Journal of Applied Sciences Research, 3(11): 1630-1636, 2007

Effect of Nitrogen Forms on Nitrate Contents and Mineral Composition in Lettuce Plants in Sandy and Calcareous Soils

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Abstract: A pot experiment was carried out on sandy and calcareous soils to evaluation the effect of nitrogen forms on nitrate contents in lettuce plants of class (lacluca sative var. capitata) , nitrogen fertilization followed in the forms of sodium nitrate(SN), ammonium sulphate (AS) or ammonium sulphate with nitrification inhibitor (N-serve)(AS+NS) with a rate 150mg/kg soil. The results showed that ammonium sulphate with inhibitor treatment gave the least value of nitrate content in both of two soils. Ammonium sulphate + inhibitor came second then sodium nitrate treatments respectively. lettuce dry weight gave the highest value with ammonium sulphate + inhibitor, in sandy soil. On the other hand, the highest value was observed with ammonium sulphate alone in calcareous soil. Total nitrogen contents gave highest value with inhibitor treatment (AS + NS) in sandy soil. Phosphorus contents gave also similar results with the addition of ammonium sulphate. The calcareous soil recorded the highest values with ammonium sulphate without inhibitor treatment in nitrogen and phosphorus contents. Potassium contents recorded the highest value with ammonium sulphate without inhibitor in both sandy and calcareous soil. Micronutrients contents Fe, Mn, Zn and Co gave a similar behavior in sandy soil, while in calcareous soil as for to Fe and Zn recorded the highest values with (AS + NS) treatment than (AS) and (SN) treatments, respectively. Mn and Co came at a like behavior since the head contents with (SN), (AS) and (AS + NS) respectively. Head nitrogen available residual effect recorded with (AS + NS) treatment by comparison with the other treatments. The highest value of chlorophyll contents as with (AS) treatment in both of two soils. All the differences between the treatments were significant under all levels to each of iron and chlorophyll.

Keywords: Nitrogen, N-serve, lettuce plant, nitrate, mineral composition, sandy soil, calcareous soil

INTRODUCTION

One of the problems which were achieved interest recently is the nitrate accumulation in several species of plants and the effect on the general health. Nitrate it self is not toxic to creatures but at elevated levels, it causes a noninfectious disease called nitrite poisoning. Nitrite is absorbed into the blood and combines with hemoglobin to form methemoglobine, which causes a reduction in the ability of the blood to carry oxygen from the lungs to body tissues. When the blood can no longer supply oxygen to the body, the creature can suffocate. Nitrate normally is broken to nitrite (NO_2) and then to ammonia (NH_3). The ammonia is then converted to amino acids then to protein by microbes. High nitrate levels, especially when growing under adverse conditions such as drought, frost, unseasonable or prolonged cool temperatures, hail, shade, and disease, high levels of soil nitrogen and soil mineral deficiencies or herbicide damage can cause high nitrate accumulation.

Nitrate levels cycle during a 24-hour period; during the night, nitrate accumulates when photosynthesis is inactive, then during the days, nitrate is quickly converted to protein. Under normal growth conditions there is little nitrate buildup. Even though plant roots are absorbing large amounts of nitrate because protein conversion keeps pace with root absorption. However in certain conditions, this balance can be disrupted so that the roots will accumulate nitrate faster than the plant convert the nitrate to protein.

Toxic levels of nitrate can accumulate after a drought – ending rain or irrigation. Since peak nitrate levels occur in the morning, it is advisable to delay haying or grazing until the afternoon of a sunny day, Dennis *et al.*,[1], Korkmaz[2], they showed that. Greet *et al.*, [3] indicated that anion of SO_4 caused the nitrate content in the leaf blades to decrease. The same effect with anion of chloride CL^- in the lettuce plants, they return that's to the osmotic balance in the roots region. The nitrate content of lettuce can also be reduced by the partial replacement of nitrate with ammonium. The greatest reduction in nitrate content is thus found where both ammonium and chloride are present in the

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solution[4]. however, In soil – grown crops applied ammonium is relatively quickly converted to nitrate at moderate of high soil temperatures Amperger and Vilsnieier[5], and can therefore increase the effectiveness of ammonium application in reducing the nitrate content of lettuce 1984[6,7].

Concerning the effect of nitrogen Forms on iron uptake in calcareous soil, iron deficiency is commonly associated with high bicarbonate levels and high nitrate in soils has been observed. Nitrate uptake usually causes alkalinistation of the rhyzosphere due to the concomitant excretion of OH^- or HCO_3 [8,10]. When iron availability is low, nitrate uptake may lead to iron deficiency in so-called iron inefficient plants. Nitrate induced iron deficiency is assumed to occur mainly on alkaline and calcareous soils where, due to the high pH values, ammonium is rapidly nitrified. Especially when nitrogen is applied, for instance as a fertilizer, nitrate induced iron chlorosis might be a relevant problem[11]. The objectives of the present study were to examine the effect of nitrogen availability and nitrogen forms on the yield, nitrate content and iron uptake and chlorophyll ratio of lettuce plant. Further, the necessity of a nitrification inhibitor when nitrogen is continually applied in ammonium form was also examined.

MATERIALS AND METHODS

A pot experiment was carried out in a greenhouse to evaluate the effect of nitrogen forms and soil type on nitrate contents in lettuce plants (Lacluca Sativa Var Capitata L.) grown on two soils namely; sandy and Calcareous. The main characteristics of the investigated soils are presented in Table(1).Soil sample (0-30cm) were collected from AbouRwash-Giza governorate (Sandy soil) and from Nobaria- Alexandria governorate (calcarouse soil), Egypt .The soils were sieved to pass through a 2mm-sieve,5Kg of soil were placed in each of plastic pots . Nitrogen fertilizers (Sodium nitrate, Ammonium sulphate and Ammonium sulphate +nitrification inhibitor (N-serve) were added to pots to give 750mgN/pot. All treatments were received the recommended dose of K and P as Potassium dihydrogen phosphate were banded at 2cm from the pot surface at rate 200mg/kg as a source for potassium and phosphorus. Three replicates pots of each treatment were used. Nitrification inhibitor (N-serve) was added at a rate 1% of added nitrogen. The moisture content of the pot was maintained at 70% of the water field capacity along the experimental time. Iron was added at rate 5ppm with EDTA –Fe, Plants were harvested at 50 days after planting. After recording head fresh weight were cut into quarters. One of which was immediately frozen for determination of nitrate while another was reweighed and dried to constant weight at 70°C. Oven-dried plant material was analyzed for total nitrogen and nitrate nitrogen was determined by distillation using microkjeldahel distillation, Jackson,[12]. Nitrate nitrogen was determined using devarda alloy by Microkjeldahel distillation. Phosphorus was determined according to Jackson,[12]. Potassium was determined by flame photometer. Micronutrients (Fe, Mn, Zn, Co) were determined by atomic absorption. Chlorophyll concentration in the fresh leaf was determined according to Lichetoem and Weilburn,[13].

RESULTS AND DISCUSSIONS

Fresh and Dry Weight of Lettuce Plant: Data in (Table 2) showed fresh, dry weight and total nitrogen as percentage in lettuce plant as affected by nitrogen forms and soil type.

Fresh and Dry Weight as Indicator for Nitrogen Contents in Sandy Soil: Ammonium sulphate with inhibitor (N-serve) treatment gave higher fresh and dry matter (215F.W – 19 D.W) g/pot than $(NH_4)_2$ SO_4 without inhibitor treatment (212 F.W- 18.5 D.W) g/pot , while sodium nitrate treatment came in last order (158 F.W – 16.3 D.W) g/pot this came as a reflection for total nitrogen contents in lettuce plant in sandy soil. As it took the same previous order. Ammonium nitrogen (NH_4^+) with inhibitor was held on the soil granules surface a long period without conversion to nitrate (NO_3^-) which is usually exposed to losses with irrigation water faraway from the roots, however applied ammonium is relatively quickly converted to nitrate at moderate or high soil temperature. The application of a nitrification inhibitor can however effectively suppress the oxidation of ammonium by nitrousomonas bacteria[5,14]. Data in table (2) revealed that the absorption of nitrogen by lettuce plants was influenced by adopted treatments and soil type ,N-concentration in lettuce plants ranged from 2.59% from nitrate treatment to 4.2% for ammonium +N-serve treatment , it could be , therefore assumed that the addition of the inhibitor helped in providing more nitrogen in the form of ammonium which might be preferred by young lettuce plants.

The previous order reflects on N-utilization efficiency in sandy soil.

Lettuce head value of N-utilization efficiency with ammonium sulphate with inhibitor (N-serve). i.e. (62%) and without inhibitor (54%) while sodium nitrate treatment came at last order (42%) which confirm that plant matter a results for the elements uptake especially nitrogen.Head fresh and dry weight and N-contents in lettuce plant with ammonium sulphate with inhibitor

Table 1: Some chemical and physical properties of the studied soils.

Site	pH 1:25	E.C 1:5	$CaCO_3$ %	O.M %	Texture	mgN/100g	
						NH_4^+	NO_3^-
Abou-Rawash	7.92	0.185	0.71	0.26	Sandy	15	93.5
Nobaria	8.32	0.20	10.2	0.17	calcareous	23	74.8

Table 2: lettuce yield and Nitrogen contents as affected by nitrogen forms and soil type.

Treatments	Sandy Soil				
	F.W g/pot	D.W g/pot	N%	N-uptake mg/pot	N-utilization efficiency %
Control	85.2	13.0	2.59	336	-
$NaNo_3$	158	16.3	3.99	650	42
$(NH_4)_2$ SO_4	212	18.5	4.0	740	54
$(NH_4)_2$ SO_4+NS	215	19.0	4.2	798	62
L.S.D 0.05	13.5	1.3		27.3	
Calcareous soil					
Control	60	11.5	2.78	320	-
$NaNO3_3$	147	15.6	3.98	620	40
$(NH_4)_2$ SO_4	173	18.5	4.0	740	55
$(NH_4)_2$ SO_4+NS	134	15.5	3.90	605	63
L.S.D 0.05	13.2	3.62		24.6	

$$\text{N - utilization n efficiency \%} = \frac{\text{N - uptake - N control}}{\text{N add}} \times 100$$

treatment (184 F.W – 19.5 D.W) g/pot in calcareous soil. That reflection for nitrogen contents where was the most concentration in this treatment (760 mg/pot) by comparison with the other treatments and thus N-utilization efficiency as a percentage recorded highest value with this treatment. Na NO_3 treatment came the last order as fresh and dry weight (147 F.W – 15.6 D.W) g/pot so nitrogen contents as a percentage or N-uptake (3.98%), (620 mg/pot) this a result reflected on N-utilization efficiency as a percentage (62%). Ammonium sulphate without (N-serve) inhibitor treatment gave the second values to fresh and dry weight (173 F.W – 18.5 D.W) g/pot or total nitrogen, N-uptake and N-utilization efficiency (3.80% - 700mg/pot- 55%) respectively.

May by chemical properties of calcareous soil as soil pH, E.C, rate of calcium carbonate (10.2%) and the little activity biological due to lack enhance the inhibitor efficiency as nearness the results between treatment of sodium nitrate and ammonium sulphate with inhibitor where the differences between them were no significant. Bundy and Bremner[15] showed that the effectiveness of N-serve as inhibitor for nitrification in soil depends greatly upon soil type and this compound is most effective with light – textured soils. Hendricheson and Keeny[16] obtained data indicating just the opposite nitrapyrin (N-serve) was inhibitorier to the nitrified population as soil pH increased from 5.7 to 7.4.

Nitrate Contents in Lettuce Plant: Data in table (3) indicate No_3-N in fresh weight, dry weight and NO_3-N as a percentage from total nitrogen, as affected by nitrogen forms and soil type in lettuce plants

Nitrate contents in fresh and dry weight recorded the least values with ammonium sulphate with (N-serve) inhibitor treatment in sandy (192 F.W – 884 D.W) ppm and calcareous soils (142F.W – 825 D.W) ppm, reflect that in NO_3-N as a percentage of total nitrogen as gave the least values in both two soils (2.1%) in lettuce plant. Ammonium suplate without inhibitor treatment came in the second order of where NO_3-N contents (222 F.W – 1310 D.W) ppm in sandy soil and (224 F.W – 890 D.W) in calcareous soil as a NO_3-N% (3.27 – 2.22) in two soils respectively. Sodium nitrate treatment recorded highest value as NO_3 – N contents in fresh and dry matter by comparison with the other treatments in sandy and calcareous soil. All differences between the treatments were significant which to lead to very important by the nitrogen forms

Table 3: Nitrate contents (ppm) in lettuce plants as affected by nitrogen forms and soil type.

Treatments	Sandy Soil			Calcareous soil		
	NO_3-N F.W	NO_3-N D.W	NO_3-N %	NO_3-N F.W	NO_3-N D.W	NO_3-N %
Control	152	280	1.08	161	280	1.00
$NaNO_3$	255	1816	4.55	367	918	2.30
$(NH_4)_2$ SO_4	222	1310	3.27	224	890	2.22
$(NH_4)_2$ SO_4+NS	182	840	2.00	113	656	1.68

$$NO_3 \text{ - in dry weight \%} = \frac{NO_3 \text{ - N in dry weight \%}}{\text{N add}} \quad X100$$

- NO3-N in dry weight % = (NO_3-N in dry weight % × 100) / N uptake
- Safe level of NO_3-N in fresh leaves 200 ppm.
- Safe level of NO_3 – N in dry leaves 1000 ppm.

Table 4: Some macronutrients contents (mg/pot) in lettuce plants as affected by nitrogen forms and soil type.

Treatments	Sandy Soil			Calcareous soil		
	D.Wg/pot	P-uptake	K-uptake	D.W g/pot	p-uptake	k-uptake
Control	14.0	53.2	682	11.5	38	557
$NaNo_3$	16.3	81.5	773	15.6	29	669
$(NH_4)_2$ SO_4	18.5	120	1297	18.5	37	918
$(NH_4)_2$ SO_4+NS	19.0	135	848	15.5	32	671
L.S.D 0.05	1.3	3.14	25.2	3.62	3.83	22.2
L.S. D 0.01	2.4	4.71	32.2	5.03	4.98	30.8

fertilization that gives the least values of nitrate accumulation in plants. Van der Boon *et al.,*[4], Tusun,[17]. They reported that nitrogen form as ammonium application reduces plant nitrate content .Richard San and Hard Grave,[18], Vaughon,[19] showed that little or no effect of ammonium fertilizers applied to soil-grown crops at planting is found unless a nitrification inhibitor is also applied. Although continual application of ammonium throughout the experimental period reduced crop nitrate content in the present study, the effect was greatly enhanced by the simultaneous application of (N-serve) inhibitor. This suggests that part of the applied ammonium was oxidizaed to nitrate in the absence of (N-serve) inhibitor. Ammonium suplate with inhibitor (N-serve) treatment gave NO_3-N concentration under safe level in sandy soil, while the all treatments in calcareous soil recorded the values under the safe level i.e. (918-890-656) ppm in dry matter respectively, NO_3-N contents in lettuce plant under the safe level i.e. (1000) ppm NO3-N in dry weight with ammonium sulphate with inhibitor (N-server) as NO_3-N contents i.e. 656 ppm of the standard safe level in dry weight. Simpson[20] recommended that no spinach should be given to children under 3 months of age, that spinach used for infant feeding must contain no more than 0.07% nitrate-N on a dry matter weight basis, and that prepared spinach be kept refrigerated. Knauar[21] estimated the maximum safe level of nitrate-N to be 0.1% of the dry matter.

Phosphorus Uptake in Lettuce Plant: Table (4) indicated the contents of phosphorus and potassium uptake in lettuce plant as affected by nitrogen forms and soil types.

Head value of phosphorus uptake was recorded with ammonium sulphate with inhibitor (AS+Ns) than ammonium sulphate without inhibitor (AS). Ammonium nitrogen (NH^+_4) in (As+Ns) treatment stand in soil a long period which adsorbed on granules surface soil, that's enhance phosphorus available for plant absorbed while in AS treatment ammonium nitrogen (NH^+_4) converted rapidly to nitrate as exposed to losses by leaching with irrigation water for a way about obtainable of roots may be due to no enhance for more phosphorus uptake. Least value as phosphorus uptake with sodium nitrate treatment by comparison with the other treatment). All differences between the treatments were significant under all levels.

Phosphorus in calcareous soil suffers the fixation problems to reason rising of pH soil (8.32) and calcium carbonate (10.2%) due to unavailable phosphorus for

Table 5: Some micronutrients contents (ppm) in lettuce plans as affected by nitrogen forms and soil type.

Treatments	Sandy Soil			Calcareous soil		
	Mn	Zn	Co	Mn	Zn	Co
Control	122	23	12	89	15	87
$NaNO_3$	135	24	28	93	25	90
$(NH_4)_2$ SO_4	177	25	39	85	27	82
$(NH_4)_2$ SO_4+NS	182	27	51	79	29	82
LSD 0.05	10.3	1.49	1.54	9.3	1.48	6.23

Table 6: Iron and total chlorophyll contents in lettuce plants as affected by soil type and nitrogen forms forms.

Treatments	Sandy Soil		Calcareous soil	
	Fe- (ppm)	Chlorophyll mg/gm	Fe- (ppm)	Chlorophyll mg/gm
Control	1140	0.57	1102	0.43
NaNO3	1537	0.67	1233	0.62
(NH4)2 SO4	1700	0.82	1273	0.73
(NH4)2 SO4+NS	1800	0.67	1966	0.71
L.S.D 0.05		0.11		0.12
L.S.D 0.01		0.13		0.15

plants. All the values were the least in calcareous soil than sandy soil. Head value as phosphorus uptake recorded with (AS) treatment while (AS+NS) treatment came at second order may be due to a little biological activity in calcareous soil. Sodium nitrate (SN) treatment came the last order by comparison with the other treatments may be cause the nitrate exposed to losses by several methods such as dentrification, volatilization and leaching

Potassium Uptake by Lettuce Plants: In sandy soil, (AS) treatment recorded the highest value as potassium uptake (1297 mg/pot) comparison with the other treatments. Ammonium sulphate with inhibitor. (AS+NS) treatment came at the second order (848 mg/pot) as the inhibitor (N-Serve) efficiency lead to inhibitate of ammonium nitrogen without convert to nitrate to a long run period which cause increasing the competition between ammonium and potassium on granules surface soil may resolve at ammonium uptake benefit without potassium. Sodium nitrate (SN) treatment came in the last order where potassium uptake i.e. (773 mg/pot). All differences between the treatments were more significant under all levels.

The same trend in sandy soil occur with calcareous soil as (AS) treatment recorded highest value (918 mg/pot) follow it both (AS+NS) and (SN) treatments but the differences between them were no significant may be due to calcareous soil properties of rising calcium carbonate i.e. (10.2%). Mathers *et al.,*[22] studied the effect of nitrification inhibitor on the relative uptake of K, Ca and Mg by winter wheat growing in pots in greenhouse and found that nitrapyrin inhibited nitrification of the added NH_4. The plant also had lower concentrations of K, Ca and Mg. the concentration of K was higher during periods of rapid growth.

Micronutrient Contents in Lettuce Plant: Data in table (5) indicated the some micronutrients contents in lettuce plants as affected by nitrogen forms and soil type. (AS+NS) treatment recorded the highest value as Mn uptake i.e. (182ppm) follow it (AS) treatment i.e. (177ppm), the differences between them were significant as continuous ammonium nitrogen in soil a longest period by efficiency of nitrapyrin inhibitor seemed to enhance Mn available and more uptake, while (SN) treatment gave the least value as Mn uptake i.e.(135ppm) may be the nitrate depress of Mn uptake in sandy soil. While in calcareous soil all the values as Mn uptake came the lesser than sandy soil and reversible the trend previously may be due to the calcareous soil properties and rising of calcium carbonate.

Zinc contents in lettuce plants in both sandy and calcareous soils showed the same trend of Mn uptake and the differences between the treatments were not significant and all the values were similar in both the two soils.

(AS+NS) treatment recorded the highest value of Co (cobalt) followed by (AS) treatment while (SN) treatment gave the least value in sandy soil. All the differences between the treatments were significantly, where the continuation of ammonium in the sandy soil was similar to (AS+NS) treatment and encourage more uptake of cobalt. In calcareous soil (SN) treatment recorded the highest value as cobalt uptake in lettuce plants was (90 ppm) while the other two treatments gave similar values as cobalt uptake, worthy of observation that control treatment recorded the higher value than nitrogen treatments and that's means that the calcareous soil properties and condition it may enhance and promote of cobalt uptake to overlook about the nitrogen forms of uses. Selim,[23] reported that the effect of the inhibitor was more pronounced at low rather than at high N-doses. On the other hand, its decrease of other cations. The effect on micronutrient elements is not noticeable since the plant requirements of such elements are quite low.

Iron and Chlorophyll Contents in Lettuce Plant: Data in (Table 6) indicated that nitrogen addition resulted in an increase in iron content as compared with control either in sandy or calcareous soils. Forever, using N-serve with AS produced higher values in Fe-content in sandy and calcareous soils. Also, it is noticed that these increases of Iron are associated with pronounced increase in chlorophyll content in leaves of lettuce plant. Fe-content seemed to be affected by different N-sources and nitrification inhibitor. Data revealed that application of calcium nitrate increased concentration of FE by 34% as compared to control. concluded that supplying the plant with As in present of nitrapyrin (N-serve) for preventing nitrification reduced Fe chlorosis even when ammonium was less than 20% of total mineral N in the soil, suggesting that NH4 uptake by plant and consequence of hydrogen (H) efflux accurs from the root soulbilizing enough Fe near the root to overcome the chlorosis[24].

Data showed that application of calcium nitrate increased the total content of chloroplly by about 17.5%. in sandy soil recorded the highest value of total chloroplly in lettuce plant compared with calcareous soil in all treatment. AS+Ns treatment was more effective in increasing the total content of chloroplly by about 52%(0.87mg/g) as compared with control (0.57mg/g).

Conclusion: Ammonium nitrogen fertilizers with nitrification inhibitor (N-serve) treatments to prove certain efficiency in a little nitrate contents in plant and increasing in total nitrogen ratio, mineral composition and dry matter. Also more save of add nitrogen at form of nitrate fertilizers which exposed to losses through several methods such as denitrification, volatilization and leaching.

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