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ORIGINAL ARTICLES

Evaluation of Integrated Nutrient Management Practices for Lettuce Production under Drip Irrigation System

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ABSTRACT

It is well known that trophic interactions between plant cover and soil organisms are key factors in ecosystems balance. In present study, the effect of combination among mineral, organic and bio-fertilizers on quality and productivity of lettuce under drip irrigation conditions have been studied. The combination effect of fertilizers on soil microbial enzyme activities also was studied. The highest total yield of lettuce is 31.96 and 31.84 Mg ha^{-1}, respectively during the two growing seasons. The best yield components were obtained with using combination of mineral, organic and bio-fertilizers (50 % mineral + 50 % organic + biofertilizers). Whereas, the using of organic and biofertilizer treatments recorded the lowest content of nitrate in leaves 0.82 and 0.81 g kg^{-1} DW, respectively, were observed in both seasons. The highest soil dehydrogenase, alkaline phosphatase and nitrogenase activities were 64.2 and 51.0 µg TPF g^{-1} dw h^{-1}, 22.3 and 19.9 µg ρNP g^{-1} h^{-1} and 83.7 and 81.2 µL C_2H_4 g^{-1} dw h^{-1} respectively in both seasons and after 30 days from lettuce transplanting were achieved by using of the combination of fertilizers (50 % mineral + 50 % organic + biofertilizer). The results of this study suggest that, it could be depended on the biological or organic farming to produce a better food and the integrated fertilization program should be followed in agriculture under drip irrigation even in clay loam soil.

***Key words*:** Lettuce, drip irrigation, biofertilizers, organic-mineral fertilizers, nitrate content, enzyme activities

Introduction

Lettuce (*Lactuca sativa* L.) is the most popular vegetable according to the highest consumption rate and economic importance throughout the world. It is considered as an excellent nutritive source of minerals and vitamins as it consumed as fresh green salad. An important problem facing lettuce production is nitrate accumulation. 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) 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 intensive application of nitrogen fertilizers carried out by the Egyptian farmers which results in unbalancing 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 methemoglobinemia (Sanchez *et al,* 2001). Presence of NO_2 in blood might result also in the formation of nitrosamines, which are carcinogenic (Ahmed *et al.* 2000).

Also, increase in N fertilizer led to increase in nitrate content of the crop tissues without significant increase in yield (Custic *et al.* 1994). Hence, increasing the use of chemical fertilizer led to high cost in vegetable production and creates pollution of their agricultural environment as well as affects the soil fertility; therefore it has become essential to use untraditional fertilizers as supplements or substitutes for chemical nitrogen fertilizer. Both bio-fertilization and organic fertilizer may be the solution of pollution decrease and high cost of chemical fertilizer to increases in our exporter. Many investigations presented the effect of untraditional fertilizers on grown lettuce such as Hosseny and Ahmed (2009) who showed that compost manure at high rate of nitrogen was associated with low nitrate concentration in lettuce. Ahmed *et al.* (2000) found that lettuce plant treating with *Azotobacter chroococcum* and *Azospirillium lipoferum* as a biofertilizer resulted in significant increases in shoot height, number of leaves and fresh weight. While, there were significant decreases when treated with *Azotobacter chroococcum*, *Azospirillium lipoferum* and *Bacillus megaterium*. A significant decrease in nitrate accumulation was noticed when the plant treated with biofertilizers. The benefits of bio-fertilizer on grown lettuce were presented by many searches such as (Chabot *et al.* 1996 and Noel *et al.* 1996).

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Fertilizers should be applied in a form that becomes available in synchrony with crop demand for maximum utilization of nitrogen from fertilizers (Boyhan *et al.* 2001). The method of fertilizer application is very important in obtaining optimal use of fertilizer. It is recommended that fertilizer should be applied regularly and timely in small amounts (Neeraja *et al.* 1999). This will increase the amount of used fertilizer by the plant and reduce the lost amount by leaching (Rajput and Patel, 2006). It is becoming increasingly clear that conventional agricultural practices can not sustain the production base, a healthy plant soil system for too long. While, to augment crop productivity, agronomist heavily depends on chemical fertilizers (Khan *et al.* 2009).

Drip irrigation is often preferred over other irrigation methods because of the former's high water-application efficiency on account of reduced losses, surface evaporation and deep percolation. Because of high frequency water application, concentrations of salts remain manageable in the rooting zone (Mantell *et al.* 1985). Root of plant is an important consideration for efficient management of mobile nutrients such as nitrate-nitrogen (NO_3-N) (Patel and Rajput, 2002). Boswell *et al.* (1985) reported that NO_3-N is relatively unreactive and therefore, susceptible to movement through diffusion and mass transport in the soil water (Rajput and Patel, 2006). NO_3-N is very mobile and if there is sufficient water in the soil, it can quickly move through the soil profile. Careful application of nitrogen and water should be able to minimize the amount of nitrogen moving below the root zone (Drost and Koenig, 2001).

Integrated nutrient management (INM) is a well-accepted approach for the sustainable management of soil productivity and increased crop production (FAO, 2008). Management practices that sustain lettuce production and improve of soil as well water quality are needed. So, the objective of the present study was to investigate the response of lettuce to mineral fertilizers and organic manure combination with and without biofertilizer under drip irrigation conditions and its effect on soil microbial enzyme activities. Hence, integrated program of fertilization for lettuce production could be evaluated.

Material and Methods

Experimental design:

This experiment was carried out during two successive seasons of 2010 and 2011 at the Experimental Farm, Faculty of Agriculture, Moshtohor, Benha University to study the effect of mineral fertilizers, organic manure and biofertilizers as well their interactions on the growth, yield components and nitrate content of lettuce (*Lactuca sativa* var. *capitata cv.* Great lakes) grown under clay loam soil and drip irrigation system. Lettuce plants were transplanted at the first week of October in the two growing seasons and harvested after 10 weeks from transplanting. The experimental treatments were arranged in a randomized complete blocks design and included ten treatments with four replicates as tabulated in **Table 1**. Transplanted plants were inoculated by dipping for about 30 minutes in a solution consists of biofertilizer cultures and 40 % (w/v) sucrose solution and then took place in the permanent field.

Table 1: Experimental design.

Treatments	Description
T1	100 % Mineral-N (Control)
T2	100 % Mineral-N + Biofertilizer
T3	75 % Mineral-N + 25 % Organic-N
T4	75 % Mineral-N + 25 % Organic-N + Biofertilizer
T5	50 % Mineral-N + 50 % Organic-N
T6	50 % Mineral-N + 50 % Organic-N + Biofertilizer
T7	25 % Mineral-N + 75 % Organic-N
T8	25 % Mineral-N + 75 % Organic-N + Biofertilizer
T9	100 % Organic-N
T10	100 % Organic-N + Biofertilizer

Biofertilizer solution was containing 500 ml of N_2- fixing free living bacterial cultures (*Azotobacter chroococcum;* 8.4×10^{11} CFU ml^{-1} and *Azospirillium lipoferum* D178; 7.2×10^{11} CFU ml^{-1}) and 500 ml of phosphate dissolving bacterial culture (*Bacillus megaterium;* 8.3×10^{11} CFU ml^{-1}). The biofertilizer cultures were prepared by strains reserved in the Agriculture Botany Department (Microbiology Branch), Faculty of Agriculture, Benha University, Egypt. The biofertilizers solution are divided into two parts. The first one added during soil preparation with compost and the second was used for soaking to transplants. Nitrogen, phosphorus and potassium fertilizers were added as NH_4NO_3 (33.5 % N), Ca $(H_2PO_4)_2.CaCO_3$ (16% P_2O_5) and K_2SO_4 (48 % K_2O). The organic manure obtained from Moshtohor village and phosphate fertilizer were added for experimental plots during soil preparation. Meanwhile, NH_4NO_3 and K_2SO_4 fertilizer were added weekly within the drip irrigation system. Phosphorus (77 kg P_2O_5 ha^{-1}) and potassium (58 kg K_2O ha^{-1}) fertilizers were added to all treatments. The added rates of compost and ammonium nitrate fertilizers were calculated on basis of N % (55 kg N ha^{-1}) in both of them. Data of chemical composition of compost manure is given in **Table 2**.

Table 2: Chemical analysis of compost.

Parameter	Unit	Value
pH	-	7.6
EC (1:5 extract)	dS m^{-1}	3.1
Organic matter	%	33.57
Total-N	%	1.21
C:N ratio	-	10.36 : 1
Total-P	%	0.91
$N-NH_4$	mg kg^{-1}	274.7
$N-NO_3$	mg kg^{-1}	50.1

Soil and plant analyses:

Table 3: Physical and chemical analyses of the experimental soil before transplanting.

Soil texture				pH	EC (dS m^{-1})	O.M (%)	$CaCO_3$ (%)
Sand (%)	Silt (%)	Clay (%)	Texture				
19.3	20.9	59.8	Clay loam	7.9	2.16	1.41	1.53

Soil available macronutrients (mg kg^{-1})			Total content of soil trace elements (ppm)						
N	P	K	B	Zn	Mn	Cu	Cd	Ni	Pb
22.5	9.1	120	15.15	89.73	935	64.65	0.154	60.56	9.16

Aqua Regia was used to digest soil sample for total contents of the investigated trace elements (Cottenie *et al.* 1982).

Random soil samples were taken before lettuce transplanting for biological, chemical and physical analysis as described by Chapman and Pratt (1961) and Jackson (1965). The experimental farm soil was clay loam soil texture with pH 7.9. The enzymes activity of dehydrogenase (DHA), alkaline phosphatase (AlP) and nitrogenase (N_2-ase) were measured using method of Schinner et al (1997) before cultivation and after 30 and 60 days from transplanting in the rhizosphere.

At harvest, four plants from each plot were randomly taken to evaluate vegetative growth characteristics, i.e., plant height (cm), plant diameter (cm), number of leaves/plant, total fresh and dry weight of leaves per plant and fresh yield (Mg ha^{-1}). Nitrate content in lettuce leaves was determined according to the method described by Cataldo et al. (1975).

Statistical analysis:

All obtained data were recorded on plot basis and statistically analyzed according to the randomized complete block design in factorial arrangement using, Duncan's Multiple Range Test at 5 % level to compare between treatment means as described by Gomez and Gomez (1984).

Results and Discussion

Lettuce vegetative growth, yield and it's components:

In present work, the effect of mineral, organic and bio-fertilizers on lettuce productivity under drip irrigation system have been studied. The following vegetative growth: plant height, head diameter, number of leaves per plant, fresh and dry weight of leaves per plant as well as total yield per hectare were tabulated in **Table 4**. The highest values of vegetative growth characteristics were recorded with using 50 % mineral + 50 % organic + biofertilizer (T6), i.e., 15.0 and 14.6 cm, 13.0 and 12.6 cm, 30.6 and 30.1 per plant, 404.5 and 393.7 g $plant^{-1}$, 35.8 and 33.8 g $plant^{-1}$, and 31.96 and 31.84 Mg ha^{-1}, respectively for first and second seasons. In general, the lowest values of vegetative growth characteristics were recorded with using the organic and biofertilizers (T10), i.e., 10.1 and 10.4 cm, 9.9 and 9.7 cm, 22.0 and 22.7 g $plant^{-1}$, 154.4 and 150.4 g $plant^{-1}$, 18.6 and 20.2 g $plant^{-1}$, and 12.26 and 12.22 Mg ha^{-1}, respectively for 2010 and 2011 seasons and with significant difference as compared with all other treatment in head diameter, fresh weight of leaves per plant and total fresh yield per hectare as shown in both seasons. The highest total yield of lettuce and most yield components were obtained using combination of mineral, organic and bio- fertilizers (T6: 50 % mineral + 50 % organic + biofertilizer) with significant difference as compared with the control (T1: 100 % Mineral) as shown in the second season only in the total yield and in both seasons in dry weight of leaves.

The superiority of using 50 % of the required N in the organic form and 50 % in the mineral form on vegetative growth may be due to the favorable effect of the mineral nitrogen on the activity of microorganisms responsible for organic fertilizer analysis in the soil (Follett *et al.* 1981). The positive effect of biofertilizer in this case indicated that there were no competition between plants and microorganisms for mineral-N uptake, hence, compost the source of organic-N was well decomposed, this explanation is in harmony with *Rai* (2006).

Table 4: Effects of different mineral, organo- and bio-fertilizer combinations on vegetative growth, yield and yield components of lettuce.

Treatments	Plant height (cm)		Head diameter (cm)		Leaves No. /plant	
	2010	2011	2010	2011	2010	2011
T1	12.5 d	12.1 c	10.7 de	10.9 d	26.8 b	24.5 c
T2	12.6 d	12.3 c	11.2 c	10.8 de	27.1 b	27.0 b
T3	11.8 e	11.9 c	10.9 cd	10.5 ef	25.0 c	24.1 c
T4	13.1 c	13.4 b	11.2 c	11.5 c	27.6 b	28.0 ab
T5	14.1 b	13.6 b	11.8 b	12.1 b	28.3 b	29.5 a
T6	15.0 a	14.6 a	13.0 a	12.6 a	30.6 a	30.1 a
T7	11.5 ef	11.4 d	10.4 e	11.0 d	24.6 cd	23.4 c
T8	11.1 f	11.1 d	10.6 e	10.8 de	23.0 de	23.4 c
T9	10.5 g	10.1 e	10.5 e	10.2 f	22.0 e	22.7 c
T10	10.1 g	10.4 e	9.9 f	9.7 g	22.0 e	22.7 c

Fore more details about treatments: T1 to T10 see Table 1
Values followed by the same letters are not significantly different by LSD's test at 0.05 level

Table 4: Conti.

Treatments	Leaves fresh weight (g /plant)		Leaves dry weight (g /plant)		Total yield (Mg ha^{-1})	
	2010	2011	2010	2011	2010	2011
T1	402.1 a	389.6 a	27.4 d	27.8 cd	31.58 a	27.28 d
T2	351.3 b	336.0 b	26.7 d	26.7 cde	28.03 b	24.31 e
T3	326.2 b	321.6 b	25.9 e	26.1 cde	25.65 c	29.78 c
T4	398.0 a	387.9 a	28.4 c	28.8 bc	31.48 a	31.36 b
T5	400.0 a	393.7 a	30.4 b	31.5 ab	31.70 a	31.60 ab
T6	404.5 a	388.2 a	35.8 a	33.8 a	31.96 a	31.84 a
T7	280.4 c	288.9 c	24.9 f	27.5 cd	22.62 d	22.43 f
T8	282.9 c	279.8 c	24.4 f	24.3 de	21.74 d	20.40 g
T9	195.0 d	182.3 d	23.0 g	23.5 ef	16.42 e	15.03 h
T10	154.4 e	150.4 e	18.6 h	20.2 f	12.26 f	12.22 i

Fore more details about treatments: T1 to T10 see Table 1
Values followed by the same letters are not significantly different by LSD's test at 0.05 level

The role of biofertilization strains in production of phytohormones and/or improving the availability and acquisition of nutrients or by both, may explain the encouraged growth of inoculated plants with non – symbiotic N-fixing bacteria (Barakat and Gabr, 1998). Furthermore, *Azotobacter* and *Azospirillium* could produce IAA and cytokinins which increased the surface area per unit of root length and were responsible for root hair branching with an eventual increase in acquisition of nutrients from the soil (Jain and Patriquin, 1985). Many organic acids which are produced by rhizosphere microorganisms are effective in solubilizing of soil phosphates (Marschner, 1995).

The effect of different fertilizers on yield and yield components of lettuce compared to the control treatment can be noticed. Depending on data in Table (4). It could be also reported that application of biological or organic fertilizers alone cannot provide required N and P completely, but applying these fertilizers with the chemical fertilizer will be effective in increasing yield of lettuce. In combination of fertilizers, essential nutrient elements for plants have been provided, thus dry matter and yield will be increased. Application of organic fertilizer combined with chemical fertilizer is an important approach to maintain and improve the soil fertility, increasing fertilizer use efficiency and improving crop yield (Xu *et al.* 2008).

Considering the positive effects mentioned for organic and bio fertilizers, integrated use biological fertilizer with chemical and organic fertilizers increased yield of lettuce. Similar result in application of fertilizers combination were obtained by other scientists (Ekin *et al.* 2009 on potato plant; Efthimiadou *et al.* 2010 on sweet maize plant; Habibi *et al.* 2011 on pumpkin; Shams, 2012 on kohlrabi).

Application of organic fertilizer alone decreased the most vegetative growth characteristics as compared to application of chemical fertilizer alone. This could be attributed to deficiency mineral nitrogen in the early development of plant and nitrogen use by soil microorganisms to break down organic materials. These results agree with those of Zaki *et al.* (2008) on sweet pepper who found that the highest vegetative growth and total yield were obtained by applying 50 % organic-N + 50 % mineral-N as compared with the full dose of nitrogen in the mineral or organic form.

Nitrate accumulation in lettuce plants:

It is well known that, lettuce 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 NO_3-N availability. The accumulation of nitrate in plants depends on their genetic characteristics, nitrogen supply or methods of application, light intensity, photoperiod, temperature or water supply (Maynard and Barker, 1972). All factors influencing the nitrate uptake, translocation and assimilation processes may affect nitrate concentration in plant tissue (Maynard *et al.* 1976). Drews *et al.* (1997) reported that the content of nitrate in lettuce plants decreased as development progressed from stage A (heads beginning to form) to stage C (head development completely). They ascribed this variation to the changing ratio of outer, medium and inner leaves during heading (Marsic and Osvald, 2002). The using of organic and biofertilizer treatments recorded the lowest content of nitrate in leaves (0.82 g kg^{-1} DW) for both seasons 2010 and 2011 this result was in agreement with Hosseny and Ahmed (2009) who showed that compost manure at high rate of nitrogen was associated with low nitrate concentration in lettuce. Whereas, the highest content of nitrate in leaves was 3.62 g kg^{-1} DW as average in both seasons and obtained by using the control (100 % mineral). But the plants which received 50 % Mineral + 50 % Organic + Biofertilizer gave a medium amount of nitrates (1.67 g kg^{-1} DW) as shown in Fig. 1. That means using mineral fertilizers in lettuce production may be contributed to increase some toxic compounds in plants like nitrate. Whereas, using organic and biofertilizers help to produce the safe and best food containing the lowest content of toxic compounds like nitrate in leaves of lettuce.

In 1995, the European Commission Scientific Committee on Food (SCF) agreed to retain its earlier Acceptable Daily Intake (ADI) for the nitrate ion of 3.7 mg kg^{-1} body weight (European Commission, 1997). Maximum level of nitrates and nitrites permitted in lettuce in Romania is 2 g kg^{-1} DW (Socaciu and Stanila, 2007). Hoque *et al.* (2008) reported that a number of factors influence NO_3 and NO_2. These include the type, amount and form of N fertilizer, as well accumulation in vegetables. High nitrate levels, especially 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 (Bozkurt *et al.* 2009).

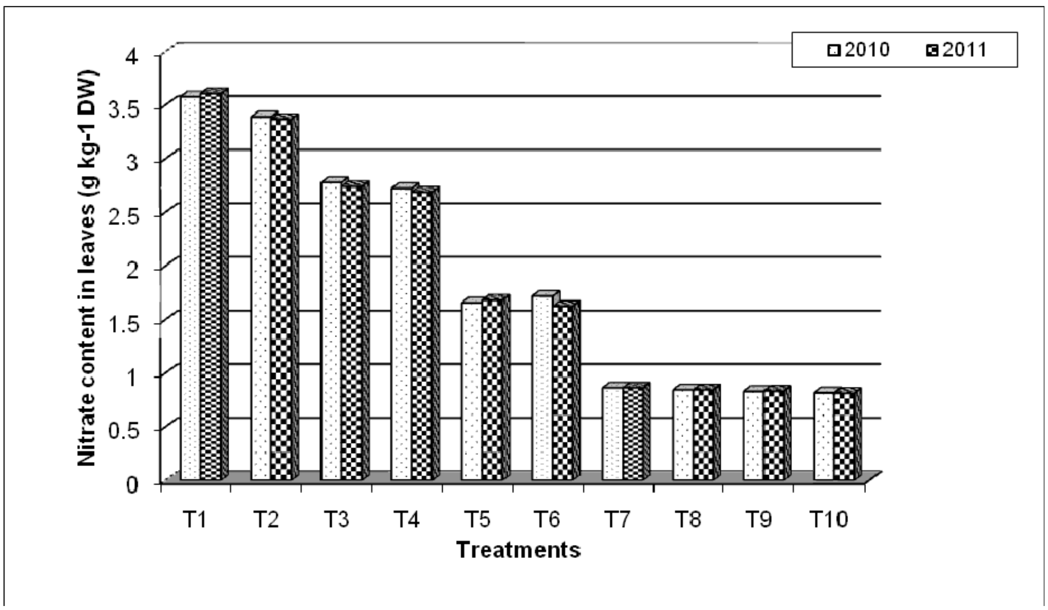

Fig. 1: Effects of different mineral, organo- and bio-fertilizer combinations on nitrate content in lettuce leaves.

Soil enzymes activity:

Soil enzymes activity are the direct expression of the soil community to metabolic requirements and available nutrients. While, the diversity of soil organisms is important, the capacity of soil microbial communities to maintain functional diversity of those critical soil processes through disturbance, stress or succession could ultimately be more important to ecosystem productivity and stability than taxonomic diversity. Acid phosphatase is released by roots and soil microorganisms, whereas alkaline phosphatase is only produced by microorganisms. Acid and alkaline phosphatase activities are often increased in rhizosphere compared to the bulk soil (Tarafdar and Claassen, 1988).

In the two growing seasons, the effects of organic (50 %) and biofertilizers tended to be stronger when applicated with additional mineral fertilization (50 %). The combination of compost and mineral fertilizer (50 and 50 %, respectively) as well as biofertilizer (T6) recorded the higher values of dehydrogenase (DH), alkaline phosphate (AlP) and nitrogenase (N_2-ase) activities (Tables 5, 6 and 7). In two seasons, the DH, AlP and N_2-ase activities were increased from 7 days to reach the highest values after 30 days from transplanting and decreased thereafter at 60 days from transplanting, this was true in two seasons. This results are agree with (Burns and

Dick, 2002) who reported that intracellular enzyme activities are short-lived because they are degraded by proteases unless they are adsorbed by clays or immobilized by humic molecules. The highest DH, AlP and N_2-ase activities were clearly observed in the treatments after 30 days from transplanting. It is clear that the increase in nitrogenous fertilizer led to decrease the nitrogenase activity, which explains superiority T6 in vegetative growth and total yield over than control treatment (T1: 100 % mineral-N), this is reflected clearly in Table 4.

Table 5: Effect of mineral, organo- and bio-fertilizer combinations on dehydrogenase activity.

Treatments	Soil dehydrogenase (µg TPF g^{-1} dw h^{-1})					
	First season			Second season		
	7 days	30 days	60 days	7 days	30 days	60 days
T1	19.5 o	31.1 hi	23.4 l	23.4 no	37.3 i	28.1 l
T2	20.8 mn	33.7 g	29.4 j	23.3 no	40.4 f	35.3 j
T3	19.7 o	31.8 ij	25.7 k	22.1 p	35.6 i	28.8 l
T4	21.4 mn	38.8 e	29.5 j	24.2 mn	43.5 e	33.0 k
T5	19.8 no	49.2 b	33.8 g	22.2 op	59.0 c	38.6 gh
T6	20.9 mn	51.0 a	37.5 e	23.4 no	64.2 a	40.6 f
T7	20.2 mn	32.4 gh	26.4 k	22.8 no	36.6 hi	29.8 l
T8	21.6 m	45.8 c	30.4 ij	25.9 m	51.8 d	36.5 ij
T9	20.4 mn	43.1 d	33.1 g	24.5 mn	59.7 bc	38.5 gh
T10	21.7 m	50.4 ab	35.9 f	24.3 mn	61.1 b	40.2 f

For more details about treatments: T1 to T10 see Table 1
Values followed by the same letters are not significantly different by LSD's test at 0.05 level

Table 6: Effects of mineral, organo- and bio-fertilizer combinations on alkaline phosphatase activity.

Treatments	Soil alkaline phosphatase (µg ρNP g^{-1} h^{-1})					
	First season			Second season		
	7 days	30 days	60 days	7 days	30 days	60 days
T1	14.4 i	14.8 hi	14.6 hi	17.3 ghi	17.8 efg	17.5 fgh
T2	14.6 hi	15.8 ef	15.4 efg	16.4 jk	19.0 cd	18.5 cde
T3	14.4 i	15.4 efg	14.8 ghi	16.1 k	17.2 ghi	16.6 hij
T4	14.6 hi	16.6 cd	16.2 cde	16.5 hij	18.6 cde	18.1 def
T5	14.4 i	15.6 efg	14.9 ghi	16.1 k	18.7 cde	17.9 efg
T6	14.7 hi	19.9 a	17.6 b	16.5 hij	22.3 a	20.5 b
T7	14.4 i	15.8 def	14.8 ghi	16.3 ijk	17.9 efg	16.7 hij
T8	14.6 hi	16.6 cd	16.2 cde	17.5 fgh	18.8 cde	19.4 c
T9	14.5 i	16.2 cde	15.0 fgh	17.4 gh	19.4 c	18.0 def
T10	14.8 hi	17.0 bc	16.6 cd	16.6 hij	20.4 b	18.6 cde

For more details about treatments: T1 to T10 see Table 1
Values followed by the same letters are not significantly different by LSD's test at 0.05 level

Table 7: Effects of mineral, organo- and bio-fertilizer combinations on nitrogenase activity.

Treatments	Soil nitrogenase activity (µL C_2H_4 g^{-1} dw h^{-1})					
	First season			Second season		
	7 days	30 days	60 days	7 days	30 days	60 days
T1	23.5 s	19.49 t	22.70 s	22.5 p	09.21 q	10.10 q
T2	20.2 t	28.07 r	18.42 t	23.1 p	27.29 o	21.43 p
T3	32.4 p	44.79 k	47.14 j	31.7 m	48.65 h	41.64 ij
T4	35.3 o	58.04 g	55.49 h	34.0 l	56.86 f	53.06 g
T5	37.5 n	69.52 d	66.37 e	35.2 l	68.78 d	65.26 e
T6	41.3 lm	83.70 a	75.20 c	39.9 jk	81.20 a	78.60 b
T7	36.3 no	60.20 f	58.90 fg	38.1 k	56.80 f	53.90 g
T8	40.2 m	78.16 b	74.46 c	39.2 k	80.82 a	72.81 c
T9	30.1 q	48.70 ij	42.50 l	27.2 o	42.50 i	38.40 k
T10	37.2 no	57.03 gh	50.37 i	29.3 n	56.83 f	50.20 h

For more details about treatments: T1 to T10 see Table 1
Values followed by the same letters are not significantly different by LSD's test at 0.05 level

The higher values of N_2-ase activity were obtained in soil treated with compost and biofertilizers mixture. This result again confirms that the organic matter plays an important role in protecting and maintaining soil enzymes in their active forms. The application of balanced amounts of nutrients and manures improved the organic matter and microbial biomass carbon status of soils, which corresponded with higher enzyme activity (Mandal *et al.* 2007). Activity of phosphatase is important in studying the phosphourus cycle because this can provide a route for P mineralization and plant uptake. However, similarity in their activities was not persistent, and sometimes even contrasting. The significantly greater activities of alkaline phosphatase activity in the compost treated soils may be due to enhance the microbial activity and perhaps diversity of phosphate solubilizing bacteria due to manure input over the years (Mandal *et al.* 2007).

It could be strongly noticed that the activity of DH, AlP and N_2-ase activity increased from lettuce transplanting and reached to the highest values after 30 days from transplanting. That means in the beginning (initial stage after 7 days from transplanting) the microbial enzyme activity did not enough active. After 30 days from transplanting, the activity reached the maximum values, this may be due to the enough nutrients from the decomposed organic fertilizer for soil microbial enzyme activities. After 60 days from transplanting, the enzymes activity decreased, where this may be due to the shortage of nutrients in the rhizosphere, complete decomposed organic fertilizer or the effect of rhizosphere on soil microbial enzyme activities. Results of this study suggest that, it could be depended on the biological or organic farming to produce a better and safe food and the integrated fertilization program should be followed in agriculture. The higher records of DH, AlP and nitrogenase activities were achieved with using the organo- and bio-fertilizers compared with chemical fertilizers. The stimulatory effect of organic fertilizer on the survival of *Azotobacter* and *Azospirillium* might have been directly exerted through its effect on the growth and proliferation of the bacteria, thereby creating a favorable habitat for better survival of the inoculated bacteria (Kumar *et al.* 2009).The highest activities of above mentioned three enzymes were recorded with T6 (50 % mineral + 50 % organic + biofertilizer) which gave the highest vegetative growth and total yield as shown in Table, 4.

Conclusion:

Based on obtained data it could be concluded that, the highest yield components were obtained using combination of mineral, organic and bio-fertilizers (50 % mineral + 50 % organic + biofertilizers). Using of organic and biofertilizer treatments recorded the lowest content of nitrate in lettuce leaves. The highest dehydrogenase, alkaline phosphatase and nitrogenase activity were observed after 30 days from lettuce transplanting and achieved using combination of fertilizers (50 % mineral + 50 % organic + biofertilizer). That means using fertilization program consists of 50 % of mineral-N as NH_4NO_3 plus 50 % of the organic-N as compost beside biofertilizer (nitrogen-fixing bacteria, i.e., *Azotobacter* and *Azospirillium*) could be used for lettuce production. The previous combination of fertilizers had led to less the accumulation of nitrate in lettuce leaves, which did not exceed over the international permissible limit. From the applied side, the previous study could be used to produce safe and healthy lettuce for exportation. Rationalization of consumption of mineral fertilizers and thus reducing the nitrogen pollution of soil and ground water under a modern irrigation systems in the land of Nile Delta of Egypt. The results of this study suggest that, it could be depended on the biological or organic farming to produce a better food and the integrated fertilization program should be followed in agriculture under drip irrigation even in clay loam soils.

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