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Reducing Nitrate Accumulation in Lettuce (Lactica Sativa L.) Plants by Using Different Biofertilizers

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ABSTRACT

This study was carried out in the two successive seasons, 1997-1998 and 1998-1999 to evaluate the effect of four different commercial biofertilizers; rhizobactrien, nitrobien, microbien and biogien on the growth, yield, chemical composition and nitrate accumulation of lettuce (*Lactuca sativa* L., cv. Dark green) plants. No significant effects could be detected on most of the studied growth characters (shoot height, number of leaves, as well as fresh and dry weights of shoots) as well as yield of the plants treated with rhizobactrien or biogien when compared with the control-untreated plants. However, significant increases were recorded by the plants treated with nitrobien, while the plants treated with microbien recorded significant decreases. Significant decrease in nitrate accumulation was recorded by the plants treated with all studied biofertilizers, specially those plants treated with nitrobien, biogien and rizobactrein, while the least decrease was recorded by the plants treated with microbien. A negative relationship between nitrate accumulation and concentration of phosphorus, total sugars, total free amino acids and soluble phenols were detected by the plants treated with the different biofertilizers. Nitrogen concentration tended to decrease by various biofertilizers in the 1st sample, while a reverse trend was detected in the 2nd one. However, no constant trend could be detected by potassium concentration. Thus, it can be suggested that the increase in total sugars, free amino acids and soluble phenols may be implicated indirectly in decreasing nitrate accumulation due to the use of these biofertilizers. Also, the increase of the previous mentioned organic components may enhance the use of nitrate transformation with the available carbon into plant structural growth.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is an important leafy vegetable crop in Egypt. It is considered as an excellent nutritive source of minerals and vitamins as it consumed as fresh green salad. Crop with such promising potentialities for local markets, would necessitate much research for improving its production; quantity and quality. An important problem facing lettuce production is nitrate accumulation in this crop. Tests of nitrate accumulation in Egyptian vegetables including lettuce showed considerable high values as compared to those found in vegetables grown in several European countries (Blom-Zandstra, 1989; Kheir *et al.*, 1991; Hanafy Ahmed, 1996 and Hanafy Ahmed *et al.,* 1997) in spite of the high intensity and long duration of day light in Egypt which favors nitrate reduction in plants. Nitrate accumulation in plants occurs as a result of nitrate accumulation in the soil due to the activity of soil nitrifying organisms. This could be mainly due to the intensive application of nitrogen fertilizers carried out by the Egyptian farmers which results in imbalancing nutritional status of the plants and consequently high nitrate accumulation as well as soil pollution. Nitrite may be formed from NO_3 after ingestion, causing methaemoglobinemia (Wright and Davison, 1964). Presence of NO_2 in blood might result also in the formation of nitroseamines, which are carcinogenic (Craddock, 1983).

Recent attention has been given to less pollution practices in modern agriculture. One of the ways to reduce soil pollution is the use of biofertilizers which have been recommended by several investigators to substitute chemical fertilizers (Saber, 1993 and El-Agory *et al.,* 1996). In addition, the use of biofertilizers may have an additional benefits such as nitrogen fixation, mobilizing phosphate and micronutrients through the production of organic acids and lowering soil pH (Saber, 1993). Besides, microorganisms such as *Pseudomonas, Azotobacter, Azospirillium* and mycorrhyzae can secrete growth promoting factors, e.g., gibberellins, cytokinins like substances and auxins (Brown, 1972 and Hartmann *et al.,* 1983). The following biofertilizers were used in this study: nitrobien, rhizobactrien, microbien and biogien. Usually biofertilizers contain one or more of the following; symbiotic and/or non-symbiotic N-fixing bacteria or phosphorus dissolving bacteria such as *Bacillus megatherium*.

The aim of this study was to increase the quality and quantity of lettuce fresh head yield by using different commercial biofertilizers. Also, this study includes attempts to reduce nitrate accumulation in lettuce plants as affected by different commercial biofertilizers as well as studying the effect of these biofertilizers on the chemical composition of this plant.

MATERIALS AND METHODS

Two field experiments were conducted during the growing seasons 97/1998 and 98/1999 at the Agricultural Experimental Station, Faculty of Agriculture, Cairo University, Giza, Egypt. Soil chemical analysis were: available nitrogen=144.2 ppm, available phosphorus=28.2 ppm and available potassium=170.0 ppm. Soil chemical analyses were conducted according to Jackson (1967). Lettuce (*Lactuca sativa* L.) cv. Dark Green transplants were planted on December 20, 1997 and November 20, 1998 in clay loamy soil with four replicates. Transplants were planted with 20 cm apart on both sides of rows with 70 cm width. Treatments included control (untreated-plants), rhizobactrien (*Azospirillium sp.* and *Azotobacter sp.*), microbein (N-fixing + phosphorus dissolving bacteria), nitrobien (*Azospirillium sp.* and *Azotobacter sp.*), and biogien (*Azotobacter sp.*). Roots of lettuce transplants were dipped into the biofertilizer prepared solution (according to the Ministry of Agriculture recommendation) immediately before transplanting. An additional 2kg/fed. of the mean biofertilizer was mixed with about 25 kg soil and added to the soil at two weeks after transplanting. Plots (10 m^2) were fertilized with ammonium sulphate (20.5% N) at the rate of 100 kg /fed. which splited into two equal doses at 3 and 6 weeks after planting, respectively. Calcium superphosphate (15.5% P_2O_5) at 150 kg/fed. and 50 kg/fed. potassium sulphate (48% K_2O) fertilizers were added to the soil as one dose before transplanting as recommended. Two samples were harvested at 70 and 100 days after transplanting. In both samples, plant height (cm), number of leaves/plant, fresh and dry weights (g/plant) as well as fresh head yield (ton/fed.) characters were recorded. Fresh head lettuce yield were harvested at 100 days after transplanting. Statistical analysis as complete randomized block design was carried and treatments mean were compared to the control-untreated plants using the least significant difference (L.S.D. 0.05) according to Snedecor and Cochran (1980).

Nitrate determination was carried out in the cell sap of the midrib of lettuce leaves as recommended by Alt and Full (1988) and Szwonek (1988). The "Merckoquant" test strips (E. Merck, Darmstadt, Germany) were used on plant sap for the estimation of nitrate status in the midrib of lettuce leaves using garlic press according to Prasad and Spiers (1982).

Determinations of total nitrogen, phosphorus and potassium were carried out on the ground dry material. The samples were digested in a mixture of sulfuric acid, salicylic acid and hydrogen peroxide according to Linder (1944). For the determination of total N, the modified "Microkjeldahl apparatus of Parnas and Wagner as described by Pregl (1945) was used. Phosphorus was determined colorimeterically using the chlorostannus reduced molybdophosphoric blue color method in sulfuric acid system as described by Jackson (1967). Potassium was determined using the flamephotometer.

Determination of total sugars, total free amino acids and total soluble phenols were carried out on the fresh material of the leaves of *Lactuca sativa* L. in the two successive samples only in the 2nd season.

Hot ethanol extract of lettuce leaves was used for the determination of total sugars using the phosphomolybdic acid method (A.O.A.C., 1975). Total free amino acids were determined using ninhydrin reagent (Moore and Stein, 1954). Total soluble phenols were estimated by using the Folin-Ciocalteu colorimeteric method (Swain and Hillis, 1959).

RESULTS AND DISCUSSION

Growth and yield:

Concerning plant growth characters; plant height, number of leaves/plant fresh and dry yield of lettuce plants data are presented in Table 1. Plant height was increased by all treatments except for plants treated with microbien in both samples of lettuce plant. Microbien significantly decreased lettuce plant height in both seasons and samples except for the 2nd sample of the 1st season. On the other hand, nitrobien treatment recorded the highest and significant increases in plant height in both samples and seasons compared to the control-untreated plants, while rhizobactrien treatment recorded non-significant increase in this connection.

Number of leaves/plant was slightly decreased in both samples in the 1st season as a result of microbien treatment, however, these decreases were significant in the 2nd season as compared to their control values. Nitrobien treatment resulted in the highest increase in the number of leaves/plant and was significant in the 2nd sample of the 1st season and in the two successive samples of the 2nd season.

Lettuce fresh weight/plant recorded significant increases when plants were treated with nitrobien as compared to the control-untreated plants in both samples in the two successive seasons. However, microbien treatment significantly decreased fresh weight/plant in the two successive samples as compared to the control-untreated values in both seasons except in the 1st sample of the 1st season where it did not reach the significant level. Similar trend was obtained for the dry weight/plant in both seasons.

All treatments except for microbien, resulted in increasing the fresh yield of lettuce heads, however, these increases were not significant except for nitrobien treated plants in both seasons. Nitrobien treatment increased lettuce total head fresh yield by 30% and 36% in the 1st and 2nd seasons, respectively.

Generally, no significant effects could be detected on most of the studied growth characters (shoot height, number of leaves, as well as fresh and dry weights of shoots) as well as yield of the plants treated with rhizobactrien or biogien when compared with the control-untreated plants. However, significant increases were recorded by the plants treated with nitrobien, while the plants treated with microbien recorded significant decreases.

Table 1: Plant height (cm), number of leaves, fresh and dry weights (g/plant) as well as fresh head yield (ton/feddan) of lettuce plants as affected by some commercial biofertilizers.

Treatment	First season								
	1st sample				2nd sample				
	Plant height	No. of leaves	Fresh weight	Dry weight	Plant height	No. of leaves	Fresh weight	Dry weight	Fresh yield
Control	26.0	25	233	19.2	38.0	36	723	50.1	10.3
Rhizobactrien	30.3	28	354	23.9	40.0	41	773	51.9	10.6
Microbien	22.3	22	179	14.3	36.7	35	448	30.5	9.2
Nitrobien	31.5	29	381	29.4	44.0	44	881	54.2	13.4
Biogien	28.8	23	276	20.5	39.0	37	681	49.9	11.0
L.S.D. (0.05)	3.2	6.0	76	5.8	4.8	7.0	105	3.3	0.95
Second season									
Control	28.0	33	423	31.9	35.9	39	683	43.1	10.8
Rhizobactrien	31.0	28	470	35.0	37.8	40	650	43.9	11.2
Microbien	24.0	25	245	20.0	29.5	31	561	39.3	9.5
Nitrobien	35.5	37	700	47.2	41.9	45	900	52.5	14.7
Biogien	33.3	31	563	37.7	38.3	34	750	44.7	11.8
L.S.D. (0.05)	2.7	3.9	161	12.0	2.5	5.8	99	4.6	1.2

The present data are in agreement with those reported by Talaat (1995) on lettuce plants. Moreover, El-Akabawy *et al.* (2000) mentioned that cotton seed yield increased significantly through the use of the biofertilizer nitrobien. This treatment excelled the remaining treatments including rhizobactrien, phosphorine as well as control. They also mentioned that nitrobien produced higher plant dry weight and N uptake. Also, Yousry *et al.* (1978) working on pea plants, pointed out that inoculation with *Bacillus megatherium* increased plant dry matter by 10.9%. Shahaby (1981) found that tomato plants inoculated with *Azospirillium* and *Azotobacter* increased dry matter by 44% and 55.1%, respectively during the summer season. Hamam (1986) working on soybean, reported that the best results in plant height, number of leaves/plant was obtained by the addition of 80 kg N/fed. or the addition of 20-40 kg N/fed. plus inoculation of seeds with strains of *Bacillus sp.* Bashan *et al.* (1989a) working on pepper and Bashan *et al.* (1989b) working on eggplant reported that inoculation of seedlings with *Azospirillium brasilense* stimulated plant growth. Cohen (1980) found that both dry weight and nitrogen contents in corn increased by 50-100% when corn seeds were inoculated with *Azospirillium* before planting. The same results were obtained by Mohandas (1987). The same author found that inoculating seedlings of tomato with *Azotobacter* resulted in high increase in leaf area, dry weight, nitrogen & phosphorus contents and yield. Similar results were obtained by Sundaravelu and Muthukrishinan (1993) on radish plants. In this respect, Schank *et al.* (1981) and Okon (1984) mentioned that N_2 fixation was not the sole cause of growth response in diazotrophs-inoculated plants. Thus, it has been suggested by many workers that other factors may also contribute to growth enhancement and yield production such as: a- formation of growth-promoting substances, e.g. auxins, gibberellins and cytokinins (Brown, 1972 and Hartmann *et al.,* 1983), b- synthesis of some vitamins, e.g. B_{12} (Mishustin and Shilnikova, 1969 and Okon, 1984), c- increasing amino acids content (Schank *et al.,* 1981), d- increasing water and mineral uptake from the soil (Lin *et al.,* 1983 and Sarig *et al.,* 1984). This could be ascribed to increases in root surface area, root hairs and root elongation as affected by a*zotobacter* as mentioned by Sundaravelu and Muthukrishinan, (1993), e- increasing the ability to convert N_2 to NH_4 and thus make it available to plant, and f- enhancing the production of biologically active fungistatical substances which may change the microflora in the rhizosphere and affect the balance between harmful and beneficial organisms (Apte and Shende, 1981). Similar results and suggestions were reported by Hanafy Ahmed *et al.* (1997) on jew's mallow and radish plants.

Chemical analysis:

Nitrate accumulation data as affected by different commercial biofertilizers treatments are presented in Table 2. All biofertilizer treatments decreased nitrate concentration in the midrib of lettuce leaves in both samples and seasons, except for microbien treatment in the 2nd sample of the 2nd season, as compared to the control-untreated plants. This decrease in nitrate concentration was significant in all treatments except for microbien in the 1st sample and rhizobacterien treatment in the 2nd sample of the 1st season where this decreases were not significant.

All biofertilizer treatments resulted in significant decreases in total nitrogen in the leaves of lettuce plants as compared to the control treatment except for microbien treatment where this decrease was not significant in the 1st sample of the 2nd season (Table 2). Conversely, in the 2nd sample in both seasons, all biofertilizer treatments recorded a reverse trend; significant increases were detected only in the 2nd season. In this respect, it can be suggested that increasing total nitrogen concentration in the leaves of lettuce plant under these biofertilizer treatments is not necessarily accompanied by increasing nitrate accumulation in the plant. This might be due to the utilization and conversion of nitrogen from the simple form such as nitrate to more complicated one such as proteins and amino acids. Similar suggestion was reported by Hanafy Ahmed (1996) on lettuce plant.

Phosphorus concentration in the leaves of lettuce plants was increased as compared to the control-untreated plants, except for rhizobactrien (insignificant decrease) in the 1st sample of the 2nd season (Table 2). Nitrobien treatment recorded significant increases in phosphorus concentration in the leaves of lettuce plants in the 2nd sample of the 1st season and 1st sample of the 2nd season as compared to the control-untreated plants. In this respect, it can be suggested that, the decrease of nitrate accumulation due to increasing endogenous phosphorus concentration in biofertilizers treated plants may have a role in nitrate reduction. In this connection, Yoneyama (1988) reported that inorganic P within plant is necessary for metabolism and storage, but high concentrations inhibit enzyme reactions and is accompanied by less nitrate uptake and accumulation. However, there was no significant differences in phosphorus concentration in the leaves of lettuce plants as compared to the control-untreated plants in the 2nd sample of the 2nd season. In this respect, Maynard *et al.* (1976) mentioned that phosphorus was found to have no or little effect on nitrate accumulation.

Potassium concentration in the leaves of lettuce plants significantly increased as a result of rhizobactrien treatment, whereas both biogien and nitrobien treatments recorded significant decreases when compared to the control-untreated plants in the 1st sample of the 2nd season (Table 2). No significant differences in potassium concentration were recorded by all biofertilizer treatments, except nitrobien treatment which recorded a significant increase in the 2nd sample of the two successive seasons. Furthermore, it is important here to mention that, there is a positive relationship between the high values of potassium concentration and all of the studied growth characters when lettuce plants were treated with nitrobien. In this respect, potassium may play a role on the synthesis of endogenous plant hormones (Hanafy Ahmed, 1986 and 1997), effect on chlorophyll synthesis, carbohydrate formation and leaf moisture content as well as through the effect of potassium on stomatal behavior (Mac-Robbie, 1988 and Huang *et al.,* 1991). In addition, a negative relationship between potassium concentration and nitrate accumulation could be detected as a result of different commercial biofertilizers treatments. In this respect, Hanafy Ahmed *et al.* (1997); Ni WuZhong *et al.* (1997) and Wang Xiu Fung and Ito (1998) mentioned that increasing potassium fertilizer level resulted in reducing nitrate accumulation in some vegetable crops. Furthermore, Ali *et al.* (1985) reported that nitrate reductase activity in the leaves and stems of rice plants supplied with K was higher than in those plants deficient in K. However, Drlik and Rogl (1992) working on carrots, reported that the soil K resources had no effect on nitrate accumulation. A similar relationship was reported by Blom-Zandstra *et al.* (1988) on lettuce plants.

Table 2: Total nitrogen (N), phosphorus (P) and potassium (K) concentrations (mg/g D.W.) in the leaves of lettuce plants as well as nitrate concentration (ppm) of the midrib of lettuce plants as affected by some commercial biofertilizers.

Treatment	1st sample				2nd sample			
	N	P	K	Nitrate	N	P	K	Nitrate
First season								
Control	--	--	--	3300	29.8	6.3	26.2	3433
Rhizobactrien	--	--	--	2600	31.2	7.5	25.2	2900
Microbien	--	--	--	2860	31.6	8.2	25.4	2600
Nitrobien	--	--	--	2630	29.8	9.1	29.5	2333
Biogien	--	--	--	2730	32.4	8.2	25.3	2800
L.S.D. (0.05)	--	--	--	534	N.S.	2.41	2.84	628
Second season								
Control	39.8	6.6	30.4	3467	31.5	10.9	30.8	3800
Rhizobactrien	32.8	5.9	33.9	2500	35.0	9.3	30.4	1315
Microbien	37.7	6.9	31.8	2800	45.0	10.9	29.6	3800
Nitrobien	36.1	10.4	28.0	1750	37.8	10.4	31.2	1363
Biogien	33.6	7.7	24.8	968	40.3	11.3	30.8	1375
L.S.D. (0.05)	3.08	1.68	1.18	195	2.93	N.S.	N.S.	197

Total sugars, free amino acids and total soluble phenols data are presented in Table 3. Total sugars concentration in the leaves of lettuce plants was increased by all biofertilizer treatments in both samples as compared to the control-untreated plants. This increase reached the significant level by the plants treated with microbien and biogien in the 1st sample as well as rhizobactrien, microbien and nitrobien in the 2nd sample as compared to the control-untreated plants.

Total free amino acids concentration in the leaves of lettuce plants was increased by all biofertilizer treatments in both samples except for rhizobactrien (insignificant decrease) in the 1st sample. This increase reached the significant level only by biogien treatment in the 1st sample and all treatments in the 2nd sample.

Total soluble phenols concentration in the leaves of lettuce plants was significantly increased in both samples as compared to the control-untreated plants except for rhizobactrien in the 1st sample.

It might be suggested that simple organic molecules such as sugars, free amino acids and total soluble phenols may act as an osmoticum for the regulation of plant osmosis. These simple organic molecules or solutes can replace nitrate in the cell vacuoles. In the present work, there was a negative correlation between nitrate accumulation and the organic compounds of sugars, free amino acids and total soluble phenols. In this respect, Reinink *et al.* (1987) assumed that a higher percentage of dry weight was associated with higher concentration of organic solutes in lettuce vacuole. The same results were confirmed by Hanafy Ahmed *et al.* (1997) who reported that biofertilizers treatments can lower nitrate concentration in jew's mallow and radish plants while sugars, amino acids and several nutrient concentrations were higher. In this respect, Hanafy Ahmed (1996) suggested that the increments in sugars and free amino acids concentrations in lettuce under $CaCl_2$ treatments may play as an osmoticum. In addition, it can also be suggested that the increase in both total free amino acids and sugars concentrations which were detected by proline treated-plants may be implicated indirectly in decreasing nitrate accumulation by lettuce plants. In this respect, the availability of sugars will affect the need for nitrate as an osmoticum (Blom-Zandstra *et al.,* 1988). The same authors working on lettuce, pointed out that NO_3 accumulation was inversely related to the accumulation of sugars and organic acids. Furthermore, Muller and Touraine (1992) and Imsande and Touraine (1994) mentioned that increasing the concentration of certain amino acids in the phloem sap causes an inhibition of nitrate uptake. In addition, Van der Boon *et al.* (1988) pointed out that organic N compounds of low molecular weight could be used as osmoticum by lettuce.

As mentioned previously, bacteria in biofertilizers can produce different compounds such as organic acids, plant growth promoters (auxins, gibberellins and cytokinins) as well as nitrogen fixation, dissolving phosphorus and production of organic acids in the soil wich consequently lowering soil pH. The ultimate resultant of those factors is improving plant growth through nutrient uptake. In this respect, Thibodeau and Minotti (1969) mentioned that the application of the organic acids salts; acetate, citrate and particularly oxalate, accelerated the growth of lettuce plants. Hanafy Ahmed (1996) reported that the organic acid citric increased sugars concentration in lettuce. Moreover, Hanafy Ahmed (1996) working on lettuce mentioned that the highest value of yield (ton/feddan) was recorded by plants sprayed with citric acid: mainly due to increases in both fresh and dry weights of head. However, the lowest yield was detected by the plants sprayed with salicylic acid, mainly due to decreases in height of plant, number of leaves as well as fresh and dry weights.

Table 3: Total sugars, free amino acids and soluble phenols concentrations (mg/g F.W.) in the leaves of lettuce plants as affected by some commercial biofertilizers.

Treatment	1st sample			2nd sample		
	Sugars	Amino acids	Soluble phenols	Sugars	Amino acids	Soluble phenols
Control	4.17	4.23	0.87	7.30	2.10	0.31
Rhizobactrien	4.52	4.02	0.76	9.53	5.20	0.76
Microbien	5.96	4.65	0.98	8.83	2.33	0.35
Nitrobien	4.97	4.84	0.90	9.64	3.00	0.68
Biogien	6.44	4.99	1.30	7.70	5.78	0.63
L.S.D. (0.05)	1.34	0.70	0.07	0.86	0.18	0.04

Increasing total sugars, free amino acid and total soluble phenols may use the accumulated nitrate in plant and enables it to use more carbohydrates for plant structural growth. In this respect, Blom-Zandstra *et al.* (1988) confirmed this relation and indicated that when a plant accumulated nitrate as an osmoticum, more carbohydrates could be used to increase the dry matter production. In this connection, Reinink *et al.* (1987) assumed that a higher percentage of dry weight was associated with a higher concentration of organic solutes in lettuce vacuole, which reduced the need of the plant for nitrate.

It is well known that biofertilizers can lower the amount of added chemical nitrogen fertilizer to the soil and consequently mitigation of pollution. In addition, the beneficial effects of biofertilizers such as production of organic acids (lowering soil pH) and production of plant growth regulators may contribute to a better plant growth and yield through enhancing nutrient uptake (Ibrahim and Abdel-Aziz, 1977). In the present work, biofertilizers may decrease nitrate accumulation in lettuce plants, however, further studies are needed to study the effect of these biofertilizers on nitrate accumulation under different levels of soil nitrogen fertilization. Biofertilizers increased the concentration of simple organic molecules such as sugars, free amino acids and total soluble phenols which played a role in regulation of plant osmosis and consequently better plant growth and yield.

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خفض تراكم النترات فى نبات الخس باستعمال بعض المخصبات الحيوية .

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الملخص العربى

أجريت تلك الدراسة خلال موسمى 1998/97 و1999/98لتقييم فاعلية اربعة مخصبات حيوية تجارية (رايزوباكترين ونيتروبين وبيوجين وميكروبين) على النمو والمحصول والتركيب الكيماوى وكذلك تراكم النترات فى نباتات الخس صنف دارك جرين. لم يكن هناك أى فروق معنوية لمعظم صفات النمو (ارتفاع النبات، عدد الاوراق، بالاضافة الى الوزن الطازج والجاف للنبات) اضافه الى المحصول للنباتات المعاملة الرايزوباكترين أوالبيوجين عند مقارنتها بالنباتات الغير معاملة . وعلى العكس من ذلك فقد أظهرت معاملة بالنيتروبين زيادة معنوية بينما أظهرت معاملة الميكروبين نقصا معنويا فى تلك الصفات. كان هناك نقصا فى تراكم النترات نتيجة لكل معاملات المخصبات الحيوية تحت الدراسة وخصوصا النباتات المعاملة بالنيتروبين والبيوجين والرايزوباكترين. بينما سجل اقل نقص نتيجة المعاملة بالميكروبين. كان هناك ارتباط سلبى بين تراكم النترات وتركيز كل من الفوسفور والسكريات الكلية والاحماض الامينية الحرة الكلية والفينولات الذائبة نتيجة المعاملة بالمخصبات الحيوية تحت الدراسة. أما تركيز النتروجين الكلى فكان منخفضا فى العينة الاولى نتيجة المعاملة بالمخصبات الحيوية تحت الدراسة بينما زاد تركيزه فى العينة الثانية. بينما لم يكن هناك اتجاه ثابت لتركيز عنصر البوتاسيوم. ومن ثم يمكن ان يعزى نقص تركيز النترات الى زيادة تركيز السكريات الكلية والاحماض الامينية الحرة الكلية والفينولات الذائبة عند استعمال تلك المخصبات الحيوية. كذلك فان زيادة المركبات العضوية السابقة ربما تؤدى الى استعمال النترات مع الكربون المتاح وتحويلهما الى مركبات بنائية تزيد من نمو النبات.