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Some plant extracts retarde nitrification in soil

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ABSTRACT

An incubation experiment was conducted to evaluate the effect of aqueous extracts of 17 plant materials on nitrification inhibition of urea- N in soil as compared with chemical inhibitor Dicyandiamide (DCD). Plant materials used in study were collected from different areas of Basrah province, south of Iraq. Aqueous extracts were prepared at ratio of 1:10 (plant material:water) and added at conc. of 0.05, 0.10 and 0.20 ml g^{-1} soil to loamy sand soil. DCD was added to soil at rate of 50 µg g^{-1} soil . Soil received urea at rate of 1000 µg N g^{-1} soil. Treated soils were incubated at 30^{O}C for 40days. Results showed that application of all plant extracts, except those of casuarina, date palm and eucalyptus to soil retarded nitrification in soil. Caper, Sowthistle ,bladygrass and pomegranate extracts showed highest inhibition percentage (51,42,40 and 40% ,respectively) and were found to be more effective than DCD (33%). Highest inhibition was achieved by using those extracts at conc. of 0.1 ml g^{-1} soil after 10 days of incubation . Data also revealed that treated soil with these plant extracts significantly increased amount of NH_4^+–N and decreased amount of NO_3^-–N accumulation in soil compared with DCD and control treatments. Results of the study suggested a possibility of using aqueous extracts of some studied plants as potent nitrification inhibitor in soil.

Key words: nitrification inhibitor, plant extract , inorganic nitrogen

IZVLEČEK

NEKATERI RASTLINSKI IZVLEČKI UPOČASNJUJEJO NITRIFIKACIJO V TLEH

V inkubacijskem poskusu je bil ovrednoten vpliv 17 vodnih rastlinskih izvlečkov na inhibicijo nitrifikacije dušika v urei primerjalno s kemijskim inhibitorjem diciandiamidom (DCD). Uporabljen rastlinski material je bil nabran na različnih območjih province Basrah v južnem delu Iraka. Vodni izvlečki so bili pripravljeni v razmerju 1:10 (rastlinski material:voda) in dodani v koncentracijah 0.05, 0.10 in 0.20 ml g^{-1} ilovnato-peščenim tlem. DCD je bil dodan tlem v razmerju 50 µg g^{-1} tal, urea pa v razmerju 1000 µg N g^{-1} tal. Tretirana tla so bila inkubirana pri 30^{O} C 40 dni. Rezultati so pokazali, da je uporaba rastlinskih izvlečkov upočasnila nitrifikacijo v tleh, razen pri izvlečkih kazaurine, dateljeve palme in evkalipta. Izvlečki kaprovca, škrbinke, trave (*Imperata cylindrica* (L.) P.Beauv) in granatnega jabolka so se izkazali za bolj učinkovite inhibitorje (51, 42, 40 in 40 % inhibicija) kot DCD (33 %). Največja inhibicija je bila dosežena z uporabo izvlečkov v koncentraciji 0.1 ml g^{-1} tal, pri inkubaciji 10 dni. Rezultati so tudi pokazali, da se je v tleh obravnavanih s temi izvlečki značilno povečala količina NH_4^+–N in zmanjšala količina NO_3^-–N v primerjavi s tlemi, obravnavanimi z DCD in kontrolo. Rezultati te raziskave nakazujejo možnost uporabe izvlečkov preučevanih rastlin kot potencialnih inhibitorjev nitrifikacije v tleh.

Ključne besede: inhibitorji nitrifikacije, rastlinski izvlečki, anorganski dušik

1 INTRODUCTION

Prilled urea is the main source of N fertilizer applied to soil. In tropical agriculture, it accounts for about 49% of total fertilizer N use (Byrnes and Freney , 1995). Urea applied to soil, is hydrolyzed by urease enzyme to form NH_4^+ which is subsequently converted to nitrate (NO_3^-)through nitrification process (Kiran and Patra,2003) . The NO_3^- is subject to losses either through percolation of soil water or as nitrogen gases or nitrogen oxides through denitrification process (Mikkelsen et.al.,1978;Katyal et al.,1985). Excessive loss of N due to NO_3^- leaching or loss through

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denitrification in addition to other ways of N losses from soil environment results in very poor recovery of applied nitrogen (Yadav and Mohan,1982). To increase nitrogen fertilizer use efficiency, several approaches have been tried. These include: use of slow release fertilizers (Malhi et al., 2003), addition of salts and acids with urea (Sloan and Anderson , 1995) and use super granules urea (Shah and Wolfe,2003). In addition to that, several chemicals such as N–serve(nitrapyrin), dicyandiamide(DCD) and many other chemicals have been applied to retard urea hydrolysis or nitrification in soil (Kiran and Patra,2003) . In spite of the encouraging results obtained with the use of these chemicals in retarding urea hydrolysis and nitrification their use is limited to experimental one due to high cost, and risk of adverse effect on beneficial soil micro flora (Vyas et al.,1993) and risk of soil and water pollution(Kiran and Patra,2003). Erickson et al. (2000) reported that plant in mature stages produce numerous organic compounds that can inhibit autotrophic nitrifiying organisms, even at low concentration in soils. Other workers reported experimental evidence for roles of root exudates and leachates of plants under climate vegetation inhibit nitrification in soil (Paavolainen et al., 1998; Jafari and Kholdebarin,2002). On the other hand, Purchase(1974)and Johnson and Edwards(1979)found no evidence of nitrification inhibition from root exudates or variety of plant extracts. Literatures reviewed above showed inconsistent results of the effect of plants extract on nitrification in soil. Hence, a comprehensive study was conducted to investigate effect of aqueous extracts of 17 natural plant materials on urea N transformations in soil as compared with the synthetic chemical nitrification inhibitors (i. e. DCD). The purpose of this paper is to report effect of these extracts on nitrification of urea – N.

2 MATERIALS AND METHODS

Soil and plant materials :

Soil used in the experiment was loamy sand collected from tomato field, located at AL-Burjsia area, Basrah province, south of Iraq. The soil classified as Entisol; Typic Torripsamment. Soil samples were collected from surface layers (0–30cm), air dried and sieved (2 mm). Some physical and chemical properties of the soil were determined following procedures described in Page et al. (1982) and presented in table (1).

Plant materials used in study were collected from different areas from Basrah province and described in in table (2). Selected plant materials were cleaned, air dried and grounded to pass 1mm sieve then kept in plastic bags at room temperature (25°C) and humidity (35%) until use. To get aqueous extract, 10 g of ground dry material was mixed with 100 ml of distilled water and horizontal shake for six hours. The homogenate was filtered through tissue paper to separate large particles, and then the filtrate was filtered further using Whatman filter paper No. 1. This process was repeated several times to collect enough quantity of extract. The filtrate was used as stock solution.

Table 1. Some physical , chemical and biological properties of soil used.

Prop.	Symbol		Value
pH (1:1 in water)	———		8.05
E. C.	dS m^{-1}		2.30
$CaCO_3$	g kg^{-1}		75.00
CEC	Cmole ($^{+}$) kg^{-1}		3.40
P (NaHCO3)	mg kg^{-1}		5.60
Total N	g kg^{-1}		0.03
Organic C	g kg^{-1}		0.40
Organic matter	g kg^{-1}		0.70
C:N Ratio	———		13.3
Urease activity	µg NH_4^+/g Soil/2h		2.3
NH_4^+ – N	µg g^{-1}		1.57
NO_3^- – N			0.61
NO_2^- – N			0.00
Ca^{+2}	m *M* L^{-1}		5.40
Mg^{+2}			3.00
Na^+			6.50
K^+			1.02
HCO_3^-			2.00
$SO_4^=$			8.50
Cl^-			7.00
$CO_3^=$			0.00
Loamy Sand	Sand	g kg^{-1}	866.00
	Silt		51.96
	Clay		82.04

Table 2. : Plants used in study

Common name	Latine name	Sampling part	Sampling date
Zizyphus	*Ziziphus mauritiano* Lam. CV. Zaitoni	leaves	Oct.
	Ziziphus spina – christi (L.) Willd.	leaves	Oct.
Colacynth	*Citrullus colocynthis* (L.) Schrod.	Fruits	Nov.
Caper	*Capparis spinosa* L.	Seeds	Oct.
Casuarina	*Casuarina equisetifolia* L.	Stem bark	Jan.
Bead tree	*Melia azedarach* L.	Fruits	Oct.
Pomegranate	*Punica granatum* L.	Peels	Jan.
Cotton	*Gossypium herbaceum* L.	roots	Jan.
Bermudagrass	*Cynodon dactylon* (L.) Pers.	rhizomes	Feb.
Bladygrass	*Imperata cylindrica* (L.) Beauv.	rhizomes	Feb.
Sowthistle	*Sonchus oleraceus* L.	Total shoot	Mar.
Wheat	*Triticum aestivum* L.	bran	Jul.
Date palm	*Phoenix dactylifera* L.CV. Zehdi	Leaves Fiber	Dec. Feb.
Oleander	*Nerium oleander* L.	leaves	Apr.
Eucalyptus	*Eucalyptus camaldulensis* Dehnh.	leaves	Apr
Myrtus	*Myrtus communis* L.	leaves	Apr.

Nitrification inhibition :

Sample of sieved soil was washed with enough 0.01 *N* KCl to leach out the inorganic forms of nitrogen present in soil. Leached soil was air dried, then 5 g of dried soil was placed in plastic containers (capacity 20ml). Soils in plastic containers were treated with solutions contain urea(at rate of 1000 µg N g^{-1} soil) and test solutions (at rates of 0.05, 0.10 and 0.20 ml gm^{-1}soil) . To compare the effect of plant extracts on nitrification on soil with synthetic chemical inhibitor (i. e. dicyandiamide, DCD), set of containers was treated with solution contain 250 µg of DCD (50 µg DCD gm^{-1} soil) and urea at rate of 1000 µg N g^{-1} soil.

Soil of control treatments was treated with solution contains only urea at the same rate as that of other treatments. The moisture content of all treatment was maintained at field capacity during the study period. Treatments were triplicated and incubated at 30°C. Set of samples was withdrawn at 10 days and other at 40 days after amendment of extracts and urea . Soils were extracted with 2 *M* KCl , then the extracted amount of NH_4^+ , NO_2^- and NO_3^- were determined following procedure of Bremner and Edwards(1965).Percentage inhibition of nitrification was calculated according to Bremner and McCarty (1988) :

$$\% \text{ inhibition} = \frac{C - T}{C} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

$T = NO_2^- + NO_3^-$ in treated soil
$C = NO_2^- + NO_3^-$ in control soil

E.C. and pH determination

To reveal the effect of plant extracts on soil electrical conductivity(E.C.) and acidity (pH), fifty grams of soils amendment with plant extracts(that showed most effect on nitrification) and urea at rate of 1000 µ N g^{-1} were placed in plastic containers, then incubated at 30° c for 40 days. Soil moisture was adjusted to field capacity during incubation periods. Set of samples was withdrawn after 2,4,8,10,25,and 40 days after incubation and soil E.C. and PH were determined.

Statistical analysis :

The experiment was designed as factorial experiment with three variables (plant extract × extract concentration × incubation period) with three replicates. The results were analyzed using analysis of variance carried out by $SPSS_{11}$ (Agyrous,2005). Differences among means were compared using revised LSD test.

3 RESULTS

Nitrification inhibition :

Data in table (3) show that application of all plant extracts, except those of casuarina, date palm and eucalyptus, to soil reduced nitrification of urea – N during incubation periods of 10 and 40 days. However, the persistence of the inhibitory effect of these extracts on nitrification decreasing with increasing incubation time from 10 to 40 days. Data indicated that degree of nitrification inhibition in soil differs with source and concentration of the extracts used . Retardation of nitrification caused by extracts of caper, sowthistle, bladygrass and pomegranate were higher than that of DCD treatment. The highest retardation was achieved by using extracts of the plants at concentration of 0.1 ml g^{-1} after 10 days of incubation. The inhibition percentages were 51,40 ,40 and 42% for caper, pomegranate, bladygrass and sowthistle, respectively as compared to 33% for DCD treatment . Statistical analysis of treatments is shown in table (4).

Inhibition effects of other plant extracts were either lower (Zizyphus, bermudagrass and oleander) or did not significantly differ (sowthistle, colacynth, bladygrass and bead tree) from that of DCD treatment.

Table 3: (%) inhibition of nitrification in soil treated with different concentrations of plant aqueous extracts after 10 and 40 days of incubation.

Conc. (ml gm $^{-1}$ soil) / Plant	After 10 days of incubation				After 40 days of incubation			
	0.05	**0.10**	**0.20**	**mean**	**0.05**	**0.10**	**0.20**	**Mean**
Zizyphus (CV. Zaitoni)	0	15	30	15	0	0	18	6
Zizyphus (Willd)	0	39	38	25.6	0	10	15	5
Colacynth	26	34	34	31.3	21	16	5	14
Caper	46	51	46	47.6	5	21	16	14.00
Casuarina	0	0	10	3.3	5	0	5	3.3
Bead tree	36	31	32	33	26	16	5	15.6
Pomegranate	34	40	14	29.3	26	32	32	30.0
Cotton	0	36	38	24.6	0	16	5	7
Bermudagrass	39	24	31	31.3	6	16	21	17.6
Bladygrass	39	40	40	39.6	0	10	16	8.6
Sowthistle	40	42	36	39.3	0	5	0	1.6
Wheat	0	37	30	22.3	0	0	0	0
Date palm (leaves)	0	35	29	21.3	0	0	0	0
Date palm (fiber)	0	0	0	0	0	0	0	0
Oleander	0	20	28	16.0	5	10	5	6.6
Eucalyptus	0	6	0	2	0	0	5	1.6
Myrtus	36	35	33	34.6	5	0	5	3.3
DCD			33	33	10	10	10	10
Mean	18.2	28.7	27.8	24.9	6.6	9.0	9.05	8.21

L.S.D. 0.01

plant extracts(p) =2.81, Conc. of extract(c) =1.14, incubation time (t) =*

p×c = 4.87, p×t = 3.98, c×t = 1.62, p×t×c = 6.89

C.V = 9.25

Inorganic N accumulation :

Table (4) shows the amount of inorganic N (NO_2^-, NO_3^- and NH_4^+) accumulated in soils treated with DCD or aqueous extracts of caper, sowthistle, bladygrass and pomegranate after 10 and 40 days of incubation as compare to control treatment. Data in the table indicated that treated soils with these plant extracts significantly increased amount of NH_4^+- N accumulated in soils comparing with that of DCD. Caper, sowthistle, bladygrass and pomegranate maintained 220.97, 207.57, 193.84 and 212.14 mg NH_4^+- N kg $^{-1}$ soil as compared with 182.14 mg NH_4^+-N kg $^{-1}$ soil at DCD treatment after 10 days of incubation. The amount of $NH4^+$-N accumulated in control soil at that time was 149.73 mg NH_4^+ - N kg $^{-1}$ soil. However, the amount of NO_3^- – N produced in soils treated with these plant extracts was lower than these of DCD or control treatments. Plant extracts or DCD effects on NO_3^- – N produced was much lower at 40 days than 10 days of incubation . No NO_2^- – N was detected at any of the treatments involved in the study.

E.C. and pH :

The effect of caper , sowthistle , bladygrass and pomegranate extracts on soil E.C. and pH as compared with control are presented in (Fig. 1 and

Fig.2). Fig. (1) shows that plant extracts increased E. C. of treated soils from 2dSm^{-1} at control treatment to about 4dSm^{-1} at early period of incubation. However, effect of all plant extracts on E.C. decreased as time of incubation increased. Fig. (2) shows that soil pH of all treatments were close to that of control treatment during the incubation period (40 days) and was in the range of 7.9 to 8.3.

Table 4: NH_4^+, NO_2^- and NO_3^- (mg Kg $^{-1}$ soil) released from soil treated with plant aqueous extracts after 10 and 40 days of incubation .

After 10 days of incubation				**After 40 days of incubation**		
treatments	**NH_4^+–N**	**NO_2^- – N**	**NO_3^- – N**	**NH_4^+–N**	**NO_2^- – N**	**NO_3^- –N**
Control	149.73	0	46.64	21.45	0	17.72
Caper	220.97	0	22.95	24.66	0	13.99
Sowthistle	207.57	0	27.05	22.39	0	16.79
Bladygrass	193.84	0	27.99	22.39	0	15.86
Pomegranate	212.14	0	27.98	23.63	0	12.12
DCD	182.14	0	31.25	21.45	0	15.86

RLSD0.01 C.V

NH_4^+ -N = 8.16 15.40

NO3- N = 2.3 12.33

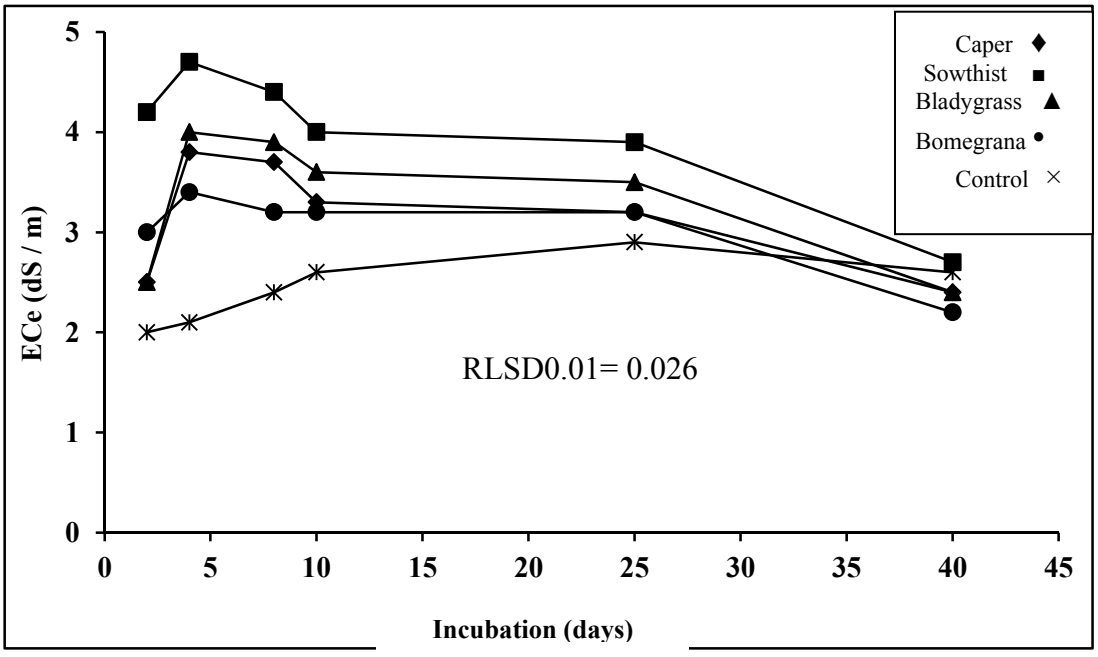

Figure 1: Effect of plant aqueous extracts on soil E.C. at different incubation periods.

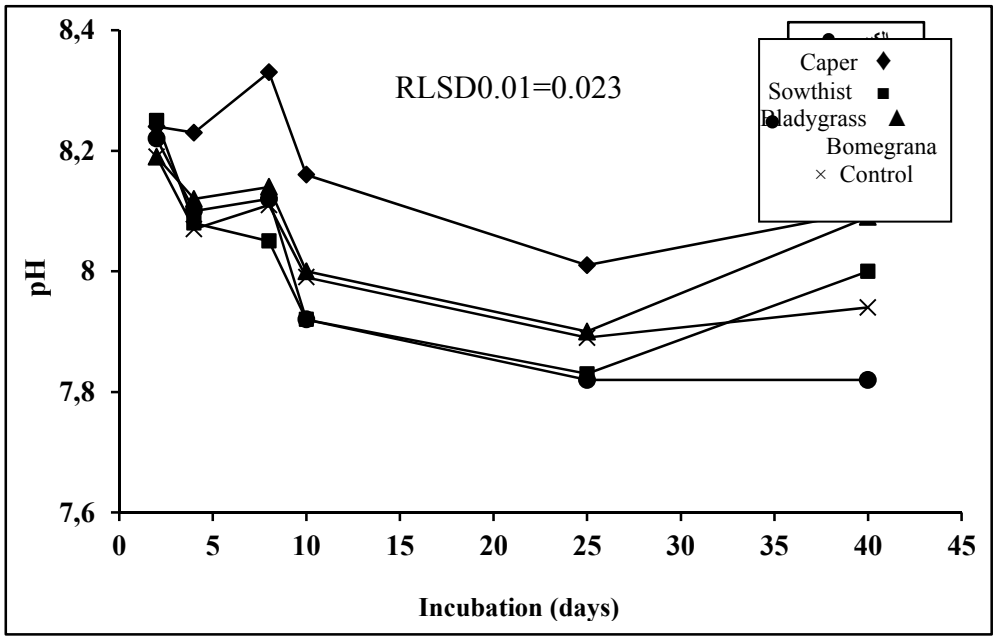

Figure 2: Effect of plant aqueous extracts on soil pH. at different incubation periods. (L.S.D 0.01 = 0.023)

4 DISCUSSION AND CONCLUSION

Nitrification inhibition and inorganic N accumulation:

Several chemical such as DCD , N–serve(DowElanco, USA) , and other have been tried to reduce urea hydrolysis or nitrification in soil in order to increase N–fertilizer efficiency . However, use of such chemicals may have adverse influence on soil micro flora and soil and water pollution(Trenkel,1997) . As early as 1952, Steiven reported presence of naturally occurring substances mostly in higher plants when introduced into soil delay nitrification. Since then interest in using of organic compounds produced and released by plants to control nitrification in soil has increased. However, inconsistent results of effect organic compounds on nitrification have been reported . In this study, selected plants or parts of plants were tested for their effect on nitrification of urea–N in soil. Results of the study showed application of most of studied plants indicated possibility of retarding nitrification of urea–N and the persistence of the inhibitory effect decreased with the time, however, degree of nitrification inhibition differs with source and concentration of the extracts used . Data of Hardy and Sevasithamparam (1989) showed negative effects of added eucalyptus bark on soil microorganism decreased with time. Organic compounds in soil could be volatilized, leached, or converted to non–toxic products as time elapse (Alexander , 1985) . Comparing with chemical nitrification inhibitor (i. e. DCD) retardation of nitrification caused by extracts of plants under study were higher/lower than, or did not differ from that of DCD.

Nitrification inhibitory properties of plant materials such as Karenj (*Pongemia glebra*) neem (*Azadirachta indica*) and tea *(Camellia sinensis*) have been reported (Kiran and Patra ,2003). White (1991) and Paavalainen (1998) reported that introducing water or ethanol extracts of plants contain phenolics, monoterpenes, and other organic compounds into soil exert allelopathic effect on nitrification in soil . Ito and Ichikawa (1999) suggested that *D. adscendens* roots release substances that inhibit not only the growth of other plants and Rhizobium nodulation, but also the nitrifiers activities in soils .On other hand, Kholdebarin and Oertli (1992) and Bremner and McCarty (1988,1993) revealed that any decrease in the amount of NO_3^-- N produced

during nitrification in the presence of cotyledon powder or climax vegetation could be due fixation, volatilization and immobilization of nitrification substrate by organic materials such as phenolics or other compounds released into soil , rather than to their effect on nitrifying bacteria . Whatever, might be the mechanism of their action aqueous extracts of caper , sowthistle , bladygrass and pomegranate plant used in this study showed higher inhibitory effect on urea – N nitrification in soil than that of chemical inhibiter (i.e. DCD). Data in table (5) supported this conclusion by showing that the amount of NH_4^+-N accumulated in soil treated with these plant extracts was significantly higher and the amount of NO_3^-- N accumulated was significantly lower than those of DCD or control treatments at early period of incubation .

E.C. and pH:

Since soil E.C. and pH are among factors controlling nitrification in soil (Alexander, 1985) , the effect of studied plant extracts on these parameters as compared with control were studied (Fig. 1 and Fig. 2) . Results in Fig. (1) Showed that, treating soils with plant extracts increased soil E.C. during early periods of incubation, however, this effect decreased as time of incubation increased. Data in table(3) showed that persistence of the inhibitory effect of plant extracts used in this study decreased with increasing incubation time from 10 to 40 days. Hence, inhibitory effect observed in this study could partly due to increased salinity of treated soils caused by plant extracts during early periods of incubation. Kumar and Wagenet (1985) and Jarallah (1998) reported negative correlation between salinity and nitrification in soil. On other hand, other studies showed that increasing soil salinity from 3 to 12 dSm^{-1} (Jabari,1989; Al-Rashdi et al.,1991) and increased salt concentration up to 0.01 M (Agrawal et al., 1971) or 0.22% (Laura, 1979) increased nitrification in soil.

In conclusion, the results obtained in this study suggested a possibility of using aqueous extracts of some plants as potent substitute for chemical compounds to retard nitrification process in soil thereby, reducing the risk of environmental pollution associated with using chemical compounds as nitrification inhibitors.

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