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Influence of different source of nitrogen compounds on some growth characters of pepper (*Capsicum annuum* L.) and some antioxidants enzymes activity under water culture.

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Abstract: *This experiment was carried out using water culture technic to investigate the influence of form and concentration of nitrogen fertilizers in growth and activity of three antioxidants enzyme such as super oxidase dismutase (SOD) and peroxidase (POD) and catalase (CAT) and non enzymatic antioxidant the chlorophyll.The experimental showed that the response of pepper plants depend highly significantly upon the concentration and the form of nitrogen fertilizer. The highest root dry weight was found when 25 mm.L^{-1}ammonium phosphate plus 75 mm.L^{-1} ammonium nitrate. The experimental unit included eight plants and the number of treatment was seven and replicated three times. The experimental design adapted in this experiment was CRD .Treating pepper plants with 100 mm.L^{-1} ammonium nitrate alone gave the highest leaves dry weight. The influence of form and concentration of nitrogen fertilizer on N,P,K on the leaves as well as Fe and Mn was differ from treatment to other treatment .The three enzymes SOD, POD, CAT was characterized by the highest activities in control treatment as a results of nitrogen deficiency which causes an accumulation of radical oxygen species (ROS), while the activity of the antioxidant enzyme was significantly decreased in the single and mixed treatment with nitrogen treatment and the enzymatic activity was different from plants part and other (roots and leaves).*

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I. Introduction

The losses of macro and micro nutrients were one of the types of the abiotic stress. The plants subjected to these stresses will shows an increase in (ROS) in plant cells as a byproduct for the biological activities in plants (Ahmadizadah et al, 2011). The increasing in the concentration of (ROS) will highly causes spoilage of membrane and cell contents and DNA which considered as oxidative or reducing agent and it can oxidizing many compounds such as NADPH and reducing the cytochrome and minerals ions (Gill and Tuteya, 2010). The increasing in ROS will inhibit the ability of cell to overcome the damage in photosynthesis II (Halliwell and Gutteridge, 1985). Many studies in different plants show that the type and concentration of nitrogen fertilizers influenced the abilities of plant to absorb the micro and macro elements (Barker and Bryson, 2007). The plants differ highly significantly in there needs to nitrogen elements as NH_4 or No_3or both and the root system needs less nitrogen elements as NH_4rather than No_3.

Halvin*et al* (2005) reported that mixing fertilizers as No_3 and NH_4 and fertilizing plant will significantly increased the dry weight of plants. Pepper plants characterized by weak rooting system which will influence the shoot ⁄ root ratio and the plant growth and reduce fruit yield (Matlob*et al,* 1989). Many research workers studied the changes in the concentration of mineral elements or the changes in the root system and vegetative growth and the biochemical changes when fertilizing plants with NH_4 or No_3 or both of them (Binford*et al*,1992). Due to the lack in literature about the activity of enzyme system under the different level or different form of nitrogen element.This study illustrates the distribution of antioxidant enzymes between the root and vegetative systems under different level of No_3 or NH_4or No_3 + NH_4.

II. Material and Methods

This water culture experiment was conducted in the green house - department of soil science and water resources- College of agriculture – university of Baghdad to investigate the influence of concentrations and types of nitrogen fertilizers which is added to the water culture media in growth of pepper plants and the activities of antioxidants enzymes.

Pepper seed Karizma cultivars was planted in special planting trays contain 209 cups for planting on 25-9-2013. The seedlings of pepper plants was transplanted after reaching 4 true leaves to a plastic container with a capacity of 3 L on 1-11-2013 in which a growing solution as shown in table(1) .

Table (1) the growing solution used in water culture media

A. the macronutrients solution (solution A)	
Type of minerals	**Concentrations MM.L^{-1}**
$CaCl_2.2H_2O$	200
K_2SO_4	100
$MgSO_4.7H_2O$	50
KH_2PO_4	10
B. the micronutrients solution (solution B)	
Type of minerals	**Concentrations MM.L^{-1}**
H_3PO_4	3.0
$CuSO_4.2H_2O$	0.1
$MnSO_4.2H_2O$	0.25
$Na_2MoO_4.2H_2O$	0.02
$CoSO_4.7H_2O$	0.04
$ZnSO_4.7H_2O$	0.3

The nitrogen treatments were as follows:
1. Nitrogen as $(NH_4)_2SO_4$at 50 and 100 MM.L^{-1} represented by **T_2, T_3**.
2. Nitrogen as $(NH_4)\ NO_3$at 50 and 100 MM.L^{-1} represented by **T_4, T_5**.
3. The treatment with $(NH_4)_2SO_4$ and $(NH_4)\ NO_3$ at 25 MM.L^{-1} for each of them and represented by **T_6**.
4. The treatment with $(NH_4)_2SO_4$ at 25 MM.L^{-1} and $(NH_4)\ NO_3$ at 75 MM.L^{-1} and represented by **T_7**.
5. The control treatment (**T_1**).

The experimental unit included eight plants and the number of treatment was seven and replicated three times. The experimental design adapted in this experiment was CRD and the results were analyzed using (SAS, 2001) with windows 2007, the average of the results was compared using least significant differences at 5% level. The nutrient solution was changed every morning as shown in the experimental treatments and adjusts the PH of the solution at 6.5 during the growing of the pepper plants.

The plants were removed from the nutrient solutions on 15-1-2014. The plants was separated to vegetative and rooting parts and dried at room temperature and the fresh weight was taken. The plant parts were dried using electrical oven at 70 c^o and the dry weight was taken for both parts.

The studied characters

A. Vegetative characters: plant height using measuring tapes,leaf area using Digimaizer (Sadik*et al*, 2011), leaf chlorophyll content by Goodwin, 1976, the dry weight of the roots and vegetative parts using sensitive balance.

B. The nutrient elements in the leaves: the nutrient elements such as N, P, K, Fe, Mn were determined by taken 0.2 g of plant parts and digested using H_2SO_4 and at ratio of 5:3 (Eresser and Parson , 1979). After the completing of the digestion , the nitrogen was estimated using micro-Kjeldail apparatus (Jackson, 1985), the phosphor using spectrophotometer at 882 nanometer and potassium using flam photometer and Fe and Mn using atomic absorption spectrophotometer.

C. The enzymatic activity for (SOD, POD, CAT) in the leaves and roots: Three gram of vegetative part or root was taken and mashed separately 0.1 molar from potassium phosphate with pH 7.0 at a ratio 1:4 v: w. the sample was mashed using electrical blender then separated using Moseley cloth then the filtrate was centrifuged at 10000 rpm for 30 minute at 4 C^o and the filtrate kept in refrigerator at 2 C^o (Pitotti*et al*, 1995). The enzymatic activities for SOD was estimated and reported by (Beyer and Fridowich, 1987) and for POD as shown by (Neizh, 1985) and CAT by using spectrophotometer as shown by (Aebi, 1974).

III. Results and Discussion

Table (2) Influence of the concentration and types of nitrogen fertilizer which added to the water culture of pepper plants on the vegetative growth

Treatments	**Plant height cm**	**Leaf area Dec^{-2}**	**Chlorophyll content mg.100 g^{-1}**
Control (T_1)	10.23	26.94	173.50
T_2	13.04	34.88	305.90
T_3	15.11	38.14	385.48
T_4	15.86	44.82	485.22
T_5	24.27	48.62	501.61
T_6	12.81	39.46	345.90
T_7	22.06	42.84	475.33
L.S.D 0.05	**2.88**	**7.12**	**41.72**

Table (2) showed a significant influence of nitrogen fertilizers on plant height in spit of a significant difference between the treatments. Treating pepper plants with 100MM NH_4significantly increased the height of plant up to 15.11 cm as compared with control treatment which gave 10.23 cm, while the treatment with NO_3 at 50 and 100 MM increased significantly height of plant and leaf area and leaf chlorophyll content to (15.86 cm, 44.82 $Dcim^2$, 485.22 mg.100 g^{-1}) and (24.27 cm, 48.62 $Dcim^2$, 501.61 mg.100 g^{-1}) for both concentration respectively as compared with 10.23 cm , 26.93 $Dcim^2$, 173.50 mg.100 g^{-1} for the control treatment.

The reason of significantly superiority of fertilizing pepper plants with NH_4 or $NO_3$and the adding of both fertilizer (NH_4 and NO_3) may be due to the increasing of the percentage of nitrogen in the leaves (table 3) and also increasing leaves chlorophyll content (Latique et al, 2013), and consequently increasing the leaf area per plants and then increasing the bioactivities in plant and increasing the cell division and cell enlargement in merestimatic tissue in plant (Taize and Zeiger , 2010), this will causes an increases in growth of plant such as height of plant, leaf area and increasing dry matter in plant (table 4).

Table (3) Influence of the concentration and types of nitrogen fertilizer which added to the water culture on the N, P, K, Fe and Mn in pepper leaves

Treatments	N (%)	P (%)	K (%)	Fe (%)	Mn (%)
Control (T_1)	0.58	0.06	1.15	53.11	54.61
T_2	1.18	0.09	1.41	68.67	57.52
T_3	2.21	0.16	1.55	83.73	60.96
T_4	2.31	0.08	1.40	85.19	55.77
T_5	2.60	0.11	1.59	87.29	59.81
T_6	2.29	0.10	1.52	77.38	56.35
T_7	2.33	0.10	1.73	81.89	67.97
L.S.D 0.05	**0.28**	**0.04**	**0.28**	**13.70**	**N.S**

The results in table (3) showed that the concentration of N.P.K in the plant from the control treatment express a nitrogen deficiency stress. The concentration of N.P.K was increased by increasing the concentration of nitrogen fertilizer in the nutrient solution and the highest concentration was in the ammonium nitrate treatment at 100 MM (2.60 %) while no changes in the concentration of phosphor and potassium because they added to the nutrient solution was prepared and the same was with Fe and Mn. Treating pepper plants with $(NH_4)_2So_4$ at 25 $MM.L^{-1}$ plus (NH_4) No_3 at 75 $MM.L^{-1}$ (T_7) highest nitrogen content in the leaves reached to 2.33 % as compared with a single treatments or treatment with $(NH_4)_2So_4$ and (NH_4) No_3 at 25 $MM.L^{-1}$ (T_6). The concentration of Fe was not changed significantly between the treatments while they significantly superior than the control treatment.While the experimental treatments did not effect on the leaf manganese content.The reasons were due to the fact that $(NH_4)_2So_4$ will acidity the media of the roots and make the Fe available to the roots (Sims, 1986).

Table (4) Influence of the concentration and types of nitrogen fertilizer on vegetative and root dry weight of pepper plants.

Treatments	vegetative dry weight (g)	root dry weight (g)
Control (T_1)	9.19	0.55
T_2	12.76	2.12
T_3	13.16	3.28
T_4	15.64	3.05
T_5	16.64	3.27
T_6	14.39	2.75
T_7	17.38	3.36
L.S.D 0.05	**2.83**	**0.55**

As shown in table (4) the treatment with $(NH_4)_2So_4$at 25 $MM.L^{-1}$ plus (NH_4) No_3 at 75 $MM.L^{-1}$ (T_7) had the highest dry weight of vegetative parts (17.38 g) as compared with (9.19g) in control treatment. The same treatment had the highest root dry weight at 3.36 g while the control treatment gave 0.55 g. pepper plants fertilized with (NH_4) No_3 at 100 $MM.L^{-1}$ (T_5) gave the highest dry weight of vegetative parts and roots (16.64 and 3.27 g) respectively.

Table (5) Influence of the concentration and types of nitrogen fertilizers in mineral solution on the activities of antioxidants enzyme on vegetative parts and root of pepper plants.

Treatments	SOD		POD		CAT	
	leaves	roots	leaves	roots	leaves	roots
Control (T_1)	45.86	42.98	197.65	276.16	8.23	8.12
T_2	31.52	28.00	165.42	232.86	6.12	6.55
T_3	15.25	15.22	150.66	202.26	5.67	6.00

T_4	17.26	18.73	160.11	186.92	5.73	6.19
T_5	13.49	12.53	137.30	163.21	3.88	3.43
T_6	16.00	17.16	166.94	185.75	5.46	5.88
T_7	13.23	10.73	123.35	151.65	3.17	2.65
L.S.D 0.05	**4.55**	**18.85**	**48.32**	**30.63**	**2.80**	**0.67**

The results in Table (5) show that nitrogen treatments reduced the content of leaves and rootsof the activities of antioxidants enzyme, especially treatment $(NH_4)_2So_4$ at 25 MM.L^{-1} plus (NH_4) No_3 at 75 MM.L^{-1} (T_7)as it was given 13.23, 123.35 and 3.17 mg.$protien^{-1}$ for SOD, POD and CAT in leaves respectively, and its gave 10.73, 151.65 and 2.65 mg.$protien^{-1}$ for SOD, POD and CAT in roots respectively, while the control treatment gave the highest value of these enzymes for both leaves and roots.

The enzyme SOD was recognized as the first line of defense to the plants from stresses (Sairam*et al*, 2002) by reducing the harmful of ROS which take many forms and the most dangerous one to the plant was the increasing of H_2O_2concentration. The mechanism of action of SOD was changing H_2O_2 to the OH plus H_2o (Nadell*et al*, 2011) to reduce the harmful effects of ROS, while the concentration of SOD was decreased when the nitrogen deficiency stress was overcomes by the addition of nitrogen fertilizers. The enzyme POD also changing H_2O_2 to the OH by removed OH which can be considered as ROS (Nadell*et al*, 2011). When the plant subjected to a stress this will increased the activity of CAT enzyme to scaving the harmful effects of ROS (Abedi and Packniyat, 2010). The mechanism of action of CAT was to complete the action of SOD and POD to change the removing of H_2O_2 in plant cells to H_2o and O_2 (Wilken *et al*, 1995).

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