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INFLUENCE OF NITRATE AND AMMONIUM NITROGEN SOURCES ON GROWTH, NUTRIENT UPTAKE AND PHOTOSYNTHETIC ACTIVITY OF LUPIN *(Lupinus albus L. var termis Forsk*

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ABSTRACT

A water culture experiment was carried out on lupin plants, to study the influence of different nitrate and ammonium ratios on growth, nutrient uptake and photosynthesis process, in respect of chlorophyll content and carbonic anhydrase activity (CAA). Nitrogen was applied in five NO_3 /NH_4 ratios : (100 : 00), (75 : 25) ,(50 : 50), (25 : 75) and (00:100). Results revealed that both shoots and roots dry weights wwere significantly increased as the portion of NO_3 in the growth medium increased. Also P and Mg concentrations in the shoots were increased with increasing NO_3. While, K and Ca in the shoots and K in the roots were increased with increasing NH_4 level. 75% of applied N in NH_4 form caused a decrease in Zn and Cu concentrations in the shoots. In contrast, Fe increased and no specific trend was observed for Zn , Fe and Cu in the roots. P uptake by shoots was not significantly affected, while P uptake by roots was significantly affected. Highest K uptake in shoots and roots was observed when NO_3 was the sole nitrogen source. Ca uptake by shoots and roots significantly increased when portion of NH_4 increased. Zn, Fe and Cu uptake by roots were increased significantly, when NH_4 portion increased. In addition, Zn and Cu uptake by shoots of lupin plants were increased significantly when plants were fed with nitrogen all in NO_3 from. Chl. a and Chl. (a + b) showed the highest value when NO_3 was the sole source for nitrogen, except Chl b. which showed the maximum value when NO_3 was only 25% from applied nitrogen. In addition, CAA was significantly increased when 100% NO_3 was used in the growth root medium.

INTRODUCTION

There is evidence that crop plants supplied with both NH_4^+ and NO_3^- in a combination forms grew better and yielded more than those supplied with either NH_4^+ or NO_3^- alone (Wang and Below 1992). In addition, the changes in pH value of the rizosphere zone induced by presence of NH_4^+ or NO_3^- caused a major effect on the availability and absorption of cations .For example, large amounts of applied nitrogen in NO_3 form resulted in an increase of K, Ca and Mg uptake (Hebrer and Below 1989, Wang and Below 1992, 1995). In contrast, many authors have been reported that NH_4^+ nutrition reduced the uptake of cations, such as K, Ca and Mg (Kirkby 1968, and Cox and Reisenauer 1973). While, presence of NH_4^+ in the growing medium resulted in increases of the uptake of P and S by plant tissues (Blair *et al.,* 1970, Gashaw and Mugwira 198 l). However, there is insufficient evidence that reduction in the uptake of cations is the sole cause of NH_4^+ toxicity to plants when it present in high concentration in the growing medium(Kirkby 1968).

Although, presence of NH_4^+ and NO_3^- together in a combination form in the growth root medium is better for growth of many plants, since CO_2 assimilation in terms of carbonic anhydrase activity (CAA) and phosphoenol pyrovate carboxylase (PEPC) activities were positively affected by the presence of both inorganic nitrogen forms (NH_4^+ and NO_3^-) in the growth medium (Haynes and Goh 1978) .While, in some other plants, CO_2 assimilation was found to be higher in NH_4^+ supplied plants than those supplied with NO_3 (Hall *et al.,* 1984). In this connection, Salsac *et al*., (1987) reported that the energy requirement for the assimilation of NO_3 is 20 - 21 ATP/mol NO_3^-, whereas NH_4^+ requires only 5 mol ATP/mol NH_4^+.

The objective of this work was to study the response of lupin plants to NO_3^- and NH_4^+ as sources for nitrogen and their influences on growth, nutrients uptake and photosynthetic activity, when they grown hydroponically.

MATERIALS AND METHODS

Seeds of lupin *{lupinus albus L. var termis* (Forsk) }were thoroughly washed and soaked in tap water, then in deionized water for 2 h. Seeds were germinated for 3 - 4 days on filter paper then seedlings were transferred on the fifth day to plastic vessels 1 liter volume filled with Hoagland and Arnon nutrient solution (1950) containing NH_4^+ and $N0_3^-$ from two sources Ca $(NO_3)_2$ and/or $(NH_4)_2SO_4$ - Nitrogen ratios of NO_3: NH_4; 100:00, 75: 25, 50:50, 25:75, 00:100 were examined, respectively. The nutrient solution was changed three times weekly and the pH adjusted at 5.5. Three weeks after germination plants were harvested and divided into shoots and roots. Dry weights of both organs were determined. Nutrients concentration were analysed according to Chapman and Pratt (1978). Chlorophyll content was estimated in the third fully mature leaf according to Maclachlan and Zalik (1963). Carbonic anhydrase activity was evaluated according to Gibson and Leece (1981). Leaf sample were prepared for assay carbonic anhydrase activity CAA (EC 4.2.1.1) as follows:-Leaf samples (1g) were ground with pestle and mortar in ice-cold medium (10 ml) which contained 0.01 M Tris-glycine buffer (pH 8.3) and 5% sucrose. After centrifugation at 8.000 r.p.m at 4°C for 20 minutes, the supernatant was removed and used for the enzyme assay.

Data were subjected to analysis of variance and the least significant differences (L.S.D) at 5% probability was used for comparison among means according to Snedecor and Cochran, (1967).

RESULTS

Plant growth:

Dry matter accumulation of both shoots and roots was significantly decreased with decrease of NO_3 : NH_4 ratios in the growth medium (Table 1). Shoots of lupin plants accumulated more dry matter when grown in NO_3 form. The same trend was observed in accumulation of dry matter in roots. However, no significant differences between all NO_3:NH_4 ratios were observed.

Table I: Effect of NO_3/NH_4 ratios on dry weight of shoots and roots and their ratio of lupin plants grown in hydroponics

NO_3/NH_4	Dry weight (g/pot)			Shoot/Root ratio
	Shoot	Root	Total	
100/00	1.39 c	0.45 d	1.84 d	3.09 a
75/25	1.13 b	0.33 c	1.46 c	3.42 c
50/50	1.03 b	0.32 c	1.35 b	3.22 b
25/75	0.98 b	0.30 b	1.28 b	3.26 b
00/100	0.82 a	0.25 a	1.07 a	3.28 b

***Columns designated by the same letter are not significantly different at P<0.05.**

Nutrients concentration

Tables 2 and 3 show that both P and Mg concentrations in the shoots were markedly increased, as the portion of NH_4 in the growth root medium increased. However, no specific trend for such elements in roots were observed. In contrast, the concentrations of K and Ca in shoots and Mg in roots were decreased , since Ca-concentration in roots increased toward NH_4 in the nutrient solution, while Mg exert no specific trend to N-form in the nutrient solution.

Table 2: Effect of NO_3/NH_4 ratios on shoot and root macronutrient concentrations in lupin plants grown in hydroponics

NO_3/NH_4	Shoot (%)				Root(%)			
	P	K	Mg	Ca	P	K	Mg	Ca
100/00	0.23	3.60	0.41	1.14	0.13	4.50	0.39	0.44
75/25	0.30	2.40	0.49	0.87	0.14	3.80	0.26	0.46
50/50	0.32	2.30	0.53	0.81	0.15	2.00	0.27	0.79
25/75	0.34	1.80	0.59	0.71	0.13	1.96	0.23	0.88
00/100	0.37	1.40	0.63	0.64	0.14	1.79	0.31	0.78

Concerning micronutrient concentrations in both shoots and roots of lupin plants, it was found that Zn and Cu concentrations in the shoots started to decline when NH_4 portion in the nutrient solution reached to 75%. While, Fe in shoots tended to increase with 75% NH_4 . Zn, Fe and Cu concentrations in roots showed no specific trend to N-form in the nutrient solution. Although, higher micronutrient concentrations were observed in the roots compared to shoots, since, concentrations of Zn, Fe and Cu reached 7.5,2.5 and 9 fold those found in shoots, respectively.

Table 3: Effect of NO_3/NH_4 ratio on shoot and root micronutrient concentrations in lupin plants grown in hydroponics

NO_3/NH_4	Shoot (mg/kg)			Root (mg/kg)		
	Zn	Fe	Cu	Zn	Fe	Cu
100/00	45	70	17.5	315	200	140
75/25	42	84	17.5	360	208	145
50/50	38	88	17.5	250	173	141
25/75	39	98	15.0	300	200	150
00/100	39	81	15.0	320	230	175

Nutrients uptake:

P-uptake by shoots of lupin plants was not significantly affected by the ratios between NO_3 and NH_4 in the nutrient solution. On the other hand, significant differences in P-uptake by roots were noticed, since more P-accumulated in roots with the decrease of NH_4 portion in the growth root medium and reached the maximum when NO_3 was only the unique source for nitrogen.

K-uptake of both shoots and roots of lupin plants was significantly affected with the ratios between NH_4 and NO_3 (Table 4).

Table 4:Effect of $N0_3/NH_4$ ratios on shoot and root macronutrients uptake in lupin plants grown in hydroponics

NO_3/NH_4	Shoot (mg/pot)				Root (mg/pot)			
	P	K	MS	Ca	P	K	Mg	Ca
100/00	3.21a	49.9e	5.53a	15.8e	0.58d	20.1d	1.24d	1.96b
75/25	3.28a	27.0d	5.52a	9.8d	0.46c	12.4c	0.87c	1.52a
50/50	3.30a	23.7c	5.46a	8.4c	0.48c	6.40b	0.86c	2.53c
25/75	3.33a	17.6b	5.79a	7.2b	0.39b	5.40b	0.69a	2.64d
00/100	3.37a	12.8a	5.74a	5.8a	0.35a	4.40a	0.78b	1.95b

***Columns designated by the same letter are not significantly different at $P< 0.05$**

The highest values of K-uptake by shoots and roots were obtained when plants were fed with all nitrogen in NO_3 form, and tended to decrease as the NH_4 portion in the nutrient solution increased, to reach the lowest value when NH_4 was the sole source of nitrogen for plants.

No significant differences in the amount of Mg accumulated in shoots of lupin plants (Table 4) were noticed, while the opposite results were noticed in roots, which took nearly the same trend of P-accumulation in roots.

Concerning Ca-uptake by shoots and roots of lupin plants, a significant gradual decrease was noticed in Ca- accumulated in shoots and marked increase was noticed in the roots of lupin plants, as the portion of NH_4 increased in the nutrient solution, while the highest value of Ca-accumulated in roots was observed when only 25 % of applied nitrogen was in NO_3 form.

Zn-uptake of both shoots and roots was significantly affected with N-form in the nutrient solution (Table 5). Zn-uptake by shoots was declined with increasing the percentage of NH_4 in the ratio. However, there are no significant differences in the amount of Zn-taken up by shoots of lupin plants when the percentage of NH_4 was increased from 50 % to be 100% in NH_4-form. The same trend of Zn-uptake by shoots was noticed in the roots ,while the significant differences were noticed only between 25 % and 50 % NH_4 treatments.

Table 5: Effect of NO_3/NH_4 ratios on shoot and root micronutrients uptake in lupin plants grown in hydroponics

N0$_3$/NH$_4$	**Shoot (μg/pot)**			**Root (μg/pot)**		
	Zn	Fe	Cu	Zn	Fe	Cu
100/00	62.4 c	93.7 b	24.3 c	142 d	89.3 c	62.5 c
75/25	47.3 b	94.6 b	19.7 b	119 c	68.6 b	47.8 b
50/50	39.1 a	90.6 b	18.0 b	112 c	55.4 a	45.1ab
25/75	37.2 a	93.4 b	14.3 a	90 b	60.0 a	45.0ab
00/100	35.5 a	73.7 a	13.7 a	77 a	57.5 a	43.8 a

***Columns designated by the same letter are not significantly different at P<O.05**

Fe-uptake was significantly decreased when all applied-N was in NH_4 form and no significant differences were observed between the other ratios of NO_3 and NH_4, while large amounts of Fe were accumulated in roots when plants were fed with N in NO_3^- form and significantly decreased when the portion of NO_3 was reduced from 100 % to 50 %, while decreasing the portion of NO_3 in the nutrient solution did not significantly affect the amount of Fe taken up by roots of lupin plants.

More Cu-uptake by shoots and roots of lupin plants was observed when plants were fed with all nitrogen in NO_3 form, while the opposite was true when all N was in NH_4-form.

Chlorophyll content:

It is obvious from Figure (1) that. all chlorophyll, except for Chl. b were higher when NO_3-N was the sole source for nitrogen, since Chl. b was not significantly higher when NO_3 portion in the nutrient solution reached to 25 %,while the lowest Chl. b value was observed when NO_3 portion represents 75 % of the applied amount of nitrogen.

Carbonic anhydrase activity (CAA):

Since the biochemical composition of plants is altered distinctly as N-source in the nutrient solution changed, CAA is expected to change as shown in Figure (1). Activity of CA in leaf extract of lupin plants grown in water culture containing either NO_3 or NH_4 as the sole N-source was higher than those grown in a media containing both NO_3 and NH_4. However, a higher significant CAA was detected when NO_3 was the sole nitrogen-source in the growing media compared to NH_4 one.

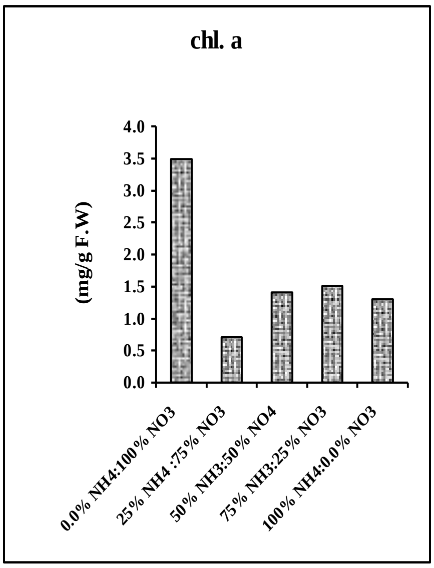
DISCUSSION

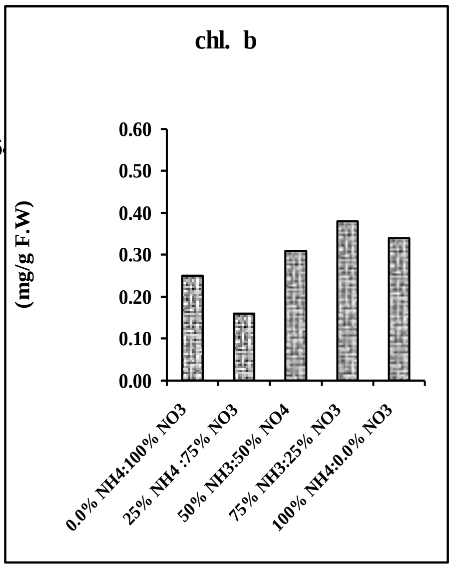
In general, all NH_4 nutrition had inhibitory effects on the dry matter accumulation of shoots and roots of lupin plants, compared to plants grown with all NO_3 (Table 1). As comparison of this study with other hydroponics studies (Polizotto, *et al.,* 1975 and Davis, *et al.*, 1986), it could be suggested that lack of growth promotion of both shoots and roots with mixed nitrogen may be related to the high concentration of NH_4, which could cause injurious effects to plants. Thus, most crop species react adversely to NH_4 nutrition through exhibiting a reduction in growth as well as foliage damage. In this connection, several studies using hydroponics system have demonstrated the injurious effect of using NH_4, as the sole source of nitrogen on growth of different crops such as bean, pea, cucumber, radish, sweet corn and tomatoes (Maynard *et al.,* 1968 and Barker and Maynard. 1972).

Nitrogen form apparently, with exceptions, affected mineral elements uptake by shoots and roots of lupin plants, since all studied elements; (except P and Mg of shoots) were significantly reduced with NH_4^+ nutrition and enhanced with NO_3^- nutrition (Tables 2 and 3). These findings have been reported by several investigators for several crop species (Gashaw and Mugwira, 1981; Wilcox *et al.,* 1985; Van Beusichen *et al.,* 1988; McCrimmon *et al.,* 1992 and Wang and Below, 1995). Also, the well known stimulatory effect of NH_4 nutrition on P-uptake was not observed in this study since plants grown with all NH_4 had similar shoot P uptake as those grown with all NO_3, but decreased amounts in roots. These findings are in a harmony to those obtained by (Blair, *et al.*,1970 on corn and Wang and Below, 1992) on wheat.

Similar to previous obtained results by Kirkby and Mengel, 1967, Shaviv *et al.,* 1987 and Wang and Below, 1995, NO_3 grown lupin plants had higher cation contents (except Mg and Fe) than all NH_4 grown plants, indicated differences in the form of nitrogen translocated from the roots (Barker and Maynard, 1972 and Wang and Below, 1992).This increase in K and decrease in Mg, in the shoots of NH_4 fed plants (Table 2) may be due to greater translocation of amino-N from roots as K but not Mg reportedly accompanies amino acids during their transport (Coic *et al*., 1970).

When lupin plants were supplied with nitrogen mainly in NO_3 form, Zn and Cu contents of shoots and roots, and not Fe in shoots were higher compared to NH_4 (Tables 4 and 5). Zn and Cu accumulation in both shoots and roots was mainly due to the enhanced plant growth, as NO_3 portion in the nutrient solution increased, and not related to the concentration in shoots, since large quantities of such elements remain in the roots.





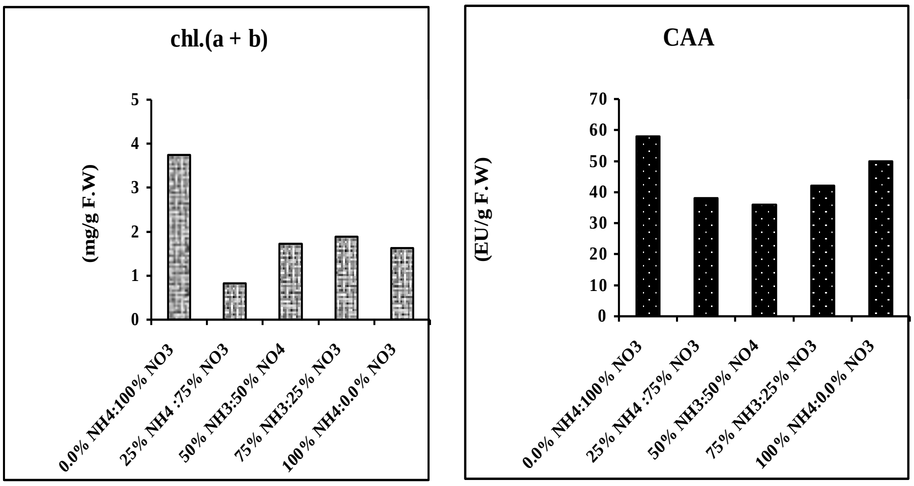


Figure (1) :Effect of NO_3 : NH_4-N ratios on chlorophyll content and carbonic anhydrase activity in leaves of lupin plants grown in hydroponics.

Despite of many reports have shown that stimulation effect of NH_4^+ fed on photosynthetic CO_2 assimilation rate, the obtained results in this study revealed that NO_3 stimulate the formation of both Chl.a and Chl. (a+b) (Figure l). This is might be due to that lupin plants have more energy to be conserved compared to other plants, since the energy required for the assimilation of one mole NO_3 is about 4 fold those needed for the assimilation of the same amount of NH_4 (Salsac, *et al.,* 1987).The stimulation effect of NO_3^-fed on pigment contents, in terms of Chl.a and Chl.(a+b) as well as on CAA may be due to the improvement caused in the uptake of the essential elements, especially Zn, which considered as an essential constituents of CA, since each molecule of CA contains 6 atoms of Zn (Botrill *et al.,* 1970). It is well known that CA catalyze the interconversion of CO_2 to HCO_3 in many photosynthetic organisms (Badger and Pfanz, 1995), since the uncatalysed interconversion speed reached 10^4 times slower than the flux rate required to support photosynthesis (Hatch and Burnell, 1990).

It might be concluded that, under hydroponics conditions, lupin plants grew better and produced more biomass with NO_3^- nutrition. This may be due to the improvement caused in the physiological performance of plants as a result of the improvement caused in nutrient uptake, pigments content and CAA . However, from a practical point of view, NH_4 injury under field condition may not be a problem as it might seen from hydroponics experiment due to many possible reasons related to the soil and root characteristics, beside N-translocation in soil as well as lupin plants are not heavily fertilized with inorganic nitrogen.

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تأثير كل من النترات والأمونيوم كمصادر للنتروجين على النمو ومحتوى العناصر وعملية البناء الضوئى فى نبات الترمس النامى فى مزارع مائية

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قسم النبات – المركز القومى للبحوث – الدقى – القاهرة

أجريت تجربة مزارع مائية على نبات الترمس باستخدام 5 نسب من النتروجين النتراتى إلى الأمونيومى وهى (100 : صفر)، (75 : 25)، (50 : 50)، (25 : 75)، و (صفر : 100) ن أ$_3$: ن يد$_4$ لدراسة تاثيرات هذه المعاملات على كل من النمو، وتركيز ومحتوى العناصر وكذلك عملية البناء الضوئى من خلال (المحتوى الكلوروفيللى) وكذلك نشاط انزيم الكربونيك انهيدريز.

وقد أوضحت النتائج أن الأوزان الجافة للمجموع الخضرى والجذرى زادت معنوياً وكذلك زاد تركيزات الفوسفور والماغنسيوم فى المجموع الخضرى مع زيادة النتروجين النتراتى فى المحلول المغذى، فى حين أن البوتاسيوم والكالسيوم فى المجموع الخضرى وفى الجذور زاد بزيادة النتروجين الأمونيومى. وقد وجد أن 75% من النتروجين فى صورة ن يد$_4$ أدت إلى نقص تركيز كل من الزنك والنحاس فى المجموع الخضرى، فى حين أن 100% من النتروجين فى صورة نترات أدت إلى زيادة معنوية فى المحتوى الكلى للزنك والنحاس فى المجموع الخضرى، وعلى العكس من ذلك زاد الحديد فى الجذور، فى حين أن الكالسيوم فى كل من المجموع الخضرى والجذرى زاد معنوياً فى وجود ن يد$_4$ كمصدر للنتروجين. وقد لوحظ أن المحتوى الكلى للبوتاسيوم فى المجموع الخضرى والجذرى أظهر زيادة فى وجود النترات بمفردها كمصدر وحيد للنتروجين.

وقد لوحظ أن تركيز كل من كلوروفيل أ وكلوروفيل (أ+ب) زاد معنويا مع النتروجين النتراتى ماعدا كلوروفيل ب الذى أظهر أعلى قيمة عند 25% نتروجين نتراتى. بالإضافة إلى زيادة نشاط أنزيم الكربونيك انهيدريز عند استخدام 100% نتروجين نتراتى فى بيئة نمو الجذور.