيوي باستخدام (Bio–health) بـ 1250غم لكل هكتار أضافة لمعاملة السيطرة. أظهرت النتائج أن زيادة ملوحة مياه الري أدت الى ارتفاع نشاط الانزيمات المقاسة (POX،CAT ، SOD، MDA) كذلك زيادة محتوى الأوراق من البرولين وانخفاض محتواها من N,P,K معنويا أدى استخدام كمبوست سعف النخيل ومخلفات الرز، الى تخفيف اضرار الشد الملحي بتقليل فعالية الانزيمات المقاسة وخفض محتوى الأوراق من البرولين وزيادة محتواها من N,P,K معنويا وكان تأثير التسميد الحيوي والكاينتين بذات الاتجاه في تقليل اثر الشد على النبات .نستنتج انه يمكن التقليل من إثر الشد الملحي نتيجة استخدام مياه مالحة من خلال استخدام التسميد العضوي والحيوي والمعاملة ببعض منظمات النمو النباتية مثل الكاينتين لتحسين نمو وحاصل النبات.

البحث مستل من أطروحة ماجستير للباحث الثاني

***Received:16/7/2017, Accepted:29/10/2017**

INTRODICTION

High salinity is one of the most widespread abiotic stress factors worldwide that severely restricts crop productivity. Salinity influences 110 million hectares in arid and semiarid regions, According to FAO, an estimated 20–30 million hectare seriously deteriorated by salinity .In addition to natural salinity, a great amount of recently cultivated agricultural area has become saline because of land clearing or irrigation, both of which led water tables to rise and concentrate the salts in soil (46). High saline environments cause osmotic stress due to the difficulties in absorbing water from the soil and ion toxicity that can negatively affect the growth of many plants. Osmotic stress and ionic toxicity cause changes in plant physiological processes, mineral distribution, enzyme activities, stomatal behavior, protein synthesis, and photosynthetic efficiency (39; 34). One of the dangerous effects of salinity stress is the oxidative degradation of lipids. It is the process in which ROS production, its "steal" electrons from the lipids in cell membranes resulting cell damage. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which lie methylene bridges (-CH2-) that possess especially reactive hydrogen atoms (28). The salinity stress conditions increased the H2O2 radicals in cells, which lead to lipids peroxidation in cell membranes (16; 7). To mitigate the effects of salt stress, plants have evolved a number of antioxidant systems to protect themselves against potentially cytotoxic levels of reactive oxygen species (ROS) such as hydrogen peroxide (H2O2), superoxide radicals (O2 •–), and hydroxyl radicals (4). Plant growth regulators are being widely used to counteract the deleterious effects of adverse environmental stresses on plants. Presoaking seeds with optimal concentration of phytohormons has been shown to be beneficial to growth and yield of some crop species growth under saline conditions by increasing nutrient reserves through increased physiological activities and root proliferation. Concerted attempts have been made to mitigate the harmful effects of salinity by application of plant growth regulators (3), Kinetin is one of the Cytokinins known to significantly improve the growth of crop plants grown under salinity, there are many researchers conducted about Kinetin ability to improve the plant growth under salt stress by deleterious of sodium toxicity and raised potassium rate inside the plant beside Kinetin role to enzyme activation (9; 7). Since chemical fertilizers are not only in short supply but also expensive, they can be supplemented with cheaper, pollution-free and renewable alternate sources of nutrients. Thus, in view of increasing nutrient demand for intensive crop cultivation, bio fertilizers of microbial origin can be a good option for sustaining productivity with more environment friendly and integrated nutrient management approach (35), Bio-health TH BS WSG is a blend of selected *Trichoderma harzianum* strains, *Bacillus subtilis*,humic acids and seaweed extract. *Trichoderma harzianum* and *Bacillus subtilis* nourish the root exudates in the rhizosphere. In latently affected plant material, for the reason that the antagonist cannot follow the parasite into the interior of the plant. Therefore, Bio-health has to be regarded as a soil conditioner or rather a plant intensifier, and not a fungicide against pathological fungi in the soil. The aim of the present work is to study manifestations of physiological diversity of lettuce under salt stress conditions, and mitigate the saline water stress by kinetin and bio- health applications, used other sources of water for irrigation, and study the antioxidant enzymes behavior in normal cases and under salt stress conditions.

MATERIAL AND METHODS

A field experiment was conducted during winter seasons of 2016–2017 at private farm of AL-Rofiat village of Babylon province, the seeds of lettuce (Nader) cultivar were planted on the first of Oct 2016., the average of rain fall, temperature and humidity (Table 1), three samples from soil were taken at depths of (0-30cm) from 20 locations of the farm randomly for the estimation the physical and chemical characters (Table 2).

Table1. rain fall, temperature and humidity

Study	TM C°	Tm C°	T C	HM %	Hm %	R mm
Oct.	35.72	16.93	25.81	52.90	12.49	0.0
Nov.	24.91	7.63	15.60	63.52	17.86	0.7
Dec.	16.78	4.24	10.19	82.72	39.43	28.9

Table 2. some physical and chemical characters of study soil.

Soil component	Texture	%
Sand		23.6
Silt	loam	36 .3
Clay		40.1
Chemical	U	
Ec	dS.m^{-1}	2.51
Ph	-	7.3
Soluble ions		
Ca^{+}	mmol /L^{-1}	31
Mg^{+}	mmol /L^{-1}	7
Na^{+}	mmol /L^{-1}	15.21
K^{+}	mmol /L^{-1}	4.35
$Co3^{=}$	mmol /L^{-1}	—
HCO	mmol /L^{-1}	5
Cl-	mmol /L^{-1}	21.15
SO4 =	mmol /L^{-1}	31.41

The seeds were sown in 216-celled Styrofoam trays filled with peat and thirty-days-old seedlings were transplanted to the permanent location of farm, each treatment had 3 replicate with 30 plants for each replicate, and the experimental unit space was 6 m^2, the water requirement limited by Gypsum block

The treatments and experimental design: The study was designed as three factors corresponding to the split –split plot design; each treatment had 3 replicate with 30 plants to each treatment, The main plot was irrigation water quality (W) as following: River water (W1) EC = 1.2 dS.m^{-1}

1. The drainage water 1 (W2) EC = 6.1 dS.m^{-1}, the chemical analysis for water quality is shown in (Table3). The sub plot was two kind of organic composts with control as following:

1.Control F1

2.Date palm fronds compost F2 (20 t .ha^{-1})

3.Rice wastes compost. F3 (20 t .ha^{-1}) The sub-sub plot was Kinetin and Bio-health fertilizers as following 1- Control T1

2.Kinetin 50 mg.L^{-1} T2

3.Bio-health 5 kg.ha^{-1} T3

The results were analyzed by (Genstat discovery 12 soft were) and the means compared according to LSD test at 0.05

Table 3. the chemical analysis for water quality.

Ec	Ph	Ca $^{+2}$	Mg^{+2}	Na $^{+}$	K $^{+}$	CO3	HCO^{-3}	Cl^{-}	SO4^{-2}
6.		32.	24.6	22.	0.	–	0. 82	34.	39.
1.	6.	4.8	3.65	3.3	0.	-	2.70	4.21	5.5

Kinetin solutions were prepared with distilled water containing 0.02% Tween-20 as a surfactant and 50 Ml of the Kinetin solution was applied to the plant foliage using a handheld sprayer in the evening after 14 days from transplanting. The control plants were sprayed with distilled water containing 0.02% Tween-20. kinetin treatments were repeated at 30 days after first spraying. bio-health application add to plants with 5 kg .ha^{-1} , 3g to each treatment divided to two installments ,1.5 gm of bio health add to 298.5 g peat with good mixing putting 10 g of this mixture down each plant with note that control treatment was had only 10 g peats.

Enzymatic assays: - Peroxidase (POX) assay: This was determined by measuring the increase in absorbance at 510

nm resulting from the decomposition of hydrogen peroxide **(38)**, The Lambda 25 UV/Vis spectrometer (Perkin Elmer) was adjusted to 510 nm. The blank was a mixture of 1.4 ml of phosphate buffer and 1.4 ml of H2O2 in the cuvette. The assay mixture contained 1.4 ml of phosphate buffer, 1.4 ml of H2O2 and 0.2 ml of the extract. The increase in absorbance at 510nm was recorded for 4 minutes. Then, ΔA240/min was calculated from the initial (45 second) linear portion of the curve.-

Superoxide dismutase activity SOD and Catalase CAT: To determine catalase activity and superoxide dismutase activity, 0.1 gm of frozen leaves and roots were homogenized in 1 ml of phosphate buffer 0.5M, pH 7 and 0.03gm of polyvenile pyrollidone (PVP) with a warring blender, the homogenate were centrifuged at 6000 rpm for 10 minutes at 4 c. The clear supernatant was used for measurement of enzyme activities with three replicates done for each assay SOD activity was assayed according to **(26)**. This method monitored the ability of SOD to inhibit photochemical reduction of pyrogallol at 420 nm. One unit of SOD activity was defined as the amount of enzyme that caused 50% inhibition The activity of CAT was assayed by using the method of **(43)** for chemicals preparation and **(2)** for CAT assay. The equations used for activity determination were:-

$$\text{SOD activity (units)} = \frac{\%inhibition \,/\, 50\% \times reaction\ volume}{total\ test\ period}$$

$$\text{Cat activity (units)} = \frac{\Delta Abs \,/\, \min \times reaction\ volume}{0.001}$$

Malondialdehyde (MDA) assay: It was estimated according to **(22)**. Reaction mix was consisting of 1 ml of crude extract with 2 ml of 0.6% TBA (thiobarbituric acid), then tubes was sealed tightly and moved to boiling water bath of 100^0C for 15 minutes. Thereafter, tubes were cooled down using ice bath, then centrifuged at 4000 rpm for 10 minutes, protein containing residue (because of TCA) was neglected, while supernatant was taken to the spectrophotometer to read the absorbance at (450, 532, 600) nm. Blank contained same materials except for crude extract that was replaced with phosphate buffer. MDA content was calculated by the following equation:

$MDA\ \mu mol.\ G^{-1} = [6.45 \times (A532 - A600) - 0.56 \times A450] \times R \times W$

Where: *A532:* Absorbance at 532 nm
A600: Absorbance at 600 nm
A450: Absorbance at 450 nm
R: Total reaction volume (3 ml)-
W: Fresh weight of sample

Determination of proline: Proline colorimetric determination preceded according to **(12; 25)** based on proline's reaction with ninhydrin ratio of 1:1:1 solution of proline, ninhydrin acid and glacial acetic acid was incubated at 100ºC for 1 hour. Thereaction was arrested in an iced bath and the chromophore was extracted with 1 ml toluene and its absorbance at 520 nm was determined spectrophotometerically .0.1 gm of shoot and root tissues was suspended with 1 ml of 3% sulfosalicylic acid and after centrifugation (10 min at 12,000 rpm) was mixed in a 1:1:1 ratio with ninhydrin acid and glacial acetic acid. The reaction and determination of proline were carried out similarly to that described above the concentration of proline in tissues were determined depending on standard curve of pure proline.

Measurement of N, P and K in leaves: Leaf Samples were dried and 0.5gm of each dry sample was taken for ash weight. Then solution of ash was made in 50ml of de-ionized water, and then dilutions were made in de-ionized water for mineral analysis. Concentration of in samples was measured using Flame Photometer according to (43), the nitrogen determination according to (20) while the determination of phosphorus us in leaves was measured according to (29).

RESULTES AND DISCUSSION

Nitrogen, Phosphorus us, Potassium in lettuce leaves:The figures 1,2,3 show a significant effect of water quality , the W2 (drainage water) led to reduction on N,P,K contents of leaves with dropping rate was (12% , 34% , 36%, respectively compared to W1 (river water), while the compost application of F1 and F2 led to increase the N,P,K contents of leaves at an boosting rate was (24% ,26% to Nitrogen), (37% and 36% to Phosphorus) and (53%, 69%) , respectively compared to F1 (control) .The Kinetin application (T1)

recorded a significant affect at a boosting rate was (9% of nitrogen), (17% of phosphorus) and (5%of potassium) while the boost rate of bio- health application was (7 %of nitrogen), (0.15% of phosphorus , no significant differences with control) and (7%of potassium) figures 1, 2, 3. The interaction between studying factors show significant values of nitrogen content in leaves , the best value in the treatment which irrigated with river water was (W1F2T2) with boost rate (44%) according to (W1F1T1) which gave the lowest value ,figure (1).The highest averages of phosphorus content in leaves at (W1F2T3) and (W1F2T2) with rising rate (40%) and (39%) respectively according to control figure 2, while the treatment (W1F3T3) and (W1F3T2) realized the highest averages of potassium content in leaves with boost rate (110%),(108%) respectively compared to control (W1F1T1) figure 3 .The results shows a significant differences among treatments under salt stress, the treatments (W2F3T2) and (W1F3T3) achieved the highest values of nitrogen and potassium contents with rising rate (69%) ,(66%) of nitrogen and (44%),(51%) of potassium, respectively compared to (W2F1T1) figure1,3, while the treatments (W2F2T1),(W2F2T2),(W2F3T2) (W2F3T3) achieved the boosting rate (82%,76%,74% and 76%) respectively compared with (W2F1T1) figure 2. the salinity led to reduce the leave contents of N,P,K The diminution of nutrients of leaves due to the high of Na and Cl concentration in irrigation water which was disturbance of nutrients accumulation, Salinity affected on nutrients uptake by two ways :the first way, direct competition of Sodium and chloride or by effecting on cell membranes permeability through membrane proteins by changing plasmalemma integrity (27; 6), these results agreed with some researches (5) that there are a decrease in the rate of N,P,K with increased salinity of water in cabbage plants , some researches (46) reported that salt stress led to reduce the NPK contents and increasing Na, lettuce leaves , (23) they explained this to the effect of sodium ions and chloride on nutrients availability and reduced their transport and absorption by roots , earlier studies reported that salinity conditions could adversely affect the biomass of lettuce (45; 15) However, when the plants were applied with both kinds of composts (palm fronds compost and rice wastes compost), had a greater N,P,K contents in leaves than untreated plants. Similar findings were observed in Cabbage in the study of some researchers (5), the rice wastes compost is superior to palm fronds compost in N and K contents in leaves significantly, while the palm fronds compost is superior to rice wastes compost in phosphorus us contents in leaves but without significant difference. There are evidences that organic manures reduce the adverse effects of various stresses on plants by affecting the uptake and accumulation of inorganic nutrients (48) The results of this study showed that (N, P, K) contents of leaves increased with Kinetin application especially under salt stress condition, the salt stress causing change in hormonal balance, including in increase the ABA content in leaves and possible decline the cytokinesis contents so the application of kinetin may be useful at these such conditions, it is necessary to consider that applied exogenous PGRs can change. The endogenous phytohormons by their uptake (33), in this concern the association between the internal mineral elements was largely effected by kinetin treatments, may also can be due to the kinetin role to reduce the injury of membranes and that improve nutrients uptake (36). The bio-health fertilizers increased the N, P, K contents in leaves but the differences were reach to significant level in P, K only. These results show that bio-fertilizers have an effect, due to facilitation the assimilation of nutrients and improve the physiological processes of plants; the positive effects produced by bio-fertilizers must bring a great amount of available nutrients to plants (13).

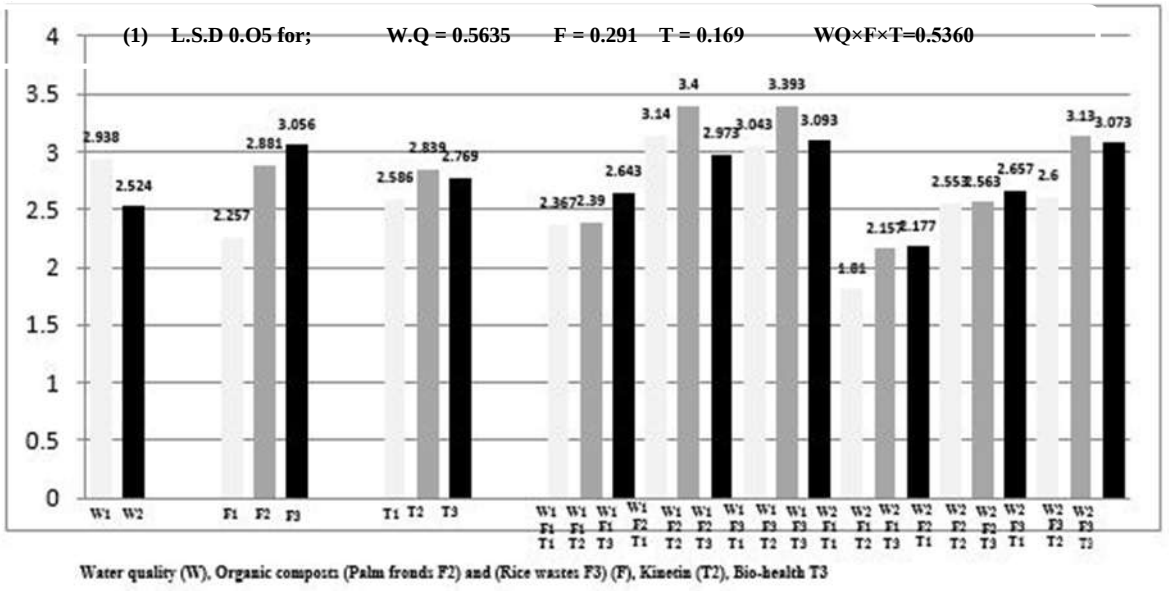

Fig 1. Effect of water quality, Organic compost, Kinetin and Bio health Nitrogen% contents in leaves

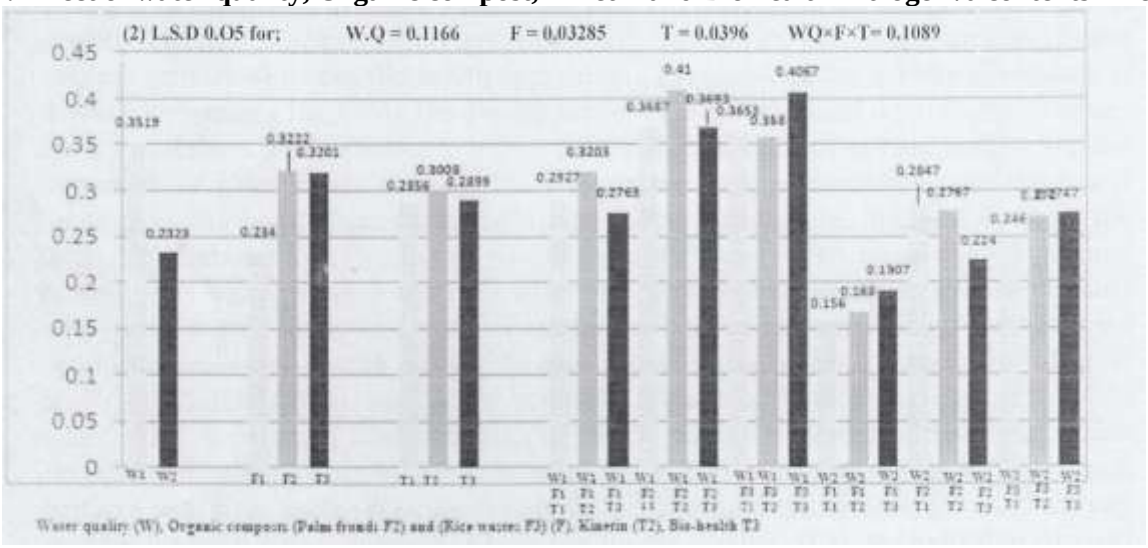

Fig 2. Effect of water quality, Organic compost, Kinetin and Bio health Phosphorus % contents in leaves

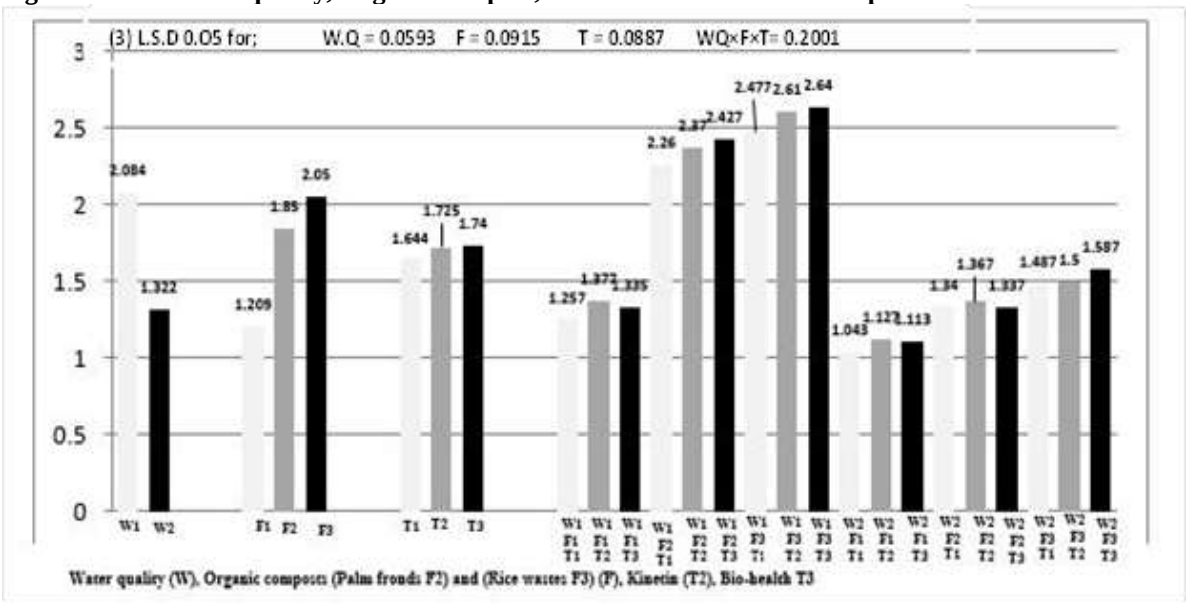

Fig 3. Effect of water quality, Organic compost, Kinetin and Bio health Potassium % contents in leaves

Anti-Oxidants Enzymes: The figure 4 shows significant differences among the treatments, the saline water (W2) increased the peroxidase activity in leaves at an boosting rate (87%) compared with treatments which irrigated with river water (W1), the organic application reduced the peroxidase activity at an dropping rate (1.3% of palm fronds compost F2) and (8% of Rice wastes compost (F3) while there are no significant differences in Kinetin (T2) and bio- health applications (T3) in peroxidase activity of leaves. The figure 4 show significant differences in peroxidase activity among the treatment especially in the treatments which irrigated with saline water , the treatments which were combine of rice wastes compost without (W2F3T1)or with Kinetin (W2F3T2) and bio – health application (W2F3T3) reduced the peroxidase activity of leaves with reduction rates (23%, 27% and 25%) respectively . While the palm fronds compost reduced the peroxidase activity with reduction rates (17%, 23% and 20%) respectively compared to saline water only. Figure 5 shows a positive relationship between the water salinity and catalase activity in lettuce leaves the saline water (W2) increased the catalase activity at an boosting rate (168%) compared to river water (W1), while the organic matter reduced the catalase activity weather palm fronds compost (F2) or rice wastes compost (F3) at an reduction rate (10%) and (14%) respectively, the result didn't show any relationship between the kinetin application (T2) and catalase activity while the Bio-health application (T3) increased the activity of catalase in leaves with boosting rate (9%).The overlap between the factors record significant differences among treatments with reference to river water the highest value of catalase activity was the treatments of palm fronds compost and rice wastes compost combined with Bio-health application ,the boosting rate was (66%) and (67%) respectively, followed by same the treatment combined with kinetin with boosting rate (49%) and (42%), respectively compared to treatments which irrigated with river water only, with regard to treatments which irrigated with saline water , the lowest value of catalase activity of treatment which combined the organic compost with kinetin weather the palm fronds or rice wastes compost , the reduction rate was (30%) and (29%) respectively , compared to treatments which was irrigated by saline water only. Superoxide dismutase activity (SOD) activity increased of treatments which irrigated with saline water (W2), at an boosting rate (121%) compared with treatments which irrigated with river water (W1), conversely the organic composts reduced the SOD activity weather the palm fronds compost (F2) or rice wastes compost (F3), the reduction rate was (6%) and (8%) respectively figure 6, with regard to Kinetin (T2) and Bio-health (T3) application ,there are no significant difference with treatments Which was not treated with its , although the kinetin and bio-health applications led to an increase the SOD activity in leaves figure 6. Of the same figure we did not notice any significant differences between overlap treatments which was irrigated by river water only, while there were significant differences between the overlap treatments which was irrigated by saline water, the highest value was an bio-health treatment untreated of compost (W2F1T3) which was irrigated by saline water (137.37 unit /mg), While the lowest values in treatments were treated with palm fronds compost which was irrigated by saline water (W2F2T1) figure 6. The results shown in Figure 7 That there are significant differences among the studying factors, the saline water was increase the SOD activity (W2) in lettuce leaves at an increasing rate (50%) compared with treatment which was irrigated by river water(W1), conversely the Organic composts (palm fronds F2 or rice wastes (F3) led to reduce the MDA activity , The rate of decline was (3%) which was not significant with reference to palm fronds compost , While it was significant for rice wastes compost with At a boosting rate was (6%).The use of kinetin (T2) resulted in a slight reduction and it was not enough significant in the SOD activity but the Bio-health application (T3) led to increase the SOD activity significantly, the boosting rate was (7%) compared with untreated treatments figure 7.The triple overlap did not appear significant differences among treatments which were irrigated by river water, while the overlap treatments which was irrigated by saline water

achieved significant differences among them, the highest values were treatments which was un treated by Bio-health application only (W2F1T3), followed by the treatments which treated by bio-health and palm fronds compost applications (W2F2T3), Their rates of elevation were (10%) and (10%) respectively , compared with treatment which was irrigated by saline water only. The lowest values of SOD activity in lettuce leaves was treatment which was treated with Kinetin application plus Rice wastes compost (W2F3T2), their rate of decline was (6%), and the difference was not enough to reach of the significant value. Results indicated that salinity stress; composts, kinetin and bio-health application had a significant effect on the activities antioxidant enzymes. The activity of POX, CAT, SOD, MDA were increased with the increase of salinity stress, the ROS-induced membrane leakage and disturbance in cellular homeostasis in salinity-stressed plants, Plants develop self defense mechanisms by producing antioxidant enzymes like superoxide dismutase, ascorbate peroxidase and catalase (1). Reactive oxygen species cause oxidative damage to membrane lipids and proteins, under saline conditions, plants produce enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), CAT and APX decrease the oxidative damage in plants. Under salinity conditions, CAT and POX complement the role of SOD in reducing toxicity caused by salinity (17). SOD catalyses the dismutation of superoxide radical anions to hydrogen peroxide and oxygen (9), some researchers (47) have reported significant increasing in activity of POX and CAT in Triticale leaves with the increase of salinity stress, salt stress induces an increase the SOD ,CAT , POX activity, frequently been correlated with salt tolerance, similarly Shams, et al., 2016 mention to that POX, CAT and SOD activity was raised with increasing of water salinity, also the MDA activity was increase with increasing of water salinity in the present study coincides with the reports of Rasool (31) in chickpea and some researchers (32) in mustard , but the increase in MDA activity in this study did not reach to significant value. the rice wastes composts significantly reduced the activity of POX, CAT, and SOD especially under salt stress while caused a slight reduction in MAD activity whilst the palm fronds compost reduced activity of POX, CAT significantly, also its achieved insignificant reduction in MDA activity, conversely the palm fronds compost induced the activity of SOD without significant difference, The rice wastes reduced the POX, CAT and SOD activity in lettuce leaves more than palm fronds, the reason may be due to Organic fertilizer apart from releasing nutrient elements to the soil has also been shown to improve other soil chemical and physical properties which enhance crop growth and development (19) ,the effect of organic matter was very distinct under salinity stress ,the organic matter can function as salt ion binding agents who detoxify the toxic ions, particularly Na+ and Cl- (41). Some researchers (11) concluded that physical, chemical and biological properties of soil in salt affected areas are enhanced by the use of Organic manure and bio fertilizer leading to improved plant growth and development, especially under salt stress. The kinetin application reduced the activity of POX, CAT, and MDA, but the reduction was not reach to significant differences, these results agreed with (21) which reported that reduction in the activities of antioxidant enzymes with kinetin application, conversely the kinetin has induce the SOD activity without significant difference as well, A possible involvement of genes in stress responses is often inferred from changes in the transcript abundance in response to a given stress trigger. An overview of the many changes in the transcript abundance of Cytokinins genes in Arabidopsis in response to environmental factors was given elsewhere (8), in the other hand the bio- health fertilizers induced the activity of POX, CAT, SOD and MDA but this reduction did not produce significant differences for POX and CAT, this result agreed with (24; 18 and 47) which they reported Arbuscular mycorrhizal fungi promote salinity tolerance by utilizing various mechanisms such as accumulation of compatible solutes and production of higher antioxidant enzymes. The kinetin application was reduce the activity of study enzymes conversely the bio health application was

induce its activity and both of them increased the growth parameters like N,P,K, in leaves , especially under salt stress, this means that both have a different strategy to increase the plant tolerance to salt stress, some researchers (14) reported that rhizosphere microorganisms, exclusively beneficial bacteria and fungi, can improve plant performance under stress environments and enhance yield. The use of PGPR may be proper in developing strategies to facilitate plant growth in saline soils, Vessy (40) reported PGPR can directly facilitating the uptake of nutrients from the environment, by influencing phytohormons production e.g. auxin, cytokinin and gibberellins.

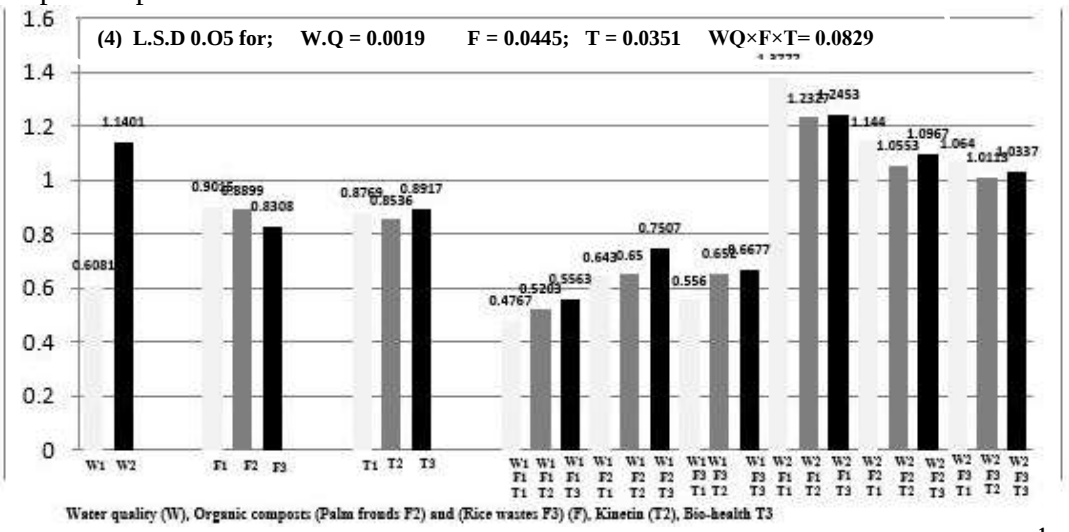

Fig 4. Effect of water quality, Organic compost, Kinetin and Bio health on POX activity Unit.mg^{-1} in leaves

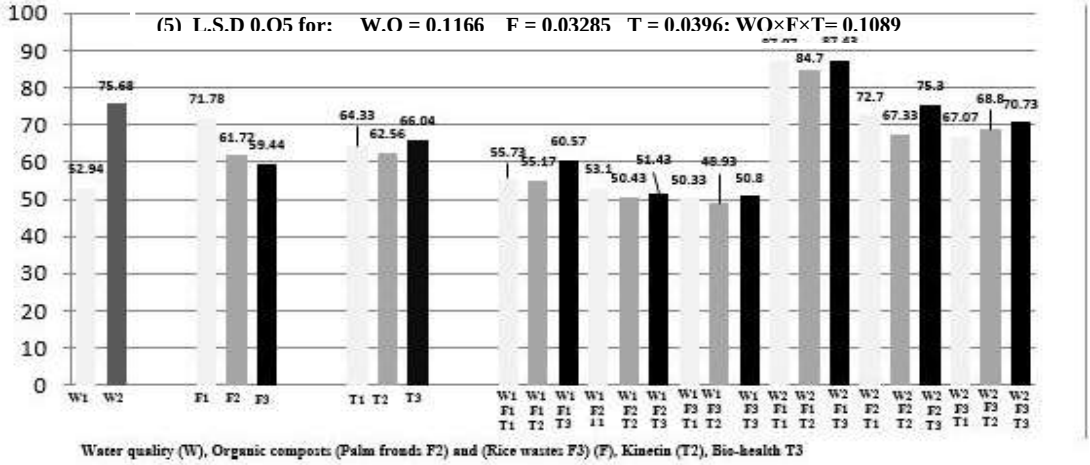

Fig 5. Effect of water quality, Organic compost, Kinetin and Bio health on CAT activity Unit.mg^{-1} in leaves

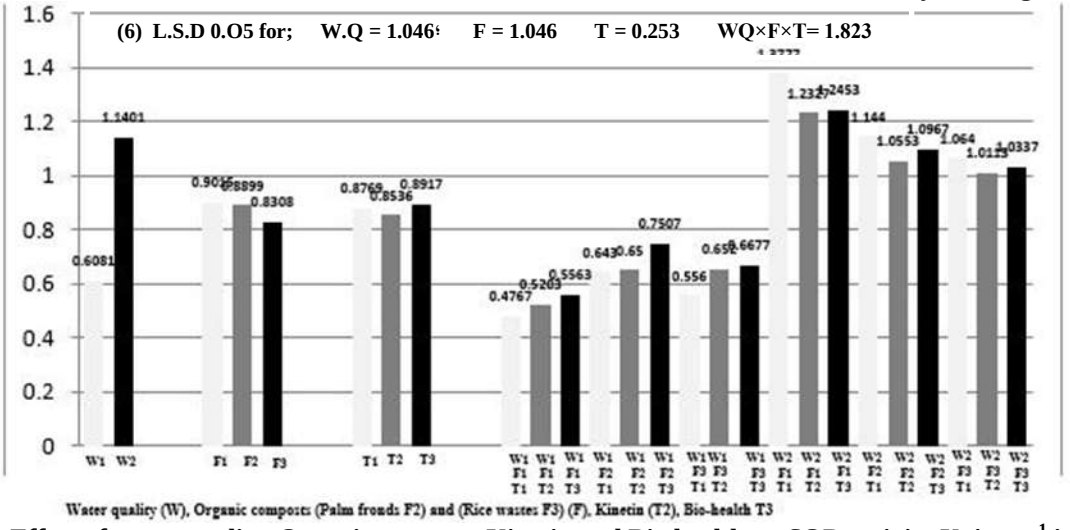

Fig 6. Effect of water quality, Organic compost, Kinetin and Bio health on SOD activity Unit.mg^{-1} in leaves

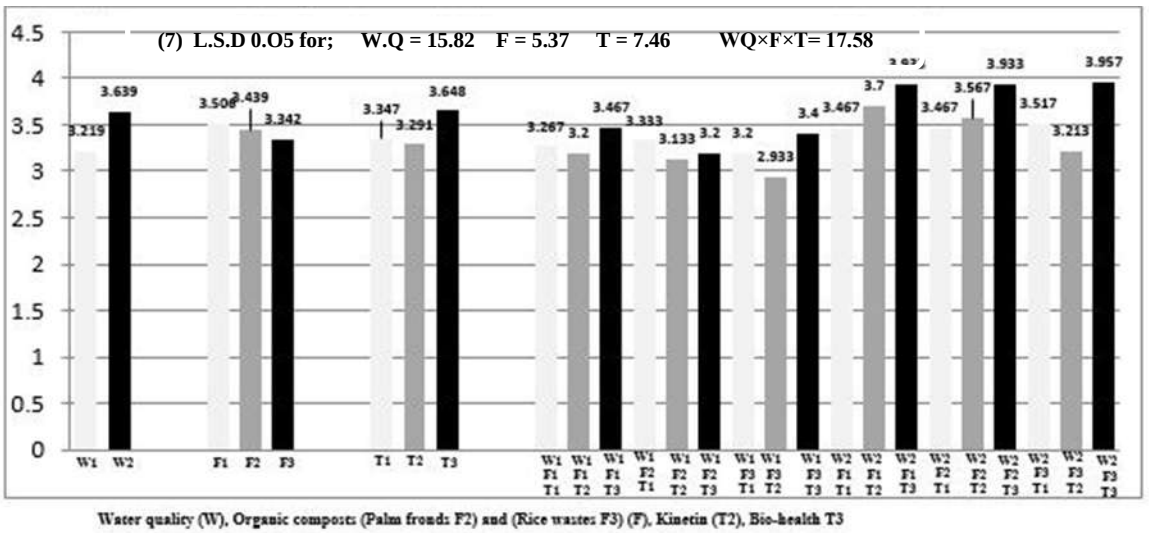

Fig 7. Effect of water quality, Organic compost, Kinetin and Bio health on MDA activity Unit.mg^{-1} in leaves

Proline contents in leaves

The treatments, which were irrigated by saline water (W2), achieved the highest levels of proline content in leaves significantly; their rates of elevation were (110%) Compared with the treatments, which were irrigated by river water (W1), figure 8.The organic manure treatments led to reduced proline content whether the palm fronds compost or rice wastes compost, their rates of reduction were (16%) and(15%) respectively figure 8, While the treatment of Kinetin was reduced the proline content, the rate of reduction was (1.8%) which was reach to significant degree, conversely the treatment of Bio-health application was increase the proline content in leaves , the rate of elevation was (1.7%) which was reach to significant degree too, compared to treatment which untreated of organic manure. The figure 8 shown a significant values among the overlap treatments in proline content of leaves, with regard to river water, the overlap treatment (W1F1T3) achieved the highest significant value at an boosting rate was (13%), While the lowest values was at (W1F3T1) with an reduction rate (10%), similarly the overlap treatments which was irrigated by saline water achieved significant differences among the treatments, the highest value at the treatments (W2F1T1) and (W2F1T3) while the lowest value of proline contents in leaves at treatments which was treated with Kinetin in both organic composts (W2F2T2) and (W2F3T2) figure 8 . Proline contents in leaves increased with salinity of irrigation water , the compatible solutes like proline are used for osmotic adjustment and for maintaining the functional state of macromolecules, probably by scavenging ROS (44),the results agreed with (39) which was fined that proline accumulates more in the leaves of plants with increasing of salt stress, The increasing of proline content during the vegetative growth phase in the salinity treatments suggests that the plants tried to stabilize their protection mechanism (37), some researches (47) which find that salinity stress increased the antioxidant enzymes activity and proline contents, , the one mechanism used by plants to scavenge the reactive oxygen species, High salt levels on plant growth increases reactive oxygen species which damage all classes of biologically important macromolecules including DNA and the generation of H2O2 and lipid hydro-peroxides which cause membrane changes, To mitigate, and repair, damage initiated by reactive oxygen, plants developed a complex like proline production (6).the organic composts (rice wastes and palm fronds) reduced the proline contents in leaves significantly ,especially under salt conditions, The reason may be due to improved growth and cell metabolism under salt stress or may improve soil water holding capacity as well as the CEC and nutrients are released slowly to crop plants two main functions of organic manures in soils are the supply of nutrients and increase in the organic matter content of soils. During the decomposition and mineralization process (36), the kinetin application reduced the proline contents in leaves while the bio

health increased the proline significantly, both have specific behavior to reduce salinity effect, kinetin may improve the membrane integrity and cellular metabolism and increased the photosynthesis activity through alleviated from oxidative stress without depend on proline production under salt condition, while the role of bio-health to increase of proline production with osmoprtectents to scavenge the ROS in cell and improved the growth under salinity. There is a possibility to mitigation the negative effect of saline water by some application like Organic composts (Rice wastes and Palm fronds), and Decomposed organic matter to solve the disruption of endohormons and lack of available nutrients under salt stress, and elevation of osmotic stress in soil solution in roots area. The Kinetin and bio-health application improved the Lettuce tolerance to the abiotic stress, which was exerted by saline irrigation water, More work concerning effects of alleviation of salinity on lettuce in arid and semi- arid zones is needed. In addition, use other applications from sources of organic matters and bio fertilization.

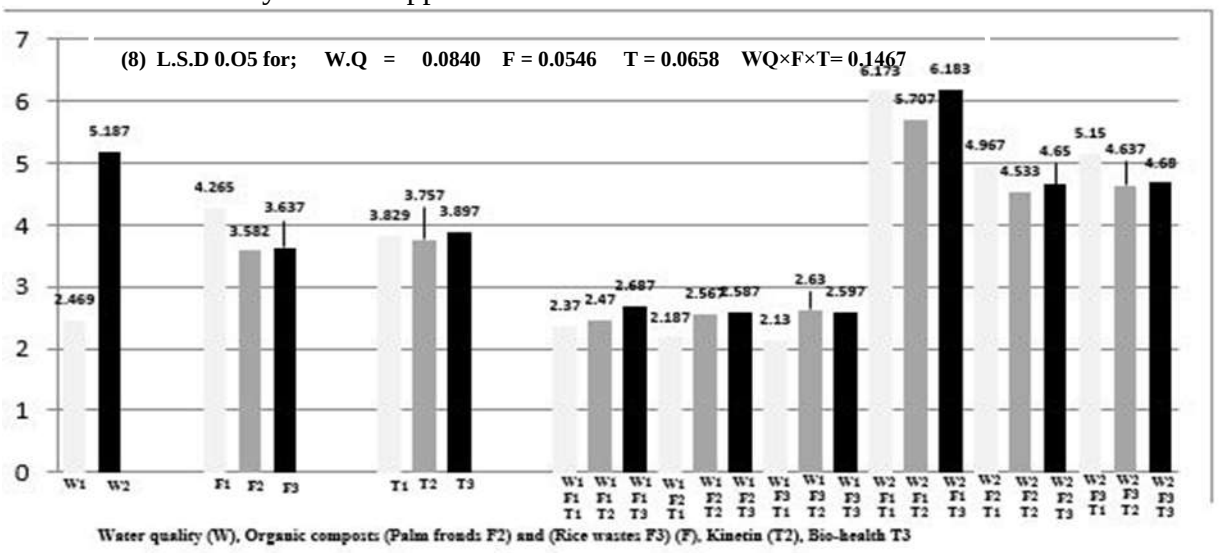

Fig 8. Effect of water quality, Organic compost, Kinetin and Bio health on Proline contents mg protrinmg^{-1} in leaves

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