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UREA AND AMMONIUM SULFATE FERTILIZERS AND HUMIC ACID EFFECT ON UREASE ENZYME ACTIVITY IN AND OUT THE RHIZOSPHERE OF *ZEA MAYS* L. CROP

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Abstract

In order to study the activity of the urease enzyme in and out the rhizosphere area of the yellow corn crop, pots experiment was carried out. It took place in the canopy of the College of Agriculture - the University of Al-Qadisiyahduring summer agricultural season of 2017. Silty clay loam soil brought from the extension farm ofNouriya, which belongs to the Department of Agricultural Extension in the province of Al-Qadisiyah. Pots were filled with soil and planted with maize cultivar of (5018).This study treated with 2 levels of humic acid (HA_1 and HA_2) (40 and 80) kg. h^{-1}, respectively, granular urea fertilizer and ammonium sulfate (U and AS) of 340 kg N. h^{-1}, control treatment and their overlaps.The experiment was conducted according to the Complete Randomized design (C.R.D) with 4 replicates. Means were compared according to L.S.D at (0.05) probability level. A laboratory experiment in which the activity of the urease enzyme in and out the rhizosphere soil for all of the study parameters after 30, 110, 90 and 60 days of planting date was also carried out.

The results are summarized as follows :

1. Urease enzyme's activity inhibited with the application of the second level of the humic acid (HA_2), but not with the first level (HA_1) application. The enzyme activity after 30 days of planting at (HA_2) itself and its interaction with granular urea fertilizer and ammonium sulfate (U + HA_2 and AS + HA_2) are (108.40, 148.15 and 153.70) µg N-NH_4^+ g^{-1} soil. $2h^{-1}$, respectively. The value of enzyme activity at (HA_1) for its substance and its interaction with granular urea fertilizer and ammonium sulfate(U + HA_1 and AS + HA_1) are (162.05 , 171.35 and 166.80)µg N-NH_4^+ g^{-1} soil. $2h^{-1}$, respectively. This inhibitory activity of the enzyme in the second level of the acid itself and with the nitrogen fertilizers continued at all periods of the study.
2. Urease enzyme activityis high in the rhizosphere area and lowin bulk soil for all treatments and periods of study. The enzyme activity in and around the rhizosphere for all study periods were (143.40, 137.61, 99.62 and 90.80) µg N-$NH_4^+$$g^{-1}$ soil. $2h^{-1}$ and (96.45 , 117.00 , 84.60 and 69.05) µg N-NH_4^+ g^{-1} soil. $2h^{-1}$, respectively.

Key words : Urease enzyme, hyomic acid, rhizosphere, urea, ammonium sulfate.

Introduction

Soil area that is affected by the vital activities of plant roots, known as rhizosphere, plays an important role in plant growth and soil fertility. Its physical, chemical and biological properties are differedthan those not affected (Bulk soil) such as the soil reaction degree, for example, its value decreases as a result of CO_2 production increases because of the respiration of microorganisms and plant roots (Al-Taweel, 2015). Recent studies have shown that this area is a place of the most biological interactions between microbiology and the biological soil system (Hinsinger *et al.*, 2006). The rhizosphere area contains root secretions that encourage the growth of microscopic biological communities as it is a source of energy andthey needed carbon for their growth. Among these secretions are organic acids, amino acids, carbohydrates and some inorganic compounds such as CO_2. It also contains enzymes that are released into the rhizosphere (Shulka and Varma, 2011). The most important of these enzymes are the hydrolysis enzymes. They play an important role in the vital transformations of the elements in the soil such as sulfur, carbon, phosphorus and nitrogen.

Nitrogen is one of the essential element in plantsnutrition since it supplies plants with two basic ions (JarAlla, 1998). It is the most naturally exposed element to the vital processes of organisms. Organic nitrogen in the soil is exposed either to volatilization in form of

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ammonia or is losing throughout washing in form of nitrate or fixed by organic and minerals alkalines in form of ammonium ion. It also synthesized within the bodies of microorganisms and plants (Tamimi, 1999). Therefore, because of the losses, chemical fertilizers applications increased. Urea is one of the most widely used nitrogen fertilizers worldwide for its high content of nitrogen (46%). It has a lowmanufacturingcost, andits raw materialsare available, easy storage and manufacturing (Watson, 2000). It is one of the most widely used fertilizers in Iraq since, it is located in the arid and semi-arid region, which is characterized by its low content of nitrogen. Urea is characterized by its high hydrolysis and its nitrogen exposure to loss in the form of ammonia gas, especially in calcareous soils.

Urease is the responsible enzyme for the decomposition of urea fertilizer.It is one of the hydrolysis enzymes that is existed in the soil. Therefore, researches worldwide have been working towards reducing the process of nitrogen loss and improve the efficiency of fertilizer application. This could be done either by using deoxyribonucleic acid inhibitors, which are rather expensiveor by using slow-dissolving nitrogen fertilizers like ammonium sulfate fertilizer containing 21% nitrogen and 24% sulfur. It is recommended to be used in soils with an alkaline reaction (Al-Nuaimi,1999). Other methods used to reduce the loss of nitrogen from fertilizers are the use of humic acids, which has recently proved to be a leading role in modern agriculture as defined by sustainable agriculture. It increases the availability of elements, especially nitrogen as it reduces the loss of its volatilization. It inhibits the activity of the urease enzyme (Bahrani, 2015). Humic acid application changes soil pH. Dong *et al.* (2008) confirm that the activity of the enzyme decreases as soil pH decreases. In accordance with their study, the enzyme activity at pH of (6, 7 and 8) was(38, 49 and 53) $\mu gN\text{-}NH_4^+.g^{-1}$ soil. $2h^{-1}$, respectively.

Inhibition of urease enzyme activityaimsnot only to reduce the process of nitrogenlosingbut also to increases nitrogen availability to plants. This is because of the chemical properties that qualify it to work as a reformer and to add nutrients to the soil (Vaughan and Ord, 1991). The basic process of enzymes inhibition is the joint polymerization of enzymes with humic molecules during wetting and absorption processes (Marzadori *et al.*, 2000a). Humic acid works oninhibiting urease enzyme activity through the polymerization and adsorption process and packaging the enzymes with humic materials (Yan, *et al.*, 2013). Soil pHdecline and the presence of humic substances lead to a change in the activity and stability of proteins, causing partial or total enzyme disruption. Urease enzyme activity is higher in the rhizosphere because of root secretions through which protecting the biological biomass and microbial activity (Gawronska, 2012). Soil urease enzyme is secreted by the existing micro-organisms and plants roots (Martens *et al.*, 1992 and Yang *et al.*, 2007). Cultivated soils have more biological biomass and high enzymatic activity in the root environment compared to other soils where enzymatic activity and biomass volume are slight (Emnova *et al.*, 2012). This is what found by Al-Taweel (2016) study, which is the activity of hydrolysis enzymes in the rhizosphere of the sunflower plant in the silty clay andsandy loam soil. Urease enzyme activity for both soils in and outside the rhizosphere were (83.6 and 51.2) and (36.7 and 22.9) $\mu gN\text{-}NH_4^+.g^{-1}$ soil.$2h^{-1}$, respectively. Studies on urease enzyme activityunder the influence of the humic acid in the area of the rhizosphere and beyond are rare.

Materials and Methods

Pots experiment was conducted in the canopy of the College of Agriculture, the University of Al-Qadisiyah. Soil was brought from the extension station in the Shafia area, which belongs to the Agricultural Extension Department in Diwaniyah.Soil samples of (0 - 30) cm depth after removal (1-2 cm) of the surface layer were taken. They were airily-dried,grinded and sifted with a sieve of 2 diameters then mixed. Some chemical, physical and biological tests were done prior to planting as shown in table 1.

This study was carried out in accordance with the Completely Randomized Design (C.R.D). The number of experimental treatments is (9) including the comparison one with four replicates, which means a total of experimental units is (36) with their overlaps :

Three levels of humic acid (0, 40 and 80) kg. h^{-1}

A single level of granular nitrogen urea fertilizer (320) kg $N.h^{-1}$

A single level of nitrogen fertilizer (ammonium sulfate) (320) $kg.h^{-1}$.

Treatment distributed as presented in table 2.

Plastic pots of 20 kg were used. They were filled with the prepared soil. Seeds of yellow corn of the cultivar of (5018) that authorized of the Department of Seed Examination and Certification in the province of Al-Qadisiyah on the 10th Jul 2017. Seeds planted at a planting rate of (8) seeds per pot. After germination, seedlings number reduced to 3 by removal the others. Phosphate fertilizer was applied in a form of triple

Table 1 : Soil chemical, physical, and biological properties ahead to planting.

Trait		Value	Unit	Method
pH		7.60	-	Black (1965b)
ECe		3.27	ds.m^{-1}	
O.M		13.60	g.kg^{-1}soil	
CEC		12.30	Cmolc.kg^{-1} soil	Papanicolaou (1976)
Available Nitrogen	$N\_NH_4^+$	7.60	mg.kg^{-1}Soil	Black (1965b)
	$N\_NO_3^-$	3.27		
Soil Separators	Sand	13.60	g.kg^{-1}soil	Black (1965a)
	Silt	12.30		
	Clay	26.40		
Soil Texture		Silty clay loam		
Bulk density		1.28	mg.m^{-3}	
Total Bacteria		12.66x10^6	mg.m^{-3}	Black (1965b)
Total Fungi		3.70x10^4	CFU.g^{-1} dry soil	
Urease Activity		26.70	µg N-NH_4^+.g^{-1} soil.2h^{-2}	Tabatabai and Bremner(1972)

Table 2 : Experimental treatments.

No.	Symbol	Treatment
1	Control	Comparison treatment (without fertilizers and acid application)
2	HA_1	Humic acid application of (40) kg.h^{-1}
3	HA_2	Humic acid application of (80) kg.h^{-1}
4	U	Granular urea fertilizer application of (320) kg.h^{-1}
5	HA_1 + U	Humic acid and granular urea fertilizer applications of (40) kg.h^{-1} and (320) kg.h^{-1}, respectively.
6	HA_2 + U	Humic acid and granular urea fertilizer applications of (80) kg.h^{-1} and (320) kg.h^{-1}, respectively.
7	AS	Ammonium sulphate fertilizer application of (320) kg.h^{-1}
8	HA_1 + AS	Humic acid and ammonium sulphate fertilizer applications of (40) kg.h^{-1} and (320) kg.h^{-1}, respectively.
9	HA_2 + AS	Humic acid and ammonium sulphate fertilizer applications of (80) kg.h^{-1} and (320) kg.h^{-1}, respectively.

superphosphate (P 20%) at a rate of (100) kg.h^{-1} once ahead to planting. Potassium sulfate (K 41.5%) was applied 2 times in a form of potassium sulfate at a rate of (120) kg.h^{-1}. The first application is done at planting date and the second with the nitrogen fertilizer (granular urea) (N 46%) and ammonium sulfate (N 21%). Granular urea was applied once again after (45) days of the first application date. Humic acid was applied in form of a powder at once ahead to planting and suitably mixed with the soil. Maize plants were treated with Corn Stem Borer, *Sesamia cretica*, pesticide at the growth stage of leaves. 10% Diaznon pesticide applied after two weeks of the first control. Maize crop was harvested on the 30th Oct 2017 at full maturity stage.

Soil samples of in and outside the rhizosphere of the maize plant were taken on (30, 60, 90 and 110) days of planting. Samples were preserved in plastic containers in a refrigerator to estimate urease enzyme activity.

The activity of the urease enzyme was estimated by placing (5) g of soil in a volumetric flask capacity (50) ml. After that, 0.2 ml of toluene and (9) ml of hydroxyl methylamino (Trismethane) of (pH = 9) and (1) ml of portable urea (0.2) molar as subject matter. Samples were incubated at a temperature of 37Ú for two hours. Afterward, (35) ml of potassium chloride solution of (2.5) molar and silver-sulfate of (100) ppm were added asinhabitation solution then the size is adjusted to 50 ml.Ammonium nitrogen amount produced by the enzyme activity isestimated using a steam distillation device according to the method of Bremner (1965) that is mentioned in Black (1965b) and by using magnesium oxide and boric acid.

Results and Discussion

Effect of humic acid and nitrogen fertilizers on the activity of urease enzyme in and outside the rhizosphere region during the study periods

Urease enzyme activity after (30) day of planting: Table 3 presented the results of the effect of humic acid and nitrogen fertilizers on urease enzyme activity in and out the rhizosphere of maize plants after (30) day of planting.The application of the first level of humic acid (HA_1) in the area rhizosphereled to a significant increase in the activity of urease of (162.05) µgN-NH_4^+.g^{-1} soil.$2h^{-1}$ compared to the control of (87.45) µgN-NH_4^+. g^{-1} soil. $2h^{-1}$. The second level of the humic acid (HA_2) made a statistical increase in urease enzyme activity more than the control. Its value was (108.40) µgN-NH_4^+. g^{-1} soil. $2h^{-1}$. The first level of the acid application had a significantly higher effect on the enzyme activity. The resultspresentedthat the first level of acid application did not inhibit enzyme activity as compared to the second level. This may due to the amount of applied acid, which did not make enough change in pH value to inhibit enzyme activity, while the second level inhibited its activity. This is consistent with the result of Marzadori *et al*. (2000b), which humic acid inhibits urease activity and inhibition ratio depend on the amount of applied acid.

The application of granular urea and ammonium sulfate led to a significant increase in the enzyme activity as compared to the control. They made values of (153.45 and 139.25)µgN-NH_4^+.g^{-1} soil. $2h^{-1}$, respectively. The reason for this is attributed to the increase of nitrogen concentration resulting fromfertilizers application. Thus, the number of microbiologythat produces the enzyme increase due to nitrogen availability.This is consistent with the study of Rihani (1987), which stated that asa number of microorganisms in the rhizosphere area increase, the activity of the urease enzyme increase as well. This is because there is a significant correlation between the activity of urease enzyme and the number of microorganisms.Urea treatment itself statistically increases the enzyme activity more the treatment of ammonium sulfate itself.The reason is might that ammonium sulfate fertilizer directly provides ammonium element, which reduces enzyme effectiveness, but granular urea is decomposed into ammonia bythe urease enzyme, so the enzyme activity increase when applied to the soil.This is similar to what Tamimi (1999) found, which is that the application of granular urea and coated urea with sulfur significantly increase the activity of the enzyme and the maximum activity was obtained with the application of granular urea. This may be attributed to the rapid response of the microorganism that secretes the enzyme when granular urea applied to the soil.

Humic acid and nitrogen fertilizers overlap are presented in this table. Humic acid application increased the enzyme activity at HA_1 that is statistically outperformed on HA_2. Application of HA_1 and HA_2 with urea made values of (171.35 and 148.15) µgN-NH_4^+.g^{-1} soil. $2h^{-1}$, respectively. The reduction in the activity with the treatment of (U + HA_2) compared to the treatment of urea may because the role of the acid in enzyme activity inhibition and reducing the hydrolysis of urea. Ammonium sulfate application with the acid increases the activity compared to ammonium sulfate treatment itself. This increase was not insignificant and the activity at both levels of humicacid (HA_1 and HA_2) and ammonium sulfate (166.80 and 153.70) µgN-NH_4^+. g^{-1} soil. $2h^{-1}$, respectively. The behavior of acid with ammonium sulfateaffecting the enzyme activityis similar to that of the acid with urea. That is, the more the acid level increase, the less the enzyme activity.This is consistent with Vaughan and Ord (1991), who attributed that to the pH value. As the pH value declining, the enzyme activity decline as well.

Treatment of (U + HA_1) made statistically more enzyme activity increase than (SA + HA_1).It may because of the type of fertilizer. Urea availability stimulates microorganisms to produce the hydrolyzed urea enzyme, while this does not occur with the availability of ammonium sulfate. This is consistent with study of Hamoud (2012) on the activity of the urease enzyme and some factors affecting urea degradation in some agricultural soils in Ramadi. (AS + HA_2) treatment increases the activity statistically over the treatment of (U + HA_2). This is due to the type of fertilizer. The activity of the enzyme in the second level additionalthe granular urea decreased as compared to the second level of the acid additional to ammonium sulfate non-insignificantly.This may be due to the effect of humic acid.Urea degradation rate decreased by inhibiting the activity of the urease enzyme. This is consistent with what is indicated by Chen *et al*. (2017), which is humic acid with urea fertilizer application reduces urea degradation rate and reduces the volatilization and loss of applied nitrogen in a form of urea. The highest urease enzyme activity in this period into the rhizosphere obtained with (U + HA_1) treatment. This is because of the fact that urea is the controlled substance of the enzyme as well as the positive effect on the microorganisms that secrete the enzyme when the first level of the humic acid was applied. It increases the number and the growth of microorganisms in the root zone of plants (Finneran *et al.*, 2002).

This table shows also the effect of humic acid and nitrogen fertilizers on the activity of the urease enzyme outside the rhizosphere region. Low enzyme activity in this area compared to the rhizosphere is shown. The significant outperforming activity of the enzyme in this region than the outside of it according to all treatments. It values at (143.40 and 96.45) µg N-NH_4^+ g^{-1} soil.$2h^{-1}$, respectively. The reason for this is due to the effectiveness of this area and to increase bioactive activity, which is a positive reflection on the enzyme activity. This is consistent with Al-Taweel (2016) study through the studying the effect of fertilization type in the activity of a number of hydrolysis enzymes including urease enzyme in and out the rhizosphere of sunflower crop.

Urease enzyme activity after (60) day of planting: Results in table 4 refers to the effect humic acid and nitrogen fertilizer application on the activity the urease enzyme in and out the rhizosphere area of corn plants after (60) day of planting. The application of humic acid led to increasing urease enzyme activity in the area rhizosphere.The first level of humic acid (HA_1) treatment made more enzyme activity than the control significantly, while the second level (HA_2) made a statistical increase in the enzyme activity as compared to the control.The activity of the urease enzyme at the first and the second levels and the comparative treatment valued at (112.45, 100.00 and 83.10) µg N-NH_4^+ g^{-1} soil. $2h^{-1}$, respectively. This may be due to an increase in the number of enzyme-suppliers microorganisms in the rhizosphere area and the positive effect of humic acid on growth and root secretions. This is consistent with what Coelho *et al.* (2016) found that the application of humic acid increases growth, roots development and the respiratory activity of the roots and microorganisms.

The results of this table indicatean increase in the activity of the enzyme as a result of the application of nitrogen fertilizer, granule urea and ammonium sulfate. Granular urea treatment and ammonium sulfate treatment significantly increase the activity of the enzyme than the control. (U) treatment statistically increase urease activity than the treatment of (SA). Its activity values are (140.15 and 136.20) µg N-NH_4^+ g^{-1} soil. $2h^{-1}$ for both of the treatments, respectively. This is consistent with what Al-Salem (1997) reached to, which isenzyme activity increases urea application.

The effect of the application of humic acid and nitrogen fertilizer on the activity of the enzyme showed a significant increase in the enzyme activity with the treatment of (U + HA_1 and AS + HA_1) over the treatment of (U + HA_2 and AS + HA_2). This means that the activity of the urease enzyme with the application of the first level of humic acid additional to the fertilizers did not cause a reduction in the enzyme activity. The reason for this perhaps the pH value since the application (HA_1) did not reduce its value to limit line that may be effective in reducing the activity of the enzyme. Marzadori *et al*. (2000a) stated that humic acid works on the inhibition of the enzyme activityby changing the pH value. Inhibition of the enzyme begins when pH is less than (7.5) and the highest rate of inhibition is it (pH =6). The inhabitation of the enzyme stops with pH of more than (7.5). Vaughan and Ord (1991) study came up with that inhibition of urease enzyme activity done through changing the pH values between (4 and 7) when humic acid is applied, while the acid had no effect in reducing the activity of the enzyme at the (pH= 7.5). Treatment of (AS + HA_1 and AS + HA_2) significantly increases the enzyme activity as compared to the treatment of (U + HA_1 and U + HA_2). This may be due to the positive effect of ammonium sulfate fertilizer on the biomass in the rhizosphere region because it supplies two important elements, sulfur and nitrogen.

Results in table 4 presented the activity of the enzyme outside the rhizosphere. It is noted that enzyme activity statistically decreases in all treatments as compared to the rhizosphere except for the treatment of (AS + HA_1) the difference was significant. Enzyme activity values in and out the rhizosphere are (137.61 and 117.00) µgN-NH_4^+ g^{-1} soil. $2h^{-1}$, respectively. This is consistent with Aswathy and Jose (2013) study of rice rhizosphere area when treated with compost levels. The clarify that urease enzyme activity increase in the rhizosphere compared tothe area outside the rhizosphere.

Urease enzyme activity after (90) day of planting : The results of the statistical analysis, shown in table 5

Table 3 : Effect of humic acid and nitrogen fertilizers in urease activity µgN-NH_4^+ g^{-1} soil. $2h^{-1}$ in and out the rhizosphere region after 30 days of planting.

Treatments		Sampling area		Treatments means
Fertilizers	Treatment	In Rhizosphere	Out Rhizosphere	
Humic acid	Control	87.45	80.25	83.85
	HA_1	162.05	76.40	119.23
	HA_2	108.40	68.90	88.65
Humic acid + granular urea	U	153.45	96.15	124.80
	U + HA_1	171.35	101.75	136.55
	U + HA_2	148.15	92.55	120.35
Humic acid + ammonium sulphate	AS	139.25	85.25	112.25
	AS + HA_1	166.80	142.25	154.53
	AS + HA_2	153.70	124.60	139.15
Area mean		143.40	96.45	—
L.S.D = 0.05		Overlap	Area	Treatments
		30.28	10.09	21.41

Table 4 : Effect of humic acid and nitrogen fertilizers in urease activity µgN-$NH_4^+$$g^{-1}$ soil. $2h^{-1}$ in and out the rhizosphere region after 60 days of planting.

Treatments		Sampling area		Treatments means
Fertilizers	Treatment	In Rhizosphere	Out Rhizosphere	
Humic acid	Control	83.10	77.65	80.37
	HA_1	112.45	106.30	109.37
	HA_2	100.00	89.60	94.80
Humic acid + granular urea	U	140.15	132.20	136.17
	U + HA_1	164.25	150.50	157.52
	U + HA_2	121.40	111.10	116.25
Humic acid + ammonium sulphate	AS	136.20	121.10	128.65
	AS + HA_1	228.95	150.60	189.77
	AS + HA_2	151.70	113.95	132.82
Area mean		137.61	117.00	—
L.S.D = 0.05		Overlap	Area	Treatments
		24.07	8.02	17.02

present significant differences in the activity of urease enzyme in the rhizosphere area of maize plants after 90 days of cultivation for all of the study parameters. The result of the application of humic acid and nitrogen fertilizer relative to the treatment of the comparison that had an enzyme activity value of (48.15)µg N-$NH_4^+$$g^{-1}$ soil.$2h^{-1}$. Humic acid application treatment of (HA_1) made a statistical increase in the enzyme activity as compared to the treatment of (HA_2). Activity values of both treatments are (78.60, 96.35) µg N-NH_4^+ g^{-1} soil. $2h^{-1}$. The first level of the acid application did not reduce the activity and may affect it positively, as a result, increasing the number of soil microorganisms in the rhizosphere area. This is consistent with what was found by Zandonadi *et al.* (2007) in their studies on the yellow corn crop, which is that humic acid application increases the biological activity in the rhizosphere region. Thisleads to enzyme activitiesto be increased. The second level of the humic acid inhibited enzyme activity. This is consistent with Yan *et al.* (2013), who stated that humic acids are important components

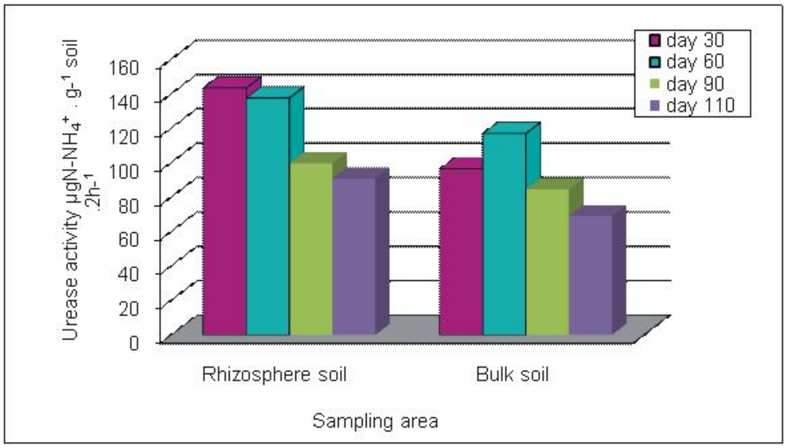

Fig. 1 : Effect of humic acid, urea and ammonium sulfate fertilizers on urease accumulative activity in and out of the rhizosphere during periods of the study.

in the soil systems and they form strong complexes with different proteins charge, which would lead to changes in the activity of enzymes through pH values. Humic acid exists in the form of charged nanoparticles in the soil solution.The reaction between soluble enzymes and soluble humic substances may be attributed to polymerization and adsorption (Polano *et al.*, 2008) and to encapsulation of enzymes in the internal structure of a humic substance (Tan *et al.*, 2008 and Tan *et al.*, 2009). The basic process involved in inhibition of the enzyme is the common polymerization of the enzymes in the humoral molecules during the moisturizing process (Burns, 1986).

Treatment of granular urea (U) statistically increases the enzyme activity over the treatment of ammonium sulfate (AS). Enzyme activity values at both treatments were (108.65 and 105.30) µg $N\text{-}NH_4^+ g^{-1}$ soil. $2h^{-1}$, respectively. The reason for this is the specialty of the urease enzyme in the decomposition of urea fertilizer and therefore its activity increases as a result as compared to the treatment of ammonium sulfate.

The decline in the enzyme activity with (HA_2) application continued with nitrogen fertilizers. The activity of the enzyme with (U + HA_1 and U + HA_2) were (138.65 and 96.35) µg $N\text{-}NH_4^+$ g^{-1} soil. $2h^{-1}$, respectively. This means that the second level of humic acid reduced the decomposition of granular urea by inhibiting the enzyme activity due to reducing pH value and controlling the hydrolysis of granular urea. This is consistent with what was Siva *et al*. (1999) and Ahmed *et al.* (2006). The activity values of the treatments of (AS + HA_1 and AS + HA_2) were (91.20, 133.90) µg $N\text{-}NH_4^+$ g^{-1} soil. $2h^{-1}$, respectively. Results showed that the activity of urease in ammonium sulfate treatment in both levels was statistically lower with acid levels compared to granular urea. The reason may the change inpH that is associated with nitrogen fertilizers decomposition. Ammonium sulfate degrading causes pHdecline more than the decline with the decomposition of granular urea. So that, the decline in the activity of the enzyme in the ammonium sulfate treatment is larger. The highest activity of the enzyme in this period was with the treatment of (U + HA_1). This previously attributed to the amount of acid and its positive effect on the biological activity of the rhizosphere area and the properties of granular urea fertilization.

The results of table 4 indicate a reduction in the activity of the enzyme in and outside of rhizosphere for all treatments as compared to the enzyme activity within the rhizosphere statistically, except for the treatment of (HA_1 and U + HA_1). This is consistent with the study of Ai *et al.* (2012) of the urease enzyme in and out therhizosphere of the wheat crop, which came up with that enzyme activity in the rhizosphere is more than outside it.This due to the increase in biogenic communities that secrete the enzyme and increase the bioactivity, which is significantly correlated with enzyme activity.

Urease enzyme activity after (110) day of planting : Results are shown in the table 6 indicates the effect of hyomic acid and nitrogen fertilizers on urease enzyme activity in the rhizosphere area and outside it of maize plants after (110) days of the planting. Humic acid application with both levels led to a significant increase in the enzyme activity into the rhizosphere area. Enzyme activity with the treatment of (HA_2) was statistically less than the treatment of (HA_1). This means the application of the second level of the acid has inhibited the activity of urease enzyme. This is consistent with the study Yan *et al.* (2013), which high concentrations of folic acid and humic acid reduce urease activity.

Granule urea treatment significantly increases the enzyme activity as compared to the control with the activity of (96.15)µg $N\text{-}NH_4^+$ g^{-1} soil. $2h^{-1}$. This is consistent with the study of JarAllah (1998) of the biological transformation of urea fertilizer, which found that the highest rate of the activity of the urease enzyme is associated with urea fertilizer compared to sulfur coated urea. Ammonium sulfate treatment significantly increases urease activity as compared to the control treatment, which valued at (86.75) µg $N\text{-}NH_4^+$ g^{-1} soil. $2h^{-1}$.This may because as a result supplying soil sulfur and nitrogen, which is positively reflected on the

Table 5 : Effect of humic acid and nitrogen fertilizers in urease activity µgN-NH_4^+ g^{-1} soil. $2h^{-1}$ in and out the rhizosphere region after (90) days of planting.

Treatments		Sampling area		Treatments means
Fertilizers	**Treatment**	**In Rhizosphere**	**Out Rhizosphere**	
Humic acid	Control	48.15	39.60	43.87
	HA_1	96.35	74.20	85.27
	HA_2	78.60	69.45	74.02
Humic acid + granular urea	U	108.15	101.75	104.95
	U + HA_1	138.65	103.50	121.07
	U + HA_2	96.35	77.15	86.75
Humic acid + ammonium sulphate	AS	105.30	100.95	103.12
	AS + HA_1	133.90	120.25	128.07
	AS + HA_2	91.20	75.60	82.90
Area mean		99.62	84.60	—–
L.S.D = 0.05		Overlap	Area	Treatments
		16.62	5.54	11.54

Table 6 : Effect of humic acid and nitrogen fertilizers in urease activity ìgN-NH_4^+ g^{-1} soil. $2h^{-1}$ in and out the rhizosphere region after (110) days of planting.

Treatments		Sampling area		Treatments means
Fertilizers	**Treatment**	**In Rhizosphere**	**Out Rhizosphere**	
Humic acid	Control	51.15	36.00	43.57
	HA_1	85.80	57.65	71.72
	HA_2	80.50	61.55	71.02
Humic acid + granular urea	U	96.15	67.00	81.57
	U + HA_1	111.45	76.10	93.77
	U + HA_2	104.65	87.60	96.12
Humic acid + ammonium sulphate	AS	86.75	69.85	78.30
	AS + HA_1	105.35	75.25	90.30
	AS + HA_2	95.40	90.50	92.95
Area mean		90.80	96.5	—–
L.S.D = 0.05		Overlap	Area	Treatments
		18.05	6.01	12.76

number of microorganisms in the area of the rhizosphere as well as to the positive effect ammonium sulfate application on the growth of plants and increase their root secretions.

The effect of the interaction between hemic acid and nitrogen fertilizer of (U + HA1), increased urease activity more than treatment of (U + HA_2) statistically. The enzyme activity with these treatments were (111.45 And 104.65) µg N-NH_4^+ g^{-1} soil. $2h^{-1}$, respectively. The impairment activity with(U + HA_2) treatment indicates a reduction in the rate of urea degradation due to the increased level of acid that reducesthe enzyme activity. This is consistent Dong *et al.* (2008) study, which concluded that the application of humic acid reduced urease enzyme and thus reducesurea decomposition and increase the efficiency of applied nitrogen fertilizer. Enzyme activity with the treatment of (AS + HA 2) declined as compared to the treatment of (AS + HA 1). These treatments values were (95.40 and 105.35) µg N-NH_4^+ g^{-1} soil. $2h^{-1}$, respectively.

The results of the table 6 indicate urea treatments and their overlap with the acid were significantly higher in enzyme activity than those of ammonium sulfate with acid. The results of the table and the figure indicate a reduction in urease activity in bulk soil as compared to the rhizosphere soil in all treatments. Enzyme activity average in bulk soil and the rhizosphere soil (69.05 and 90.80)µg $N\text{-}NH_4^+$ g^{-1} soil. $2h^{-1}$, respectively. This clearly significant decline may attribute to the vital activities that are higher closer to plant roots and to root secretions, including enzymes, that released into the soil.This is consistent with what he says Bais *et al.* (2003) that the presence of plant roots in the soil increases various biomedical communities accompanied by secretion increase of enzymes in the area of the rhizosphere compared to bulk soil.

Aggregative values of urea enzyme activity at study intervals

Fig. 1 refers to the effect of humic acid, granular urea and ammonium sulfate fertilizers on the collective urease enzyme activity for all study periods in and outside the rhizosphere regions. It is clear that urease activity the rhizosphere is more than in the bulk soil all periods of study. The reason may belong to plant root secretions that contain amino acids, sugars and organic acids and enzymes additional to the vital activity is high in the rhizosphere area. Al-Taweel and Rashidi (2016) mentioned that enzymes activity in the rhizosphere area due to plant root secreted enzymes and enzymes that are secreted by biogenic population in this area. Aggregate values of the urease activity in the four periods in this regain respectively are (143.40, 137.61, 99.62 and 90.80) µg $N\text{-}NH_4^+$ g^{-1} soil. $2h^{-1}$. Maximum enzyme activity was in the first period (after 30 days of planting) and then continuously declined. This is consistent with what Tamimi (1999) study of in the study of the activity of urease enzyme in the rhizosphere of the yellow corn, which had fife periods. The heist activity was in the first one and then declined until the last period.

Urease enzyme activity outside the rhizosphere is less than its activity in it. In bulk soil, its activity values were (96.45, 117.00, 84.60 and 69.05) µg $N\text{-}NH_4^+$ g^{-1} soil.$2h^{-1}$, respectively. The highest aggregate value of the enzyme there was in the second period. This because of the accumulation of nitrogen outside the rhizosphere area a result of fertilizers application. When controlled substance available, the enzyme is stimulated and the action begins to decrease after then. Enzyme activity gradually decreases in the third and fourth period due to substance diminishing.

In brief, humic acid of (40 kg. h^{-1}) did not inhibit enzyme activity, while treatment level of inhibited it either just by itself or with nitrogenous fertilizers. Urease enzyme activity increased in the rhizosphere soil and decreased outside it with all treatments. Maximum enzyme activity reached the highest values in the first period of the study then began to decline gradually. Therefore, humic acid application at (80 kg. h^{-1}) is recommended to have the best results of urease activity inhibition and reducing nitrogenous fertilizers degradation.

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