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Effect Different Extracts of Tribulus Terrestris on ammonia Volatilization in Loamy Soil

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Abstract--- *The study was tested by studying effect of three different plants extracts on ammonia volatilization and number of bacteria after addition extracts to soil with urea in lraqi loamy soil. Three plants extracts of (Olea europaea, lepidium aucheri, Tribulus terrestris) were tested on many isolated bacteria from soils (Bacillus cereus, Bacillus spp, Proteus mirabilis, Proteus vulgaris, Klebsiella pneumoniae, pseudomonas aeruginosa, E.coli Staphylococcus aureus). All bacteria were classificatedon (Schwartzet al, 1965) and biochemistry test to different this bacteria. The results explained that all parts of Tribulus terrestris extracts caused decreasing number of isolated bacteria from soil after addition urea and extracts to loamy soil and were reduction ammonia volatilization from soil but other plants extracts Olea europaeaand lepidium aucheri didn't cause any reduction with number of isolated bacteria and ammonia volatile after addition to soil. So this reason was exclude this extracts from using to test against ammonia volatilization but all parts of Tribulus terrestris gave a significantly difference activity of reduction ammonia volatile (p α 0.05) from soil compared with control. Seeds with alcoholic extract was the best treatments at reduction number of bacteria and volatile ammonia. Increasing concentration of extracts caused a significantly reduction (p α 0.05) with ammonia volatilization and number of bacteria.*

Keywords--- *Tribulus Terrestris, Ammonia Volatilization, Reduction.*

I. INTRODUCTION

Urea is one of organic Nitrogen fertilizers and has (46%of Nitrogen), it is the most distribution in the world among others nitrogen fertilizers, it is easy manufacture and cheap (Power and Prasad, 1997). Calcareous soils causes hydrolysis urea (Ali and Stroehlen, 1991) after addition to soils and forms ammonia gas and CO_2 after rasing Ph value and losing NH_3 gas from fertilizer unit (Bremner, 1995). Urease enzyme causes hydrolysis urea to ammonia and CO_2 and comes to soil from plants roots, and some microorganism like *Klebsiella Bacillus, Helycobacter pyloria, Proteus* and *pseudomonas.* Urease enzyme caused ammonia volatile after doing on urea substrate. In order to reducing ammonia volatilization ,There are many strategies to reducing ammonia volatile coated urea with sulpher ,using polymers (Nasima*et al*.,2010) changing time of planting, method of fertilizer application (Neghamish, 2013), using chemical inhibitors and using plants extracts and more softy to plant, soil, environmental system and reducing ammonia volatile but the chemical fertilizers cause many dangers to plant, soil and environmental system (Macadam et *al*.,2003; Frye, 2005). Some of plants extracts are decreasing ammonia volatilization from soil after addition Urea to soils (Ghosh *et al*.,2002; Neghamish and kareem, 2017). *Tribulus terrestres* is one of plants that causes reduction ammonia volatilization and reduction number of bacteria in soil after addition urea because it has many Active secondary compounds Alkaloids, flavonoids Tannic acid and saponins

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(Qasem*et al*., 2013; Mesir, 2013). The aim of research is studying effect different parts of plants extracts on reduction numbers of bacteria in soil and decreasing ammonia volatilization after addition.

II. METHODS AND MATERIALS

2.1 Prepartion plants extracts

The parts of different plants was tacked to extract by (1:10) (plant: water or alcohol). The samples of plants were dried on air and grinded and placed 10 gm of plants weight in flask size 250 ml and added 100 ml of distilled water and mixture together and shacked with mechanical shaker for 6 hours with speed 160 C/min, the mixture of plants with water or alcohol shacked directly until finishing the time of mixture, the solution was Isolated by some parts of clothes and passed on filter paper size (Whatman No .1), all parts of different plants were collected to be stock`s solution and kept in refrigerator until to use in the study.

2.2 Ammonia volatilization

Ammonia volatile was measured by Volk in 1959,method with using 20 ml of (boric acid 2%) to catch ammonia volatile and added to medication mixed with boric acid (bromo cresol green and methyl red)and was measured ammonia volatile after solving with boric acid and titration with 0.02N HCL to solution, the NH_3/area recorded according to equation.

N x Vx 14=mg NH_3/area

N: normality of HCL

V: volume of HCL

2.3 chemical and physical properties of soil

*Degree of soil reaction was assayed by using (Ph –meter) model-Hanna-Ph21. (Black, 1965).

*Electricity conductivity (EC) was determined by conductivity meter for soil (1:1), (water:soil), (Black, 1965). Table (1).

*Calcium and magnesium were done by using 0.01N EDTA according to (Page *et al*., 1982).

*Sodium and potassium were determined by using flame photometer (Page *et al*., 1982)

*Soil texture was measured by using hydrometer according to (Page *et al*., 1982),

*NH_4 was extracted by 2N HCL and using micro kjeldhal afterextraction and titration with 0.02N H_2SO_4.

*NO_3 was extracted by 2N KCL and treated by titration with sulphoric acid after using micro Kjeldhal system (Black, 1965)

*Organic matter was estimated according to walkley and black (black, 1965) after oxidation with potassium dichromate and with concentrated H_2SO_4 and calculated by multiplying organic Carbon factor (1.724).

*SO_4 was estimated according to (Black, 1965) after addition $BaCl_2$ to the extract of soil.

* Field capacity was determined with weighting methods to soil according to (Black, 1965).

* Carbonate and bicarbonate were determined by titration with naphthalene indicator and titration with acid 0.01N H_2SO_4 (Black, 1965).

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Table 1: Physical and chemical properties of soil.

Parameters	Value	Unit
Ph	8.21	-
ECe	1.59	$Ds.m^{-}$
Field capacity	28	%
Organic matter	1.23	%
Cations:		
Ca^{++}	5.50	$Meq.L^{--}$
Mg^{++}	3.50	$Meq.L^{--}$
Na^{+}	2.45	$Meq.L^{--}$
K^{+}	2.80	$Meq.L^{--}$
$NH4^{+}$	1.2	$Meq.L^{--}$
Anions:		
SO_4^{-2}	4.0	$Meq.L^{--}$
NO^{-}_3	2.1	$Meq.L^{--}$
HCO_3^{-}	2.0	$Meq.L^{--}$
CO^{-}_3	0.0	$Meq.L^{--}$
CL^{-}	6.0	$Meq.L^{--}$
Clay	20	%
Sand	45	%
Silt	35	%
Soil texture	Loam	-

III. RESULTS AND DISCUSSION

Table (2) explained effect some different plant extracts and kinds of isolated bacteria after addition plant extracts to soil, The result in the table (2) recorded effect three plants extracts of *(Olea europaea, Lepidium aucheri Bosis and Tribulus terrestris*) tested against *(Bacillus spp, Bacillus cereus, Staph. Aureus, Pseudomonas aeruginosa, klebsiella pneumonia*e, *E.coli, Proteus mirabilis and Proteus vulgaris.* The results explained these isolated bacteria did not inhibition by *Lepidium aucheri extract and Olea europaea* extract but all extracts of *Tribulus terrestris* inhibited all bacteria in this study and with all parts of plants.

Table 2: Explain type of different Plants extracts on inhibition many isolated bacteria from soil

Kinds of Bacteria	*Type of Plants extracts*		
	Lepidium aucheri	*Olea europaea*	*Tribulus terrestris*
Bacillus spp	-	-	+
Bacillus cereus	-	-	+
Proteus mirabilis	-	-	+
Proteus vulgaris	-	-	+
pneumonae Klebsiella	-	-	+
Pseudomonas aeruginosa	-	-	+
E. coli	-	-	+
Staphylococcus aureus	-	-	+

(+) Inhibition**(-)**No Inhibition

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Tribulus terrestris extracts inhibited all isolated bacteria in the studytable (2) and reduced the number of bacteria in the soil table (3) and reduced ammonia volatilization from soil table (4) because of *Tribulus terrestris* extract had many active secondary compounds in the plant like (Alkaloids, Streoids, Saponins, Flavonoids, Diogenins and Lignanamid) (Mesir, 2013), or returend to Alkaloids activity on bacteria cell and linked with amino acid (Bourke *et al*., 1992) or *Tribulus terrestris* extract contained tannic acid and lipids acid were doing antibiotic against bacteria (Lee*et al*., 1994; Al-Ali *et al* 2003;Mohammed, 2008). *Tribulus terrestris* extract have steroidal saponins (protodioscin, Terrestrinin) doing against microbes and have high toxicity (Bedir *et al*., 2002;Bedirand Khan,2000;Ibrahimand Enas, 2015). There are three type of plant extracts (*Olea europaea* , *lepidium aucheri* and *Tribulus terrestris* were testing against isolated bacteria from soils that *Bacillus. spp, Bacillus cereus, Staph. aureus, Pseudomonas aeruginosa, klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris and E. coli*). The results in table (2) explained that All parts of different plant extracts of *Tribulus terrestris* caused inhibition to all bacteria in the study but more bacteria did not inhibition by extracts of *lepidium aucheri* and *Olea europaea*, this result recorded in table (2) *Olea europaea* and *lepidium aucheri* didn't inhibition isolated bacteria in study and didn't reduce ammonia volatile a significantly from incubated soil after addition urea. The study was tested activity of *Tribulus terrestris* with difference extracts on number of bacteria after addition to incubated soil and caused decreasing with number of isolated bacteria compared with control treatment and others extracts with others plants (*Olea europaea* and *lepidium aucheri*). On other side, the difference *Tribulus terrestris* extracts caused a significantly difference at decreasing ammonia volatile compared with other Plant extracts and controltreatment during 20 days from incubation soil (P α 0.05).

Table 3: Effect of different of concentration of Tribulus terrestris extracts against number of bacteria.

Treatment	0.1ml / gmsoil	0.2ml ext./gmsoil	Mean
Control (without extracts)	100800	103200	102000A
Lepidium aucheri (hot water)	120000	70000	95000B
Olea europaea (hot water)	105000	100000	102500A
Leaves and seed with hot water	51200	36000	43600E
Extract seeds with alcohol	18000	14800	16400G
Stem extract cold water	50000	40000	45000E
Leaves and seeds extract with water and alcohol	77600	60000	68800C
Leaves and seeds alcoholic extract	57200	67000	62400D
Stem (hot water)	42000	32000	37700F
Mean	64600A	52950B	

R.L.S D (I) = 3217.8 , R.I. S. D = 1609, R.I.S. D $_{txc}$ =4551.6

t 0.05 0.05(C) 0.05

Table (3) explained effect of two concentration 0.1, 0.2 ml/gm soil and types of differents extracts from different plants on numbers of bacteria in loamy soil, the results recorded that all different parts of *Trib. terrestris* with different solvents caused a significantly reduction in probability level (P α 0.05) on numbers of bacteria compared

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with other extract from other plants like, *Olea europaea* and *Lepidium aucheri* on numbers of bacteria and compared with control treatment. The mean numbers of bacteria in loamy soil were 102000, 102500 and 95000 cfu/gm soil) to control treatment, *Olea europaea* and *Lepidium aucheri* respectively, but the mean numbers of bacteria to all parts of *Tribulus terrestris* and all types of extracts were (16400, 43600, 45000, 68800, 62400 and 37.700 cfu/gm.soil to Alcoholic seeds, leave and seed (hot water), stem (cold water), leaves and seeds (water and Alcohol), leaves and seeds (Alcoholic extract) and stem (hot water) respectively, and the reduction with mean of numbers of bacteria from soil after addition extract a significantly difference with (P α 0.05) compared with other extract and at control treatment. Increasing concentrations of all extract to all different plants were caused a significantly difference (P α 0.05) compared with control at numbers of bacteria. Increasing concentration from 0.1 ml/g soil to 0.2ml/ g soil was produced a significantly difference (P α 0.05) between the two concentration and the mean numbers to concentration 0.2 m/ g soil reached 52950cfu/ gm soil after was 64000 cfu/ gm soil to concentration 0.1ml/ g soil. Increasing ability inhibition of extract by increasing concentration returned to increasing accumulation matrial out cell and effect on cell members and transport enzyme activity (Mesir, 2013). This results are similar with some researchers (Edmunds, 1960). Increasing concentrations caused reducing mean of numbers of microbes in soil after addition *Tribulus terrestris* extracts to soil, the reason returned to increasing active compounds in the extracts and do on member of cell or linked with RNA of proteins and were more toxicity on microbes (Namba, 1988; Bourke *et al*., 1992; Lee*et al*., 1994). This result is compatible with (Mesir, 2013) and increase concentration of extracts caused toxicity on microbes in the soil.All part of plant extracts of *Tribulus terrestris* were decreasing a significant ammonia volatile and number of bacteria in loamy soil compared with control treatment and other plant extract of *Olea europaea* and *lepidium aucheri* the reasons of this decreasing returned to active secondary compounds in the plant do against negative and positive gram bacteria like (flavonoid, Al kaloids, saponins and Tannic acid) this results are according with results of many researches (Mitscher*et al*., 1999; Mohammed, 2008; Mesir, 2013 ; Neghamish and Kareem, 2017). Increasing ability inhibition of extract by increasing concentration returned to increasing accumulation matrial out cell and effect on cell members and transport enzyme activity (Edmunds, 1960) this results are similar with some researches (Mesir, 2013). Or the reason of effect different plant extract on activity number of bacteria and ammonia volatile returned to different type of plant parts or type of extract, and method of extraction(Mesir, 2013; Qasem *et al*., 2013). The different of ability between plants extracts back to types of solvent and it`s polar ability to solve active compounds of parts of plants (Kelmanson *et al*., 2000). Effect of plant extracts on bacteria differs because of thickness cell wall and it`s containing lipids and protein or effect extracts on natural and type of structure bacteria or effect on activity of compounds in parts and its effect on structure of bacteria or on number of bacteria in soil. The results explained that alcoholic extract is more effect than other extracts (hot water and cold water) because of having more active secondary compounds so heat with the alcoholic extract gave more activity compared with others extracts to the some plant. This results is compatible with (Mesir, 2013; Neghamish and Kareem, 2017). Table (4) explained effect of different extracts of *Tribulus terrestris*, *Lepidium aucheri* and *Olea europaea* on ammonia volatile after addition urea to incubated soil on 28 ^{o}C. The results showed that all extracts of *Tribulus terrestris* caused reduction in an ammonia volatile from soil but the others extracts of others plants (*Lepidium aucheri* and *Olea europaea*) did not reduce ammonia volatile from incubated soil after 20 days, some of

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extracts for parts of *Trib.terrestris* with different types of extract caused a significantly difference among them, the mean of ammonia volatile to seed and leaves to the extract of *Tribulus terrestris* changed according to kinds of extract and were 50 to hot water, 41.07 to cold water, 50.23 to water and alcohol and 35.38 to alcohol extract but alcohol extract for seed *Tribulus terrestris* 31.833mg NH_3/100gm soil after 20 days. All extracts of *Tribulus terrestris* caused a significantly difference decreasing ammonia volatile from the soil compared with control treatment and with other plants extracts (*lepidium aucheri* and *Olea europaea*). And were 61, 59.92 and 70.65 mg NH_3/100gm soil respectively. The result in the table (4) explained all *Tribulus terrestris* extracts decreased amounts of ammonia volatile a significantly (P α 0.05), while *lepidium aucheri* extract did not reduction a significantly ammonia volatile from incubated soil but *Olea europaea* extract (with hot water) caused increasing to ammonia volatile in this study all extracts that caused reduction ammonia volatile from the soil after addition. The best treatments at redusing ammonia volatile were alcohol extract of seeds and leaves of *Tribul. terrestris* and alcohol extract of seed of *Tribul. terrestris* and were 35.38 and 31.833 mg NH_3/100gm soil after 20 days respectively.

Table 4: Explained effecting different extracts of plants on volatilization of ammonia from incubated soil for 20 days

Treatment	Kinds of extract	Amounts of NH_3 mg/ 100gmsoil after20 days
Control with urea	------	61.00 B
Lepidium aucheri Bosis	Hot water	59.92 B
Olea europaea	Hot water	70.65 A
Tribulus terrestris seed and leaves	Hot water	50.53 C
Tribulus terrestris seed and leaves	Cold water	41.07 D
Tribulus terrestris seed and leaves	Water and alcohol	50.23 C
Tribulus terrestris seed and leaves	Alcohol	35.38 DE
Tribulus terrestris seed	alcohol	31.833 E

The reduction of ammonia volatile by extracts of *Tribul. terrestris* returned to *Tribul. terrestris* extracts had many secondary compounds like (kaloids, flavonoid, saponins and Tannic) this results are according with results of (Mohammed, 2008; Mesir, 2013). The secondary compounds inhibited all bacteria in study and inhibited urease enzyme on urea and prevent ammonia volatilization from incubated soil because of stopping urea hydrolysis in the soil.

IV. CONCLUSION

All parts of *Tribulus terrestris* effected on number of bacteria in soil and inhibition all bacteria in the study and was reducing ammonia volatile from fertilizer soil with urea. Compared with other different plants extracts. All parts of *Tribul. terrestris* and with different methods of extract were very active against all bacteria in the study. The seed extract of *Tribul. terrestris* by alcohol was the best treatment at decreasing ammonia volatile from incubated soil after 20 days.Increasing the size of the inoculations caused increasing reduction ammonia volatilization and decreasing number of bacteria in soil after addition to soil.

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