An Attempt for Reducing Mineral Fertilization in Lettuce Production by Using Bio-Organic Farming System

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Abstract

Overuse of mineral fertilizers causes environmental problems due to the excessive accumulation and leaching of harmful elements to the ground water. With the increased environment awareness, organic agricultural production is being more important around the world. The present study is aimed to reduce mineral fertilizing for lettuce plants without yield loss and also to improve inner head quality by using bio-organic treatments. Field trials on lettuce plants cv. 'Oviation' were evaluated during the 2006/2007 and 2007/2008 seasons. Four N sources (100% N as mineral N (control), 50% of mineral N, 100% organic N as chicken manure and 50% mineral N + 50% organic N) and three bio-fertilizers application (untreated, *Bacillus subtilis* FZB24 and yeast) were studied.

Results showed that reducing mineral N application to 50% or chicken manure application led to high reduction in nitrate content of lettuce head with little reduction of head yield as compared to mineral N fertilizer. Organically fertilized plants combined with 50% of mineral N fertilizer resulted in similar yield as 100% of mineral fertilization treatment with higher marketable yield. Both inoculation with *Bacillus subtilis* FZB24 and foliar application of yeast showed a positive effect in increasing lettuce yield and quality as compared to the untreated treatment (control). *Bacillus subtilis* FZB24 was the best for increasing fresh weight, yield and Ca content, while yeast resulted in the highest dry matter.

INTRODUCTION

Vegetables are crops of high fertilizer requirements. This may be due to both the high demand of the crops as well as the high economic value of the product, leading the farmer to apply excessive fertilizers (Schenk, 2006). Although it is considered the best fertility soil in Egypt, most vegetable crops grown in the Delta of Egypt are often over-fertilized with mineral N fertilizers. Since fertilizer costs were low as compared to the value of the product, and because of the relatively high economic value of the extra yields, farmers do not wish to risk yield loss due to nutrient shortage. Overuse of mineral fertilizers causes environmental pollution due to the excessive accumulation and leaching of harmful elements to the ground water (Ju et al., 2007). High availability of N fertilizers, not tailored to the crop requirement, can reduce the quality of the product, as a consequence of accumulation of nitrate in the edible portions, compromising its marketability (Parente et al., 2006). Therefore, N fertilization in vegetable fields has to take into account not only farming economics but also the environment and human health (Schenk, 2006).

Vegetables could provide more than 70% of the human daily intake of nitrate (Premuzic et al., 2004). Leafy vegetables particularly may accumulate nitrate when the supply of N is high (Reinink, 1991; Prasad and Chetty, 2008). Despite a short cultivation cycle, lettuce is considered one of the leafy vegetables that provides a highest intake of nitrate, since it demands high doses of nitrogen. Vegetable consumers are extremely health conscious and their preference is high quality (rich in vitamins and minerals) and chemical residue-free (nitrate and heavy metals) products (FAO, 2002). As a result, natural resources or ecologically friendly agriculture is increasingly being adopted around the world. Therefore, it is advisable to use a safe agriculture system for lettuce production. Chicken manure as organic N source contains high levels of available nutrient elements, which are essentially required for plant growth and its productivity. For vegetable crops supplied with chicken manure, the treatment proved to be essential for an increased yield and improved quality (Fawzy et al., 2006; Saleh et al., 2006). The application of rhizobacterium *Bacillus subtilis* strain FZB24 WG, was effective as a plant-strengthening agent and has been assumed to reduce the negative effects of stress for several vegetable crops (Bochow et al., 2001; Schmiedeknecht et al., 2001; Woitke et al., 2004; Saleh et al., 2005; Saleh, 2009). Dry yeast is a natural bio-substance suggested to have stimulating, nutritional and protective functions when used on vegetables. Foliar application of yeast was found to increase growth, yield and quality of many vegetable crops (Abou El-Nasr et al., 2001; Mona et al., 2005; Fawzy, 2007).

The objectives of the present study are to compare the mineral N source (conventional) with a proposed low mineral N input (50%) and using natural organic N source (chicken manure) in combination with bio-fertilization by *Bacillus subtilis* or yeast as alternative/integrated ecologically friendly fertilization strategy in field lettuce production.

MATERIALS AND METHODS

Two field experiments were conducted on alluvial silty-clay soil with 1.5% N and 2.6% OM in the Delta region, El-Menoufia Governorate, Egypt during the 2006/2007 and 2007/2008 seasons. Four-week old lettuce seedlings (cv. 'Oviation') of good quality were transplanted on 15 November. Two-factorial experiments were carried out in a split plot design with three replicates. Four N sources and the application of three bio-fertilizers were combined in twelve treatments as follows:

A: Sources of nitrogen:

1. 100% N as conventional mineral N (control) as 100 kg N/ha ammonium sulphate (20.5% N), was divided into three equal amounts and broadcasted at two-week intervals after transplanting.
2. 50% of mineral N (the half amount of conventional N treatment).
3. 100% organic N as chicken manure (3.6% N), 100 kg N/ha of chicken manure was incorporated in the soil before planting.
4. 50% mineral N + 50% organic N.

B: Application of bio-fertilizers:

1. Untreated control, foliar sprayed with water (200 L/ha).
2. *Bacillus subtilis*, strain FZB24 WG® (ABiTEP GmbH, Berlin) was inoculated into the root zone. A pure bacterial spore suspension for application was prepared by dissolving 0.2 g/L of the granulate resulting in $2\ 10^{10}$ spores/L. For root bacterization, seedling substrate was watered with bacterial spore-suspension (1.0 L/m^2) at two true leaves stage. After 45 days from transplanting, *Bacillus subtilis* FZB24 was applied as a foliar spray (200 g granular product/200 L/ha).
3. Yeast, dry yeast was applied as a foliar spray (400 g dry yeast/200 L/ha) twice at one-month intervals after transplanting.

The different sources of nitrogen (Factor A) were assigned to the main-plots, while bio-fertilizer treatments (Factor B) were randomized and occupied the sub-plots. The sub-plot area was 8.0 m^2 containing approximately 70 lettuce plants. All sub-experimental plots were fertilized with equal amounts of calcium superphosphate (15.5% P_2O_5) at rate 250 kg/ha before planting and potassium sulphate (48% K_2O) at rate 200 kg/ha after one month from transplanting. Surface irrigation was used and the standard agriculture practices were applied wherever they were necessary.

Recordings

After 75 days from transplanting, all plants were harvested and 10 heads/plot were chosen for evaluation of leaf chlorophyll content (SPAD), fresh weight (g/plant), total yield and marketable yield (kg/m^2) as well as dry matter percentage.

Chemical Analysis

Nitrate, vitamin C and Ca contents in lettuce heads were determined. Ca (%), was determined by the flame AAS (VARIAN Spectra AA 100). NO_3 (mg/kg), was determined in distilled water extracts of dried tissue by the procedure of Cataldo et al. (1975). Vitamin C was estimated in mg/kg fresh weight by using the method described in A.O.A.C. (1990).

Statistical Analysis

The treatment effects were evaluated by analysis of variance, and significant differences between treatment means were determined by Duncans's multiple range test at P<5% as reported by Gomez and Gomez (1984). For data summarizing, the combined statistical analysis for the two seasons was expressed in this paper, since the results took often the same trend in both seasons.

RESULTS

The combined analysis for two-season results is shown in Table 1 (fresh weight, dry matter percentage and yield) and Table 2 (chlorophyll, Ca, vitamin C and nitrate contents).

Fresh Weight, Dry Matter Percentage, Yield and Chlorophyll Content

Data in Table 1 indicated that, fresh weight of lettuce (g/plant) was decreased by applying 50% of mineral N or when lettuce plants organically fertilized totally by chicken manure as compared to application of 100% of mineral N (control). Whereas, the differences between fresh weight of lettuce plants fertilized by 100% of mineral N or combined application of 50% organic N source (chicken manure) + 50% mineral N source were not significant. 50% of mineral N input had no significant effect on dry matter percentage, while the percentage was increased in plants organically fertilized totally by chicken manure or partly by 50% chicken manure combined with 50% mineral N source as compared to 100% of mineral N source. Both of total yield and marketable yield (kg/m^2) behaved similarly to fresh weight of lettuce. Moreover, the combined application of 50% organic N source with 50% mineral N source ranked the first in increasing marketable yield (kg/m^2) followed by applying 100% of mineral N. The differences in leaf chlorophyll content (SPAD) among all studied treatments were not significant at 5% level.

With regard to the effect of bio-fertilizers, both of inoculation with *Bacillus subtilis* FZB24 and foliar application of yeast showed a positive effect in increasing fresh weight of lettuce (g/plant), dry matter percentage and subsequently total yield and marketable yield (kg/m^2) as compared to untreated treatment (control). The application of *Bacillus subtilis* FZB24 was the best for increasing fresh weight (g/plant), total yield and marketable yield (kg/m^2) of lettuce, while yeast resulted in the highest percentage of dry matter.

Concerning the interaction effects of N-fertilizer sources and bio-fertilizers, the application of 50% organic N as chicken manure plus 50% mineral N as ammonium sulphate in combination with *Bacillus subtilis* FZB24 resulted in the highest fresh weight (g/plant), total yield and marketable yield (kg/m^2) of lettuce. While, application of 100% organic N as chicken manure in combination with untreated bio-fertilizer control was always inferior as compared to all interaction treatments concerning fresh weight, total and marketable yield. The statistical analysis of the obtained data for dry matter percentage revealed that, interaction between treatments was not significant at the 5% level.

Ca, Vitamin C and Nitrate Contents

Data in Table 2 indicated that, Ca content was increased in lettuce plants by reducing 50% of mineral N input and/or application of organic N source (chicken manure) as compared to application of 100% mineral N source (ammonium sulphate). All N-fertilizer sources did not have any significant effect on the content of vitamin C. The nitrate content was sharply decreased in lettuce plants when they were organically fertilized totally by chicken manure or with reducing 50% of mineral N input alone or in combination with 50% organic N source (chicken manure).

With regard to the effect of bio-fertilizers, the contents of Ca and vitamin C were increased in lettuce plants inoculated with *Bacillus subtilis* FZB24 or foliar sprayed by yeast as compared to the untreated treatment (control). The application of *Bacillus subtilis* FZB24 was the best for increasing Ca content, while no statistical difference was recorded between *Bacillus subtilis* FZB24 and yeast concerning vitamin C. Nitrate content was not statistically altered by both of bio-fertilizer treatments (*Bacillus subtilis* FZB24 and yeast) as compared to untreated treatment (control).

Concerning the interaction effects of N-fertilizer sources and bio-fertilizers, the application of 50% organic N source (chicken manure) plus 50% mineral N source (ammonium sulphate) in combination with *Bacillus subtilis* FZB24 resulted in the highest Ca content in lettuce plants. Application of 100% mineral N source without any bio-fertilizer was always inferior as compared to all interaction treatments concerning Ca content. On the other hand, nitrate content was decreased in lettuce plants with reducing 50% of mineral N input in combination with *Bacillus subtilis* FZB24 or yeast as compared to application of 100% mineral N source without any bio-fertilizer application. The interaction treatments of different N-fertilizer sources and bio-fertilizers had no significant effect on vitamin C content in lettuce heads.

DISCUSSION

By reducing 50% of conventional mineral N input, lettuce fresh weight, total and marketable yield were reduced only to 91, 91 and 93%, respectively. Organically fertilized plants resulted in 87, 87 and 84% lettuce fresh weight, total and marketable yield, respectively as compared to application of 100% N source. This slight decrease is acceptable, and may be related to the nitrogen level in the alluvial soil in Delta of Egypt, which was already rather high (1.5%). The small reduction in the biomass and yield may be partly compensated by saving of the mineral fertilizers cost and the positive effect of nitrate loss (leaching down). The observed results are in agreement with those reported by Premuzic et al. (2004), Fawzy et al. (2006) and Saleh et al. (2006). The productivity of lettuce plants fertilized by 50% chicken manure in combination with 50% mineral N was not only similar to those fertilized by 100% conventional mineral N, but also had less nitrate content. As a consequence, there is a double advantage by improving the quality of product and reducing environmental impact without any yield loss risk (Premuzic et al., 2004). Although lettuce has been shown to be a high nitrate accumulating species when fertilized with nitrogen doses higher than the requirement (Blom-Zandstra and Lampe, 1985; Parente et al., 2006), no nitrate content risk under the present field study because nitrate is still less than the critical level. In relation to human health, the mineral N supply for lettuce should be low or combined with chicken manure, in order to minimize the concentration of nitrate and obtain a high concentration of vitamin C. The supply with organic N reduces the availability of nitrate in soil and reduces the accumulation of nitrate in leaves (Reinink, 1991; Premuzic et al., 2004).

Both inoculation with *Bacillus subtilis* FZB24 and foliar application of yeast showed a positive effect in increasing lettuce yield and head quality as compared to untreated treatment (control). The remarkable improvement effect of bacterization on lettuce plants can be attributed to root growth stimulant and growth-stimulating hormones of *Bacillus subtilis* FZB24, which act as plant growth and health promoter, leading to the increase of the net photosynthesis rate (Kilian et al., 2000; Idris et al., 2004). It has been hypothesized for the mode of action of *Bacillus subtilis* FZB24 by Bochow et al. (2001) that the given bacterial production of auxin and auxin precursors during root colonization induces a flush in the plant auxin synthesis. Saleh (2009) showed a positive effect for

Bacillus subtilis in increasing lettuce productivity and improving head quality even under stressed conditions, which is in agreement with our finding in the present study. The positive effect of yeast as a natural bio-substance when it is foliar sprayed on lettuce plants can be attributed to its content of hormones, sugars, amino acids, vitamins and minerals. Many investigations cleared that, application of dry yeast as a foliar spray increased growth, yield and quality of vegetables (Abou El-Nasr et al., 2001; Mona et al., 2005; Fawzy, 2007). N.R.P. 1977 stated that, the chemical analysis of yeast was protein (47.2%), arginine (2.6%), glycin (2.6%), histidin (1.4%), islysine (2.9%), lauicine (3.5%), lysine (3.8%), methionine systine (0.6%), phnyl-alanine (3%), tyrosine (2.1%), threonine (2.6%), tryptophan (0.5%) and vitamin B (2.9%).

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Tables

Table 1. Effect of reducing mineral N-fertilizer and application of organic manure in combination with bio-fertilizers on fresh weight, dry matter and yield of lettuce.

Treatments		Fresh weight (g/plant)	Dry matter (%)	Total yield (kg/m^2)	Marketable yield (kg/m^2)
N-fertilizer sources					
Mineral (M)		489a	6.88b	4.40a	3.86a
50% of M		447b	6.92b	4.02b	3.59ab
Organic (O)		426b	7.15a	3.83b	3.25b
50% M + 50% O		487a	7.13a	4.39°	4.02a
Bio-fertilizers					
Untreated Control		442c	6.99b	3.98b	3.65b
Bacillus subtilis		480a	6.98b	4.32a	4.03a
Yeast		465b	7.11a	4.18ab	3.90a
Interactions					
Mineral (M)	Control	477ab	6.81a	4.29ab	3.65bc
	Bacillus	498a	6.79a	4.48a	4.03ab
	Yeast	492a	7.05a	4.43ab	3.90ab
50% of (M)	Control	430b	6.90a	3.87bc	3.37c
	Bacillus	457ab	6.86a	4.11b	3.74ab
	Yeast	453ab	7.01a	4.08b	3.67bc
Organic (O)	Control	394b	7.13a	3.55c	2.94d
	Bacillus	461ab	7.12a	4.15b	3.57bc
	Yeast	423b	7.20a	3.81bc	3.24cd
50% M + 50% O	Control	467ab	7.11a	4.20b	3.70b
	Bacillus	504a	7.13a	4.54a	4.26a
	Yeast	491a	7.16a	4.42ab	4.11ab

Means within each column followed by the same letter are not significantly different at P<5%.

Table 2. Effect of reducing mineral N-fertilizer and application of organic manure in combination with bio-fertilizers on chlorophyll, Ca, vitamin C and nitrate contents of lettuce.

Treatments		Chlorophyll (SPAD)	Ca (%)	Vitamin C (mg/kg)	NO_3 (mg/kg)
N-fertilizer sources					
Mineral (M)		32.2a	1.37b	89a	2021a
50% of M		31.9a	1.46ab	94a	1818c
Organic (O)		30.1a	1.43b	97a	1794c
50% M + 50% O		32.4a	1.54a	99a	1961b
Bio-fertilzers					
Untreated Control		31.3a	1.38b	89b	1922a
Bacillus subtilis		32.1a	1.51a	97a	1892a
Yeast		31.6a	1.46ab	98a	1881a
Interactions					
Mineral (M)	Control	32.0a	1.27c	84a	2028a
	Bacillus	32.5a	1.44b	90a	2023a
	Yeast	32.0a	1.40bc	93a	2011a
50% of (M)	Control	31.5a	1.42bc	89a	1896b
	Bacillus	32.0a	1.52ab	96a	1782c
	Yeast	32.2a	1.45ab	97a	1775c
Organic (O)	Control	29.6a	1.38bc	91a	1791c
	Bacillus	30.7a	1.49ab	101a	1802bc
	Yeast	30.0a	1.42bc	99a	1788c
50% M + 50% O	Control	32.0a	1.45ab	93a	1972ab
	Bacillus	33.0a	1.60a	101a	1961ab
	Yeast	32.1a	1.56ab	102a	1949ab

Means within each column followed by the same letter are not significantly different at P<5%.