

# **STUDY ON FERTILIZATION TECHNIQUES FOR LOWERING NITRATE CONTENT IN LETTUCE CROP**

**By**

**SHAIMAA HAFEZ NASR EL-SAPAGH**

**B.Sc. Agric. Sci. (Soil Science), Fac. Agric., Cairo Univ., 2000**

**M.Sc. Agric. Sci. (Soil Science), Fac. Agric., Cairo Univ., 2008**

**THESIS**

**Submitted in partial Fulfillment of the  
Requirements for the Degree of**

**DOCTOR OF PHILOSOPHY**

**In**

**Agricultural Sciences  
(Soil Science)**

**Department of Soil Science  
Faculty of Agriculture  
Cairo University  
EGYPT**

**2017**



**APPROVAL SHEET**

**STUDY ON FERTILIZATION TECHNIQUES FOR  
LOWERING NITRATE CONTENT IN LETTUCE  
CROP**

**Ph.D. Thesis  
In  
Agric. Sci. (Soil Science)**

**By**

**SHAIMAA HAFEZ NASR EL-SAPAGH**  
**B.Sc. Agric. Sci. (Soil Science), Fac. Agric., Cairo Univ., 2000**  
**M.Sc. Agric. Sci. (Soil Science), Fac. Agric., Cairo Univ., 2008**

**APPROVAL COMMITTEE**

**Dr. ESMAT HASSAN ATTIA NOUFAL**.....  
**Professor of Soil Science, Fac. Agric., Banha University.**

**Dr. MOHAMEDY IBRAHIM EL-KHERBAWY**.....  
**Professor of Soil Science, Fac. Agric., Cairo University.**

**Dr. YOUSSEF ALI ABDEL-AAL** .....  
**Professor of Soil Science, Fac. Agric., CairoUniversity.**

**Dr. SAYED TAHA ABOU-ZEID**.....  
**Professor of Soil Science, Fac. Agric., Cairo University.**

**Date: 27 / 12 /2016**

**SUPERVISION SHEET**

**STUDY ON FERTILIZATION TECHNIQUES FOR  
LOWERING NITRATE CONTENT IN  
LETTUCE CROP**

**Ph.D. Thesis  
In  
Agricultural Sci. (Soil Science)**

**By**

**SHAIMAA HAFEZ NASR EL-SAPAGH**  
**B.Sc. Agric. Sci. (Soils), Fac. Agric., Cairo Univ., 2000**  
**M.Sc. Agric. Sci. (Soils), Fac. Agric., Cairo Univ., 2008**

**SUPERVISION COMMITTEE**

**Dr. YOUSSEF ALI ABDEL-AAL**  
**Professor of Soil Science, Fac. Agric., Cairo University**

**Dr. SAYED TAHA ABOU-ZEID**  
**Professor of Soil Science, Fac. Agric., Cairo University**

**Dr. AMAL LOTFY ABD EL-LATIF**  
**Associate Professor of Soil Science, Fac. Agric., Cairo University**

**Name of Candidate:** Shaimaa Hafez Nasr El-Sapagh      **Degree:** Ph.D  
**Title of Thesis:** Study on Fertilization techniques for Lowering Nitrate Content in Lettuce Crop.  
**Supervisors:** Dr. Youssief Ali Abdel-Aal  
Dr. Sayed Taha Abou-Zeid  
Dr. Amal Lotfy Abd El.Latif  
**Department:** Soil Science **Approval:** 27 / 12/ 2016

### ABSTRACT

Nitrogen is one of the essential elements for plant growth. The most common nitrogen source in hydroponics is nitrate ( $\text{NO}_3^-$ ). When nitrate uptake rate exceeded its assimilation because of excessive nitrate application, nitrate accumulation in plant tissue occurs. Vegetables, particularly leafy vegetables, accumulated nitrate readily. Some previous researches suggested that the vegetables with high nitrate in the diet will cause some health problems. The objective of this work was to study the means by which nitrate concentration can be depressed in lettuce plants, to achieve this purpose, two experiments were conducted

The first experiment was conducted to study the effects of nitrate-N/ amino acid sources ratios on the growth,  $\text{NO}_3^-$  accumulation and some macronutrient concentrations of lettuce (*Lactuca Sativa* L.) var. Sahara to select the best ratios of amino acids to  $\text{NO}_3^-$ -N for hydroponics culture of lettuce. Eighteen treatments were applied to lettuce plants. The eighteen treatments were a combination of six amino acids (AA), arginine (Arg), alanine (Ala), aspartic acid (Asp), glutamic acid (Glu), glutamine (Gln) and glycine (Gly), and three  $\text{NO}_3^-$ -N/AA-N molar ratios: (1) 100: 0, (2) 80:20 (3) 60:40. All treatments had the same total N concentration at a rate of  $12.5 \text{ mmolL}^{-1}$  in nutrient solution. The results of this experiment indicated that increasing the portion of AA-N to replace  $\text{NO}_3^-$ -N in nutrient solution decreased plant biomasses and glutamic acid had stronger decrease than the other AAs. Nitrate concentration decreased with decreasing  $\text{NO}_3^-$ -N/AA-N ratios and glutamic treatment had stronger effect than other AAs. Replacing of amino acids in nutrient solution increased essential, semi essential and non essential amino acids content of lettuce shoot compared to 100%  $\text{NO}_3^-$ -N treatment.

The second experiment was conducted to evaluate the effect of nitrogen sources and rates on growth, nitrate accumulation and macronutrient concentrations of iceberg (*Lactuca sativa*) var sahara. Using ammonium nitrate, ammonium sulfate and calcium nitrate and nitrogen rates (0,30,60 and  $90 \text{ kg N fed}^{-1}$ ) and / or without nitrification inhibitor (ammonium thiosulfate ATS). The highest shoot fresh weight and shoot dry weight values were obtained from ammonium sulfate + ATS in N rates  $90 \text{ Kg N fed}^{-1}$ . The highest accumulation of nitrate was obtained from calcium nitrate in N rates  $90 \text{ Kg N fed}^{-1}$ . The highest concentration of N% and P% were obtained from (ammonium nitrate + ATS) and (ammonium sulfate + ATS), respectively with N rate  $90 \text{ Kg N fed}^{-1}$  while the highest K % concentration was recorded from both ammonium sulfate and (ammonium sulfate + ATS) in N rates  $90 \text{ Kg N fed}^{-1}$ .

**Key words:** Lettuce, nitrate, amino acid, nitrogen and nitrification inhibitor.

## *ACKNOWLEDGEMENT*

*I wish to express my sincere thanks, deepest gratitude and appreciation to Dr. Youssief A. Abdel-Aal and Dr. Sayed T. Abou-Zeid, Professors of Soil Science, Faculty of Agriculture, Cairo University for suggesting the problems, supervision, continued assistance, and guidance through the course of my study and for their revision of the manuscript of this thesis. Sincere thanks and deep appreciation are also due to Dr. Amal Lotfy Abd El-Latif Associate Professor of Soil Science, Faculty of Agriculture, Cairo University for sharing in supervision, valuable advice and progressive criticism.*

*Grateful appreciation is also extended to all staff members of Soil Science Department, Faculty of Agriculture, Cairo University.*

*Special deep appreciation is given to my father, my mother, my brothers, my son and my friends.*

# CONTENTS

	Page
<b>INTRODUCTION</b> .....	<b>1</b>
<b>REVIEW OF LITERATURE</b> .....	<b>3</b>
<b>1. Nitrate accumulation in vegetables and its impact on human health</b> .....	<b>3</b>
<b>2. The strategies used to reduce the accumulation of nitrate in the plant</b> .....	<b>6</b>
a. Effect of different nitrogen sources and levels.....	<b>7</b>
b. Effect of plant part.....	<b>10</b>
c. Effect of season.....	<b>11</b>
d. Effect of amino acid .....	<b>12</b>
e. Effect of nitrification inhibitor .....	<b>15</b>
<b>3. Effect of amino acid on</b> .....	<b>18</b>
a. Plant growth.....	<b>18</b>
(1) Plant fresh weight.....	<b>18</b>
(2) Plant dry weight.....	<b>20</b>
(3) Yield.....	<b>21</b>
b. Amino acid content.....	<b>22</b>
c. Macronutrients concentration.....	<b>24</b>
(1) Nitrogen.....	<b>24</b>
(2) Phosphorus. ....	<b>26</b>
(3) Potassium.....	<b>27</b>
d. Effect of amino acid on enzyme activity.....	<b>28</b>
<b>4. Effect of nitrogen fertilizers on</b> .....	<b>29</b>
a. Plant growth.....	<b>29</b>
(1)Plant fresh weight.....	<b>29</b>
(2)Plant dry weight.....	<b>30</b>
(3)Yield.....	<b>30</b>
b. Macronutrients concentration.....	<b>31</b>
(1) Nitrogen. ....	<b>31</b>
(2) Phosphorus. ....	<b>32</b>
(3) Potassium.....	<b>32</b>
<b>MATERIALS AND METHODS</b> .....	<b>33</b>
<b>RESULTS AND DISCUSSION</b> .....	<b>41</b>

	<b>Page</b>
<b>1. The first experiment (nutrient film techniques experiment)</b>	
.....	<b>41</b>
a. Effect of amino acids replacing nitrate on growth of lettuce plants. .....	<b>41</b>
(1) Fresh and dry weights of shoots.....	<b>41</b>
(2) Fresh and dry weights of roots.....	<b>44</b>
b. Effect of amino acids replacing nitrate on nitrate concentration in lettuce fresh shoots. ....	<b>45</b>
c. Effect of amino acids replacing nitrate on macronutrient concentrations in lettuce shoots.....	<b>50</b>
d. Effect of amino acids replacing nitrate on amino acids content in lettuce shoots.....	<b>60</b>
<b>2. The second experiment (pots of soil experiment).....</b>	<b>66</b>
a. Effect of nitrogen forms, rates and nitrification inhibitors on growth of lettuce plant. ....	<b>66</b>
(1) Fresh and dry weights of shoot. ....	<b>66</b>
(2) Fresh and dry weights of root.....	<b>72</b>
b. Effect of nitrogen forms, rates and nitrification inhibitors on nitrate concentration in lettuce fresh shoots. .....	<b>76</b>
c. Effect of nitrogen forms, rates and nitrification inhibitors on macronutrients concentrations in the shoots of lettuce plant. .....	<b>80</b>
(1) Nitrogen concentration (%).....	<b>80</b>
(2) Phosphorus concentration (%).....	<b>84</b>
(3) Potassium concentration (%).....	<b>86</b>
d. Effect of nitrogen forms, rates and nitrification inhibitor on macronutrient contents (mg pot <sup>-1</sup> ) in the shoots of lettuce plants.....	<b>88</b>
(1) Nitrogen content.....	<b>88</b>
(2) Phosphorus content.....	<b>91</b>
(3) Potassium content.....	<b>93</b>
<b>SUMMARY</b> .....	<b>95</b>
<b>REFERENCES</b> .....	<b>105</b>
<b>ARABIC SUMMARY</b> .....	



# LISTT OF TABLES

No	Title	Page
1	Nitrogen compositions (mmol L <sup>-1</sup> ) in nutrient solutions for different treatments.....	35
2	Some physical and chemical properties of the initial soil used in the experiments.....	36
3	Shoots fresh and dry weights (g. plant <sup>-1</sup> ) in lettuce plants grown in solutions with different NO <sub>3</sub> <sup>-</sup> -N/AA-N ratios and amino acid sources.....	42
4	Roots fresh and dry weight (g. plant <sup>-1</sup> ) of lettuce plants grown in solutions with different NO <sub>3</sub> <sup>-</sup> -N/AA-N ratios and amino acid sources.....	45
5	Nitrate concentration (mg.kg <sup>-1</sup> f.w) in fresh shoots of lettuce plants grown in solutions with different NO <sub>3</sub> <sup>-</sup> -N/AA-N ratios and amino acid sources.....	47
6	Nitrogen concentration (%) in the shoots of lettuce plants grown in solutions with different NO <sub>3</sub> <sup>-</sup> -N/AA-N ratios and amino acid sources.....	51
7	Nitrogen content (mg .plant <sup>-1</sup> ) in the shoots of lettuce plants grown in solutions with different NO <sub>3</sub> <sup>-</sup> -N/AA-N ratios and amino acid sources.....	53
8	Phosphorus concentrations (%) in the shoots of lettuce plants grown in solutions with different NO <sub>3</sub> <sup>-</sup> -N/AA-N ratios and amino acid sources.....	54
9	Phosphorus content (mg . plant <sup>-1</sup> ) in the shoots of lettuce plants grown in solutions with different NO <sub>3</sub> <sup>-</sup> -N/AA-N ratios and amino acid sources.....	55
10	Potassium concentration (%)in the shoots of lettuce plants grown in solutions with different NO <sub>3</sub> <sup>-</sup> -N/AA-N ratios and amino acid sources.....	57
11	Potassium content (mg . plant <sup>-1</sup> ) in the shoots of lettuce plants grown in solutions with different NO <sub>3</sub> <sup>-</sup> -N/AA-N ratios and amino acid sources.....	59
12	Essential amino acid composition (Mg. g <sup>-1</sup> ) in the shoots of lettuce plants grown in solutions with different NO <sub>3</sub> <sup>-</sup> -N/AA-N ratios and amino acid sources.....	61

No	Title	Page
13	Semi essential amino acid composition ( $Mg. g^{-1}$ ) in the shoots of lettuce plants grown in solutions with different $NO_3^-$ -N/AA-N ratios and amino acid sources.....	63
14	Non essential amino acid composition ( $Mg. g^{-1}$ ) in the shoots of lettuce plants grown in solutions with different $NO_3^-$ -N/AA-N ratios and amino acid sources.....	65
15	Shoots fresh and dry weights ( $g. pot^{-1}$ ) as affected by nitrogen forms, rates and nitrification inhibitor.....	68
16	Roots fresh and dry weights ( $g. pot^{-1}$ ) as affected by nitrogen forms, rates and nitrification inhibitor.....	73
17	Nitrate concentration ( $mg. Kg^{-1}.fw$ ) in fresh shoots of lettuce plants as affected by nitrogen forms, rates and nitrification inhibitor.....	77
18	Nitrogen, phosphorus and potassium concentrations % in shoots of lettuce plants as affected by nitrogen forms, rates and nitrification inhibitors.....	80
19	Nitrogen, phosphorus and potassium contents ( $mg pot^{-1}$ ) in shoots of lettuce plants as affected by nitrogen forms, rates and nitrification inhibitors.....	82

## LIST OF FIGURES

No	Title	Page
1.	Shoots fresh and dry weights (gm. plant <sup>-1</sup> ) of lettuce plants grown in solutions with different NO <sub>3</sub> <sup>-</sup> -N/AA-N ratios and amino acid sources.....	43
2.	Roots fresh and dry weights (gm .plant <sup>-1</sup> ) of lettuce plants grown in solutions with different NO <sub>3</sub> <sup>-</sup> -N/AA-N ratios and amino acid sources.....	46
3.	Nitrate concentrations (mg.kg <sup>-1</sup> f.w) in fresh shoots of lettuce plants grown in solutions with different NO <sub>3</sub> <sup>-</sup> -N/AA-N ratios and amino acid sources.....	48
4.	Nitrogen concentration (%) in the shoots of lettuce plants grown in solutions with different NO <sub>3</sub> <sup>-</sup> -N/AA-N ratios and amino acid sources.....	52
5.	Nitrogen content (mg.plant <sup>-1</sup> ) in the shoots of lettuce plants grown in solutions with different NO <sub>3</sub> <sup>-</sup> -N/AA-N ratios and amino acid sources.....	53
6.	Phosphorus concentration (%) in the shoots of lettuce plants grown in solutions with different NO <sub>3</sub> <sup>-</sup> -N/AA-N ratios and amino acid sources.....	54
7.	Phosphorus content (mg. plant <sup>-1</sup> ) in the shoots of lettuce plants grown in solutions with different NO <sub>3</sub> <sup>-</sup> -N/AA-N ratios and amino acid sources.....	56
8.	Potassium concentrations (%) in the shoots of lettuce plants grown in solutions with different NO <sub>3</sub> <sup>-</sup> -N/AA-N ratios and amino acid sources.....	58
9.	Potassium content (mg. plant <sup>-1</sup> ) in the shoots of lettuce plants grown in solutions with different NO <sub>3</sub> <sup>-</sup> -N/AA-N ratios and amino acid sources.....	59
10.	Shoots fresh and dry weights (g. pot <sup>-1</sup> ) of lettuce plants grown in solutions with different NO <sub>3</sub> <sup>-</sup> -N/AA-N ratios and amino acid sources.....	71
11.	Roots fresh and dry weights (g. pot <sup>-1</sup> ) of lettuce plants grown in solutions with different NO <sub>3</sub> <sup>-</sup> -N/AA-N ratios and amino acid sources.....	75

<b>No</b>	<b>Title</b>	<b>Page</b>
12	Nitrate concentration (mg. Kg <sup>-1</sup> .fw) in fresh shoots of lettuce plants as affected by nitrogen forms, rates and nitrification inhibitor.....	<b>77</b>
13	Nitrogen concentrations (%) in shoots of lettuce plants as affected by nitrogen forms, rates and nitrification inhibitor.....	<b>83</b>
14	Phosphorus concentrations (%) in shoots of lettuce plants as affected by nitrogen forms, rates and nitrification inhibitor.....	<b>85</b>
15	Potassium concentrations (%) in shoots of lettuce plants as affected by nitrogen forms, rates and nitrification inhibitor.....	<b>88</b>
16	Nitrogen content (mg pot <sup>-1</sup> ) in shoots of lettuce plants as affected by nitrogen forms, rates and nitrification inhibitor.....	<b>91</b>
17	Phosphorus content (mg pot <sup>-1</sup> ) in shoots of lettuce plants as affected by nitrogen forms, rates and nitrification inhibitor.....	<b>92</b>
18	Potassium content (mg pot <sup>-1</sup> ) in shoots of lettuce plants as affected by nitrogen forms, rates and nitrification inhibitor.....	<b>94</b>

## INTRODUCTION

Lettuce (*Lactuca sativa* L.) is a plant of considerable agricultural and economic interest but as a leafy vegetable it accumulates large quantities of nitrate especially when grown in high  $\text{NO}_3^-$ -N availability and low radiation. The accumulation of nitrate in plants depends on their genetic characteristics as well as on many environmental factors such as nitrogen supply or methods of application, light intensity, photoperiod, temperature or water supply (Mars'ic' and Osvald, 2002)

Nitrogen is the most essential mineral nutrient that promotes sufficient plant growth and consequently yield. It is absorbed by roots either as ammonium ( $\text{NH}_4^+$ ) or nitrate ( $\text{NO}_3^-$ ) ion and incorporated in amino acids and eventually proteins (Blom-Zandstra, 1989). The most common N source in hydroponics is nitrate ( $\text{NO}_3^-$ ). Frequently farmers use excessive rates of nitrogen in vegetables to avoid N-deficiency (Porto *et al.*, 2008), ignoring environmental pollution, increase in production cost as well produce quality deterioration problems (Wang *et al.*, 2008 and Montemurro, 2010).

Nitrate in vegetables is considered to be the main source of dietary nitrate intake (Santamaria *et al.*, 1998). It has been shown that 72%–94% of the  $\text{NO}_3^-$  in the human body was derived from vegetables (Shen *et al.*, 1982 and Dich *et al.*, 1996). Some previous researches suggested that the vegetables with high nitrate in the diet could put a human into the risk of gastrointestinal cancer and methemoglobinaemia

(Bartsch *et al.*, 1988 and Slob *et al.*, 1995). Therefore, there is great concern about the nitrate content in the daily diet, especially in leafy vegetables.

The objective of this work was to study the means by which nitrate concentration can be depressed in lettuce plants. To achieve this purpose, two experiments were carried out. The aim of the first experiment was to investigate the effects of nitrate –N/amino acid-N ratios on the growth,  $\text{NO}_3^-$  accumulation, and some macronutrients concentrations of lettuce and to select the best ratios of amino acid-N to  $\text{NO}_3^-$  N for hydroponic culture of lettuce. The second experiment was conducted to evaluate the effect of nitrogen sources and rates on growth, nitrate accumulation and macronutrient concentrations of iceberg (*Lactuca sativa*) varsahara; using ammonium nitrate, ammonium sulfate and calcium nitrate and nitrogen rates (0, 30, 60 and 90 kg N  $\text{fed}^{-1}$ ) with / or without adding nitrification inhibitor ammonium thiosulfate ATS.

## REVIEW OF LITERATURE

Lettuce (*Lactuca sativa*) is an annual plant of the daisy family Asteraceae. It is most often grown as a leaf vegetable, but sometimes for its stem and seeds. Lettuce was first cultivated by the ancient Egyptians who turned it from a weed. Whose seeds were used to produce oil, into a food plant grown for its succulent leaves. Lettuce is most often used for salads, although it is also seen in other kinds of food, such as soups, sandwiches and wraps; it can also be grilled. Lettuce is the only member of the *Lactuca* genus to be grown commercially (Koike et al., 2006).

Lettuce (*Lactuca sativa* L.) is a plant of considerable agricultural and economic interest but as a leafy vegetable it accumulates large quantities of nitrate especially when grown in high  $\text{NO}_3^-$ -N availability and low radiation. The accumulation of nitrate in plants depends on their genetic characteristics as well as on many environmental factors such as nitrogen supply or methods of application, light intensity, photoperiod, temperature and water supply (Mars̃ic' and Osvald, 2002)

### **1. Nitrate accumulation in vegetables and its impact on human health.**

Nitrogen is the most essential mineral nutrient that promotes sufficient plant growth and consequently yield. It is absorbed by roots either as ammonium ( $\text{NH}_4^+$ ) or nitrate ( $\text{NO}_3^-$ ) ion and incorporated in amino acids and eventually proteins (Blom-Zandstra, 1989). The most