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Study the Effects of Humic Substances on Growth, Chemical Constituents, Yield and Quality of Two Lettuce Cultivars (cv.s. Dark Green and Big Bell)

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Abstract
An experiment was conducted at the Experimental Station of Faculty of Agriculture, Cairo University, Giza, Egypt, during two successive seasons (2012 and 2013) to study the effects of humic substances on yield and quality of two lettuce cultivars (Dark Green and Big-Bell). The experiment included 7 treatments; (T1) 50% mineral recommended NPK with humic substances extracted from biogas manure as foliar treatment (HBF); (T2) 50% NPK with humic substances extracted from the compost as foliar treatment (HCF); (T3) 50% NPK with humic substances of biogas manure as drench soil treatment (HBS); (T4) 50% NPK with humic substances of compost as drench soil treatment (HCS); (T5) 50% NPK+HBF+HCS; (T6) 50% NPK+HBS+HCF, and (T7) 100 % mineral recommended NPK (control).
Dark Green was taller than Big-Bell with two combination treatments, while Big-Bell had higher fresh weight. Meanwhile, Dark Green was double in dry weight. Treatment 50% NPK+HCF+HBS was superior in chlorophyll content in both cultivars. Big-Bell achieved the highest total crop yield particularly with foliar application of humic substances extracted from compost or biogas manure with 50%NPK. Humate of compost either by foliar or soil application with Dark Green cultivar and humate biogas by soil application with Big-Bell cultivar gave the lowest nitrate content in the leaves. Combination treatment, 50% NPK+HCF+HBS (T6) was the highest in total count of bacteria, fungi and actinomycetes, while combination treatment, 50% NPK +HCS+HBF (T5) was the best treatment in values of dehydrogenase and nitrogenase enzymes in the rhizosphere region in both cultivars. Also, the combination treatment (T6) recorded the highest level of total soluble solids in both Dark Green and Big-Bell cultivars, respectively. NPK contents in lettuce leaves were the highest with T1, T6, T4 and T5 for both cultivars. It is concluded that the best treatments were T1 (50% NPK+HBF), T2 (50% NPK+HCF) and T5 (50% NPK+HBF+HCS) which gave highest fresh yield and low levels of nitrate.

Keywords: Lettuce, humic substances, compost, biogas manure, bio-stimulators

Introduction
Lettuce (*Lactuca sativa* L.) is the most popular among the salad crops [11]. It is among the top five most commonly consumed vegetables in the united stat [2]. Lettuce is considered as an excellent nutritive source of minerals and vitamins as it is consumed as a fresh green salad [3]. Also, lettuce leaves are a rich source of antioxidants, vitamins A and C [4], and phytochemicals which are anti-carcinogenic [5]. The cultivated area of lettuce in Egypt is about 3110 hectares, which produced about 68644 tons [6]. Lettuce is a shallow-rooted crop and requires an extensive amount of nitrogen fertilizer to produce high yield [7]. N is an important factor for higher yield and average head weight of lettuce [8]. On the other hand, nitrogen is the most limiting nutritional factor for crop production in arid and semiarid lands. Thus, addition of N-fertilizer to soils has become a mandatory agricultural practice in arid regions [9]. However, the increase in the nitrogen fertilization rate enables obtaining a higher yield but at the same time conveys a risk of deteriorating yield quality resulting from an excessive nitrate accumulation. It particularly refers to leaf vegetables [10,11]. Nitrate accumulation is the main problem facing lettuce production [1]. Tests of nitrate accumulation in Egyptian vegetables, including lettuce showed considerable higher values as compared to

those found in vegetables grown in several European countries [12, 13,14]. In this trend, high nitrate in the fresh vegetables has been found to be responsible for methemoglobinemia, particularly in babies [15,16]. Also, an increase in N fertilizer led to increase in nitrate content of the crop tissues without significant increase in yield [17]. Furthermore, increasing the use of chemical fertilizers lead to the high cost of vegetable production and created pollution of agricultural environment as well as affects the soil fertility [8]. Therefore, there is an increasing interest in the use of organic N sources as fertilizers for the production of vegetable crops and particularly for the organic production of vegetables [18]. The organic fertilizers can be used to reduce the amount of toxic compounds such as nitrates produced by mineral fertilizers in vegetables like lettuce [5]. Consumers prefer fresh vegetables among the most popular organic products [19]. Liquid fertilizers or foliar feeding has been introduced into the agricultural market in recent years as an alternative to traditional solid fertilization to increase the effectiveness and efficiency of traditional solid fertilizers [20]. The foliar application of organic fertilizers can supply nutrients more rapidly than methods involving root uptake which made the local growers use foliar fertilizers to supplement soil applied nutrients to compensate for decreased root activity. These products have numerous agronomic applications, including the supply of plant nutrients, control of pests and diseases, and in management of soil health. The most commonly identifiable groups of these products are compost teas, seaweed extracts, and humic substances [21]. Humic substances may be absorbed by the roots and transported to shoots, enhancing the growth of the whole plant [22]. Also, it can be added to the soil for improvement the crop yield. There are divergent findings about humic substance effects on plants. Application of humic substances can potentially stimulate crop growth and development through the actions of plant growth-promoting hormones, including cytokinins, auxins, and gibberellins [21]. Its effects may be attributed to many factors, including the natural source and concentration of humic substances, soil pH, and plant species [22]. A benefit of humic acid due to its ability to complex metal ions and form aqueous complexes with micronutrients and also may form an enzymatically active complex, which can be carried on reactions that are usually assigned to the metabolic activity of living microorganisms [22]. So, the use of these organic substances in such soil showed a good means in that concern [24]. The major functional groups of humic acid include carboxyl, phenolic hydroxyl, alcoholic hydroxyl, ketone and quinoid [25]. There is a paucity of information on the use of humic substances as fertilizers for vegetable production and therefore the objective of the study was to assess the effects of two different sources of humic substances in single or in combined applications on the biological activity in the rhizosphere, growth, yield, and quality properties of lettuce.

2. Materials and Methods

2.1.Field trials were carried out at the Agricultural Experimental Station, Faculty of Agriculture, Cairo University, Giza, Egypt, during the two winter successive seasons of 2011/2012 and 2012/2013 using Big-Bell and Dark Green lettuce cultivars, to study the efficiency of using some organic stimulators (two different sources of humic substances) on improving plant growth, the biological activity in the lettuce rhizosphere (total counts of bacteria, fungi, actinomycets, nitrogenase, and dehydrogenase activities) and increasing the quantity and quality of yield. Lettuce seeds were planted in the nursery on 18 and 20 of November 2011 and 2012 seasons, respectively. The experiment included seven treatments with each lettuce cultivar,(T1) 50% mineral recommended NPK with humic substances extracted from biogas manure as foliar treatment (HBF); (T2) 50% NPK with humic substances extracted from the compost as foliar treatment (HCF); (T3) 50% NPK with humic substances of biogas manure as drench soil treatment (HBS); (T4) 50% NPK with humic substances of compost as drench soil treatment (HCS); (T5) 50% NPK+HBF+HCS; (T6) 50% NPK+HBS+HCF, and (T7) 100 % mineral recommended NPK (control).

Table 1: chemical analyses of the experimental soil

pH	EC dS/m	Cations meq/l				Anions meq/l			
		Ca++	Mg++	Na+	K+	CO3-	HCO_3-	Cl-	SO_4=
7.97	0.83	1.3	0.4	6.3	0.5	0.14	1.2	3.1	4.2

2.2. Transplanting was carried out on January 13th and 15th of 2012 and 2013, respectively, on both sides of the ridges. The experimental design was split-plot with three replicates, representing each cultivar in the main plots and the seven humic extract treatments to the subplots. The plot dimensions were 1.1m width, 3.4m long and contained two rows 80 cm apart, (3.7 m² plot area), and each row contained 24 plants 20-25cm apart.

2.3. Soil Properties: The experimental trial was conducted in clay soil using surface irrigation system. Chemical properties of the experimental soil were analyzed and the results are shown in Table 1

Table 2: Physical, chemical and biological analysis of compost and biogas manure samples

Physical and chemical analysis			Biological analysis		
	Compost	Biogas		Compost	Biogas
Density (kg/ m^3)	594	400	Total bacterial count (*cfu* /g x 10^6)	75.6	130
Moisture content (%)	17.7	27.74	Total actinomycets (*cfu*/ g x 10^4)	15.9	2.6
Dry matter (%)	82.3	72.26	Total fungi (*cfu*/g x 10^4)	19.5	5.7
pH (1:10)	7.51	8.11	Total coliform (*cfu*/g x 10^2)	Nd	Nd
EC dS/m (1:10)	3.75	5.97	Faecal coliform (*cfu*/g x 10^2)	Nd	Nd
Ammonia (ppm)	51.7	38.2	*Salmonella* and *Shigella* (*cfu*/g x 10^1)	Nd	Nd
Nitrate (ppm)	277.3	402.8	Nematode (larva/200g)	Nd	Nd
Total nitrogen (%)	1.36	1.61	Weed Seeds	Nd	Nd
Organic matter (%)	54.80	38.98			
Organic carbon (%)	31.78	22.61			
Ash (%)	45.20	61.02			
C/N ratio	23.4:1	14: 1			
Total phosphorus (%)	0.69	0.58			
Total potassium (%)	0.58	0.79			

Nd: not detected; C/N: Carbon / Nitrogen ratio; cfu: colony forming unit

2.4. Compost and biogas manure source:

Compost and biogas manure were obtained from Agricultural Wastes Training Center, Moshtohor, Kalubia Governorate, Egypt. The physical and chemical analyses are shown in Table 2. The main physical and chemical properties of the compost and biogas manure were determined according to the standard methods described by [26] and [27]. The plate count using the suitable serial dilutions and specific media was applied for estimation of the examined microbial groups. Nutrient agar medium [28] was used for estimating the total count of bacteria. Meanwhile, Martin's agar medium was used for fungi and Jensen's medium [29. Total and faecal coliform was counted on MacConekey's agar medium, while salmonella and Shigella were counted on SS agar medium [30]. Nematode was examined according to [31].

2.5. Extraction of humic substances:

Extractions of humic substances (HS) from compost and biogas manure were run according to the method described by [32]. Total phosphorus was determined by the method described by [33]. Total potassium was determined by flame photometrically [34]. Total nitrogen was determined according to [26]. Total acidity, phenolic and carboxylic groups were determined as described by [35]. Characteristic of humic substances are shown in Table 3.

2.6.Time and Method of Application: The mineral fertilizers (100 % recommended mineral NPK) were applied at a rate of 55kg nitrogen/fed (164 kg ammonium nitrate, 33.5% N); 22.5kg P_2O_5/fed (150 kg super calcium phosphate, 15.5% P_2O_5) and 24 kg K_2O/fed (50 kg potassium sulfate, 48% K_2O). Super phosphate was added as one dose during soil preparation, whereas ammonium nitrate and potassium sulfate were added at three equal portions, before transplanting, and after 20 and 40 days from transplanting. The humic substances extracted from mature compost and biogas manure were applied onto soil surface beside plants after 3, 6 and 9 weeks from transplanting at rates of 1.6 and 1.4 L/fed for HS-C and HS-B, respectively. The foliar treatments of humic substances were applied at the same times at rates of 0.8 and 0.7 L/fed for HS-C and HS-B, respectively. The recommended agricultural practices for commercial lettuce production were followed. Harvesting was carried out on 13 and 16 March in the first and the second season, respectively.

2.7. Data Recorded:
2.7.1. Growth Characters: A random sample of ten lettuce plants was taken from each plot to investigate the plant height (cm), head diameter (cm), head fresh weight (g), and head dry weight (g).

Table 3: Characteristic of humic substances extracted from compost and biogas manure.

Samples	Humic acid %	Fulvic acid %	Total (mmol/100g HS)			Total Macro-elements (%)		
			Acidity	Phenolic groups	Carboxylic groups	N	P	K
HS-C.	26.6	16.1	925	590	335	3.5	1.1	4.2
HS- B.	30.4	17.5	875	510	365	5.1	2.3	4.5

HS-C.: humic substances extracted from compost. HS-B. : humic substances extracted from biogas manure.

2.7.2. Chemical and biological analysis:
-Chlorophyll content: Mean of 3 readings per leaf, taken from 3 plants per plot, was measured using a Minolta SPAD-502 meter (Spectrum Technologies Inc., Plainfield, IL.).
-The percentages of total soluble solids (TSS) were measured using Digital Refractometer SR-95.
-Nutrient minerals (N, P, and K) in head lettuce were determined on dry weight basis of wrapping leaves according to [36]. Total nitrogen was determined by Kjeldahl method according to the procedure described by [37]. Phosphorus content was determined according to [38]. Potassium content was determined spectrometrically using atomic absorption spectrometer as described by [34]. Assessment of NO_3 in the lettuce heads was performed using Brucine method reported by [39].
- Dehydrogenase activities were determined in rhizosphere region during harvesting by triphenyl formazan (TPF) extraction method according to [27]. The activity of nitrogenase (µmole C2H4 / gm soil) was estimated according to the methods of [40]. The plate count, using the suitable serial dilutions and specific media, was applied for estimation of the examined microbial groups. The media included: Nutrient agar [28] for a total count of bacteria, Martin's agar medium [24] for fungi and Jensen's medium [29] for Nematode.
2.8. Statistical analyses: The results were expressed as means. Treatment means were compared using the least significant difference of the means; the significant difference (at $P < 0.05$) was evaluated by analysis of variance (ANOVA by using GenStat Discovery Edition 3.

3. Results and discussion
3.1. Plant Growth Characters
3.1.1. Plant height (cm): The two lettuce cultivars differed significantly in plant height in both seasons (Table 4). Dark Green cv was taller with 33.89 and 34.53cm in the first and second seasons, while the Big-Bell was shorter with 24.30 and 23.57cm in both seasons, respectively. Treatment T6 (50% NPK + HCF+ HBS) recorded the tallest plants in both seasons, recording 30.25 and 30.23cm, in the first and second seasons, respectively. Generally, Dark Green cultivar plants that fertilized with a combination of chemical and humic substances showed the highest values (Table 5). Plants that received either 50% NPK + HBF+ HCS or 50% NPK+HCF+HBS were taller than all other treatments (35.73 and 35.58 cm for both seasons, respectively). El-Shinawy *et al*. [41] reported that lettuce plants treated with inorganic fertilizer were taller than plants treated with buffalo manure. Results revealed that treatments of 50% NPK+HCF and 50% NPK+HCF+HBS attained the tallest plants for Big-Bell cultivar (26.25 and 25.33 cm for both seasons, respectively). This result is in line with the findings of [42], who reported that vermicompost, and FYM combined with 50 and 100% recommended dose of NPK were superior in terms of root length of carrot. The increase in plant growth could be attributed to the beneficial effects of N on stimulating the meristmatic activity for producing more tissues and organs [8]. Nitrogen plays major roles in structural proteins and other several macromolecules related to growth plants [43]. Also, may be due to containing organic manure

nutrient elements that can support crop production beside, organic matter improve the chemical and physical properties of soil [44].

Table 4: Effect of some nutrient treatments on plant height (cm), fresh weight (g) and dry weight (g) of two lettuce cultivars

Cultivars	Plant height(cm)		Fresh weigh (g)		Dry weight(g)	
	2012	2013	2012	2013	2012	2013
Dark Green	33.89	34.53	640.21	682.54	54.44	57.82
Big Bell	24.30	23.57	922.14	882.10	26.14	24.46
LSD at 0.5	0.31	2.65	90.04	N.S.	9.84	7.42
Treatments						
T1: 50% NPK + HBF	29.30	29.49	824.45	830.60	40.74	41.15
T2: 50% NPK + HCF	29.57	27.80	817.90	758.35	37.89	36.57
T3: 50% NPK + HBS	27.78	28.15	738.05	743.70	40.97	42.45
T4: 50% NPK + HCS	28.47	29.30	765.90	761.65	39.72	42.55
T5: 50% NPK + HBF+HCS	29.30	29.28	844.20	792.90	45.84	42.75
T6: 50% NPK + HCF+ HBS	30.25	30.23	750.75	836.75	40.38	42.09
T7: 100 % NPK (control)	29.01	29.10	727.00	752.30	36.54	40.44
LSD at 0.5	0.86	2.05	52.69	N.S.	8.02	6.06

HBF: Humic substances extracted from biogas as foliar, HCF: Humic substances extracted from compost as foliar, HBS: Humic substances extracted from biogas as soil treatment, HCS: Humic substances extracted from compost as soil treatment.

3.1.2. Fresh weight (g): Fresh weight was greater in the Big-Bell cultivar at the end of the experimental period, compared with the Dark Green cultivar with 922.14 and 882.10 g in the first and the second season, respectively (Table 4). Treatment T5 (50% NPK+HBF+HCS) recorded the best results in the first season (844.20 g); while T6 treatment (50% NPK+HCF+HBS) showed better results in the second season (836.75g). For Dark Green cultivar, plants treated with combinations of soil, foliar applications, and inorganic fertilizers grew better than other treatments. In the present study, the plants received both 50% NPK+HCF+HBS or 50% NPK+HCS+ HBF obtained more fresh weight than those supplied by 50% NPK+HCF, 50% NPK+HBF, 50% NPK+HCS and control (Table 5). On the other hand, the Big-Bell cultivar showed the opposite trend, where using 50% NPK with the foliar application either with HCF or HBF recorded the best results in both seasons respectively. The results are in agreement with those reported by [41] who found that fresh mass of lettuce was influenced positively by organic manure. Other researchers [45, 46], also reported the positive role of organic fertilizers compared to inorganic fertilizers on a fresh mass of amaranthus and cucumbers. Similarly [47] reported that fresh weight of lettuce was increased as N rate increased. HA and FA differently enhanced the growth of *Phaseolus vulgarus*[48].

3.1.3. Dry weight (g): One of the estimated quality features was among others the dry matter, according to [49]. The obtained results showed a significant increase in dry matter content of the leaves of the Dark Green cultivar; it gave almost double the dry weight of Big-Bell cultivar in first and second seasons, having values of 54.44 and 57.82 g, respectively. Treatment T5 (50% NPK+HBF+HCS) showed the highest dry weight in both seasons (Table 4). Both treatments of HBS and HBF+HCS combined with 50% NPK were significantly higher in dry weight with Dark Green cultivar. Plant dry weight may provide the best estimate of fertilizer efficiency response [50]. These results are in agreement with those obtained by [22], who reported that the humic compounds may be absorbed by the roots and transported to shoots, thus enhancing the growth of the whole plant. A similar observation was also reported by [51] who reported a positive response of dry mass to organic fertilizer in lettuce. Xu *et al*. [52] also, showed that vegetables grown with higher levels of organic manures grew better than those grown with inorganic fertilizers. The increments in leaf dry weight could be attributed to nitrogen concentration and its effect on the rate of photosynthesis [53]. Magkos *et al*. [54] evaluated the dry matter content of several leaf vegetables such as spinach, lettuce, chard, and white cabbage. They found that organically cultivated crops have higher dry matter content as compared to those produced inorganically.

Table 5: Effect of interaction between cultivars and nutrient treatments on plant height, fresh weight and dry weight of lettuce

Cultivars	Treatments	Plant height (cm)		Fresh weigh (g)		Dry weight (g)	
		2012	2013	2012	2013	2012	2013
Dark Green	T1: 50% NPK + HBF	33.52	34.30	584.9	673.2	50.87	55.64
	T2: 50% NPK + HCF	32.88	31.43	538.8	538.4	46.87	46.83
	T3: 50% NPK + HBS	33.25	34.80	678.1	731.3	58.97	63.60
	T4: 50% NPK + HCS	33.43	35.10	601.1	662.2	52.30	57.57
	T5: 50% NPK + HBF+HCS	34.17	35.73	745.7	696.1	64.87	60.87
	T6: 50% NPK + HCF+ HBS	35.58	35.13	646.4	785.3	56.23	60.93
	T7: 100 % NPK (control)	34.40	35.20	686.5	691.3	51.00	59.27
Big Bell	T1: 50% NPK + HBF	25.08	24.67	1064.0	988.0	30.60	26.66
	T2: 50% NPK + HCF	26.25	24.17	1097.0	978.3	28.91	26.31
	T3: 50% NPK + HBS	22.30	21.50	798.0	756.1	22.97	21.29
	T4: 50% NPK + HCS	23.50	23.50	930.7	861.1	27.13	27.53
	T5: 50% NPK + HBF+HCS	24.42	22.83	942.7	889.7	26.80	24.62
	T6: 50% NPK + HCF+ HBS	24.92	25.33	855.1	888.2	24.52	23.24
	T7: 100 % NPK (control)	23.62	23.00	767.5	813.3	22.08	21.60
LSD at 0.5	TXC	1.22	2.91	236.5	207.7	11.34	8.57

HBF: Humic substances extracted from biogas as foliar, HCF: Humic substances extracted from compost as foliar, HBS: Humic substances extracted from biogas as soil treatment, HCS: Humic substances extracted from compost as soil treatment.

Table 6: Effect of some nutrient treatments on chlorophyll content, crop yield (ton/fed) and nitrate content (mg/kg) of two cultivars lettuce heads.

Cultivars	Chlorophyll Content (Spad)		Crop yield ton/fed.		Nitrate content mg/kg	
	2012	2013	2012	2013	2012	2013
Dark Green	29.33	30.78	25.04	26.63	0.790	0.806
Big Bell	31.53	31.67	36.83	34.78	0.999	0.797
LSD at 0.5	N.S.	N.S.	4.19	N.S.	0.124	N.S.
Treatments						
T1: 50% NPK + HBF	28.54	29.17	32.95	32.52	0.775	0.660
T2: 50% NPK + HCF	28.55	30.05	32.68	30.29	0.610	0.560
T3: 50% NPK + HBS	27.53	29.99	29.54	29.72	0.785	0.630
T4: 50% NPK + HCS	30.75	29.17	30.85	30.95	0.930	0.860
T5: 50% NPK + HBF+HCS	33.54	35.14	33.50	31.77	0.970	0.830
T6: 50% NPK + HCF+ HBS	31.14	33.7	29.98	29.83	0.850	0.820
T7: 100 % NPK (control)	32.97	31.37	27.05	29.87	1.340	1.250
LSD at 0.5	4.02	4.05	6.37	N.S.	0.19	0.09

HBF: Humic substances extracted from biogas as foliar, HCF: Humic substances extracted from compost as foliar, HBS: Humic substances extracted from biogas as soil treatment, HCS: Humic substances extracted from compost as soil treatment.

3.1.4. Crop yield: The obtained result showed that crop yield of the Big-Bell cultivar was greater at the end of the experimental period, compared with the Dark Green cultivar recording 36.83 and 34.78 ton/fed in the first and the second season, respectively (Table 6). The treatment T5 (50% NPK+HBF+HCS) recorded the best results in the first season (33.50 ton/fed); whereas treatment T1 (50% NPK+HBF) showed better results in the second season (32.52 ton/fed). On the other hand, Big-Bell cultivar recorded higher total crop than Dark Green cultivar with foliar treatment (HS extracted biogas or compost) + 50% NPK recording 43.83 and 39.47 ton/fed in the first and the second season, respectively. On the other hand, in Dark Green cultivar, the foliar applications of compost or biogas, and the full dose of NPK recorded the lowest total crop, while treatments of HBF+HCS or HBS with 50% NPK showed significantly the highest yield achieving 29.83 and 29.23ton/fed in first and second season, respectively (Table 7). Similar finding was gained by [52] who showed that vegetables grown with organic fertilizers grew better and resulted in a higher total yield than those grown with chemical fertilizers. Also, similar result reported by [48] who revealed that HS extracted from compost (5 ppm+50 ppm nitrogen) achieved the highest yield with *Phaseolus vulgarus*. The present research showed that inorganic fertilizers resulted in lower yields compared to humic substances in the production of lettuce. These are in agreement with those obtained from [55] who reported that chemical fertilizers do not possess good characteristics to aggregate the soil particles. As a result, the plants produced by inorganic fertilizers showed relatively lower yield compared to organic materials. Also, as that known the decrease in yield at the highest nitrogen dose might be due to toxicity in the plant [56].

3.2. Chemical and Biological Analysis:

3.2.1. Chlorophyll content: Nitrogen is considered as the characteristic constituent of the integral part of chlorophyll molecules, proteins, and amino acids [57]. In both seasons, without significant differences, chlorophyll content of Dark Green was lower than that of Big-Bell (Table 6). Treatment T5 recorded the best results in the first and second season with values of 33.54 and 35.14 Spad. Chlorophyll concentration significantly decreased by spraying lettuce plants with humic substances from biogas, and from compost. In both cultivars, the combination treatments of HS from biogas soil treated + HS from compost foliar treated + chemical fertilizer and HS from compost foliar treated + HS from biogas soil treated + chemical fertilizer, as well as the full dose of chemical fertilizer significantly increased total chlorophyll concentration compared to the single treatments (Table 6). On the other hand, the results here presented contradict the earlier findings of [58] reporting a higher chlorophyll content at a more intensive nitrogen fertilization. Based on a study conducted on Dark Green lettuce by [59], the full dose of nitrogen fertilization (120 kg N ha^{-1}) enhanced leaf growth and photosynthesis.

3.2.2. Nitrate contents in leaves: The lowest nitrate content in vegetables is very important for human health, due to its potential transformation to nitrites, which have the highest possibility to interact with hemoglobin and affect blood oxygen transportation [60]. It is known that the content of nitrate in lettuce is limited by head size [61] as well as by nitrate content in soil [62]. Results of the present study showed that nitrate content of the Big-Bell was significantly higher than that of the Dark Green in the first season. Among all treatments, the highest value of NO_3 was obtained when the plant was received the full dose of chemical fertilizer T7 (100 % NPK) in both cultivars and in both seasons (Table 6). The nitrate contents of Dark Green plants that received 100% NPK were higher than other treatments, while HS extracted from biogas manure either foliar or soil application with 50% NPK attained lower values of nitrate being 0.59 and 0.61 mg/kg in first and second seasons, respectively. This suggests that several plant species accumulate NO_3 as a result of an excess of N uptake. Similar results were reported by [63,64] who found significant decreases in nitrate accumulation when lettuce plants were treated with bio-fertilizers. Williams [65] also, reported lower value in nitrate concentration in organically fertilized crops, particularly leafy vegetables. Data presented in Table 7 showed that nitrate contents of the Big-Bell plants, received 100% NPK were higher than other treatments, whereas treatment T2 (50% NPK + HCF) showed the lowest NO_3 content (0.54 and 0.46mg/kg) in first and second seasons , respectively. However the control treatment showed the highest NO_3 content (1.47 and 1.30 mg/kg in first and second seasons respectively). Similar findings were gained by [66] who determined a lower nitrate concentration in cabbage with organic fertilization compared with mineral fertilization. Stopes *et al*. [67] found that the peak nitrate content might be lower in organically produced vegetables including lettuce. Dapoigny *et al*. [68] concluded a relationship between light intensity and nitrate accumulation, a low light intensity during cultivation leads to an excessive nitrate accumulation, while a high light intensity, i.e. a long day, activates photosynthesis and nitrate reductase resulting in lower nitrate content in plants. Many other factors determine the nitrate content in plants, acting simultaneously during the cultivation. Therefore, nitrate content in the plant is difficult to predict [69]. Soil microorganisms are important component of terrestrial ecosystem because they play a central role in organic matter decomposition and nutrient cycling, thereby affecting soil nutrient availability and consequently primary productivity. Therefore, knowledge of the factors influencing soil microorganisms is fundamental for sustainable environmental management [70].

Table 7: Effect of cultivars and nutrient treatments on chlorophyll content, crop yield and nitrate content of lettuce heads.

Cultivars	Treatments	Chlorophyll Content (Spad)		Crop yield Ton/fed.		Nitrate content mg/kg	
		2012	2013	2012	2013	2012	2013
Dark Green	T1: 50% NPK + HBF	29.17	30.87	23.40	25.57	0.59	0.64
	T2: 50% NPK + HCF	29.13	30.40	21.53	21.50	0.68	0.66
	T3: 50% NPK + HBS	24.03	28.30	27.20	29.23	0.62	0.61
	T4: 50% NPK + HCS	28.83	24.97	24.03	27.50	0.83	0.89
	T5: 50% NPK + HBF+HCS	31.50	34.97	29.83	28.00	0.87	0.76
	T6: 50% NPK + HCF+ HBS	30.67	35.40	25.83	27.43	0.73	0.88
	T7: 100 % NPK (control)	31.97	30.57	23.43	27.20	1.21	1.20
Big Bell	T1: 50% NPK + HBF	27.90	27.47	42.50	39.47	0.96	0.68
	T2: 50% NPK + HCF	27.97	29.70	43.83	39.07	0.54	0.46
	T3: 50% NPK + HBS	31.03	31.67	31.87	30.20	0.95	0.65
	T4: 50% NPK + HCS	32.67	33.37	37.67	34.40	1.03	0.83
	T5: 50% NPK + HBF+HCS	35.57	35.30	37.17	35.53	1.07	0.90
	T6: 50% NPK + HCF+ HBS	31.60	32.00	34.13	32.23	0.97	0.76
	T7: 100 % NPK (control)	33.97	32.17	30.67	32.53	1.47	1.30
LSD at 0.5	TXC	4.01	4.02	6.21	N.S.	0.17	0.08

HBF: Humic substances extracted from biogas as foliar, HCF: Humic substances extracted from compost as foliar, HBS: Humic substances extracted from biogas as soil treatment, HCS: Humic substances extracted from compost as soil treatment.

3.2.3. Total counts bacteria: Data in

HBF: Humic substances extracted from biogas as foliar, HCF: Humic substances extracted from compost as foliar, HBS: Humic substances extracted from biogas as soil treatment, HCS: Humic substances extracted from compost as soil treatment.

revealed that the highest total count bacteria was detected with treatment T6 followed by T5, the values were 139, 141 and 110,120 x 10^7cfu/gm soil with Dark Green cultivar in first and second seasons respectively. Meanwhile, the same treatment (T6) with Big- Ball cultivar gave the same behavior with Dark Green cultivar (95 and 105 x 10^7cfu/gm soil in both seasons respectively. Also, data revealed that the highest values of total count fungi was recorded with treatments T6 and T3 with Dark Green cultivar (25 and 46 x10^5cfu/g soil), while T6 gave the highest number in Big Ball cultivar (23 and 45 x10^5 cfu /gm soil during season 2012 and 2013 respectively). In addition, total actinomycets numbers were high with treatment T6, which exhibited 92, 109 and 55, 75 x10^5 cfu

/gm soil in both cultivars and seasons respectively. It is worth that the numbers of total count bacteria, fungi and actinomycets with Dark Green cultivar were higher than that of Big-Bell cultivar. This could be attributed to type of root exudates of cultivar. Humates are known to stimulate microbial activity. Soil testing for microbial activity, levels increased 400 to 5000 times with the introduction of humate (300 ppm) into the soil. Humates added to feed rations stimulate the microbial growth and the extent can be quite large depending upon the species, the culture medium, and the environment. Beneficial bacteria and fungi reproduction created in the presence of humic acid biologically increase plant growth. The microbial activity produced by these bacteria and fungi are excellent root stimulators. Humic acid has also been shown to lower pH to a more neutral level, helping with the availability of nutrients. All these translate into healthier, stronger, and more pest-resistant plants [71]. Afifi [48] reported that humic acid extracted from compost (10ppm with 25ppm of nitrogen) was the superior concentration for their significant result in total bacterial counts, actinomycets and fungi in soil (185 x 10^5, 255 and 102 x 10^5 *cfu* / g, respectively). Also, he found that the low concentration of humic acid extracted from biogas manure with the highest concentration of nitrogen showed an enhancement in all soil microorganisms than all concentrations of Fulvic acids.

Table 8. Effect of humic substances extracted from both compost and biogas on soil biological characters.

Treatments	Microbial counts (cfu g^{-1} soil) Bacteria x (10^7)		Fungi x (10^5)		Actinomycetes x (10^5)	
	2012	2013	2012	2013	2012	2013
			Dark Green			
T1: 50% NPK + HBF	60	65	11	30	40	51
T2: 50% NPK + HCF	55	60	13	35	44	55
T3: 50% NPK + HBS	98	105	15	46	71	87
T4: 50% NPK + HCS	105	112	17	39	76	94
T5: 50% NPK + HBF+HCS	110	122	20	41	88	102
T6: 50% NPK + HCF+ HBS	139	141	25	45	92	109
T7: 100 % NPK (control)	75	98	7	25	32	48
			Big Bell			
T1: 50% NPK + HBF	21	82	12	14	20	25
T2: 50% NPK + HCF	20	88	11	16	19	23
T3: 50% NPK + HBS	38	92	14	19	36	45
T4: 50% NPK + HCS	45	75	17	23	38	56
T5: 50% NPK + HBF+HCS	51	84	18	35	48	61
T6: 50% NPK + HCF+ HBS	95	105	23	45	55	75
T7: 100 % NPK (control)	71	80	8	12	18	22

HBF: Humic substances extracted from biogas as foliar, HCF: Humic substances extracted from compost as foliar, HBS: Humic substances extracted from biogas as soil treatment, HCS: Humic substances extracted from compost as soil treatment.

Table 8: Effect of some nutrient treatments on Enzymes, total soluble solids content (TSS) % of two cultivars lettuce heads

Cultivars	Dehydrogenase TPF/gm soil		Nitrogenase µmole C_2H_4/gm soil		T.S.S. Content %	
	2012	2013	2012	2013	2012	2013
Dark Green	34.77	40.02	18.08	20.16	2.67	2.85
Big Bell	34.59	41.71	25.66	28.09	2.36	2.35
LSD at 0.5	N.S.	N.S.			N.S.	N.S.
Treatments						
T1: 50% NPK + HBF	30.05	33.61	16.35	17.75	2.34	2.40
T2: 50% NPK + HCF	28.12	32.66	12.72	13.88	2.37	2.19
T3: 50% NPK + HBS	37.24	41.71	17.11	18.99	2.55	2.84

T4: 50% NPK + HCS	37.73	43.77	30.67	33.23	2.60	2.67
T5: 50% NPK + HBF+HCS	49.42	57.32	40.42	44.22	2.59	2.55
T6: 50% NPK + HCF+ HBS	39.82	47.51	17.66	20.32	2.78	3.09
T7: 100 % NPK (control)	20.43	29.50	18.17	20.51	2.40	2.48
LSD at 0.5	6.45	6.45			N.S.	N.S.

HBF: Humic substances extracted from biogas as foliar, HCF: Humic substances extracted from compost as foliar, HBS: Humic substances extracted from biogas as soil treatment, HCS: Humic substances extracted from compost as soil treatment.

3.2.4. Dehydrogenase enzymes activities: Dehydrogenases (DHA) are intracellular enzymes that are active in living cells and are an important indicator of microbial activity of the soil [72]. As shown in Table 9, DHA activities in rhizosphere region of Dark Green cultivar were higher than that of Big-Bell cultivar in the first season, while in the second season the opposite rend was happening. The values of enzymatic activity were higher in the combination treatments particular T5 than the other treatments for both cultivars. On the other hand, the full dose of NPK (control) recorded the lowest values being 20.23, 28.89 and 20.63, 30.10 TPF/g/day for Dark Green and Big-Bell cultivars for season 2012 and 2013 respectively. This effect was reflected in lettuce yield, although reduction of recommended mineral fertilizers to 50%. These results are matched with [73,74] where they found that inoculation of tomato plants with phosphate dissolving bacteria enhanced activities of dehydrogenase compared to control. Studies of enzyme activities in soil are important as they indicate the potential of the soil to support biochemical processes which are essential for the maintenance of soil fertility [75]. The dehydrogenase activity was estimated as an indication of the respiratory activity of roots and soil microorganisms [64]. Nitrogenase activity revealed the same behaviors, treatment T5 was superior in nitrogenase enzyme values (35.95, 37.23 and 44.88, 51.2 µmole C_2H_4 / gm soil in season 2012 and 2013 respectively), followed by T4. Nevertheless, control treatment achieved increases than treatments T1 and T2 (12.13, 13.52 and 24.16, 27.5 during both 2012 and 2013 seasons respectively). Massoud et al. [76] reported that the nitrogenase and dehyrogenase activities, mixture combined of Arbuscular mycorrhizae, *Azotobacter chroococcum*, *Azospirillum lipoferum*, and *Bacillus circulans* + 50 % (HA) + 50 % NPK recorded high nitrogenase activity after 45 days, whereas, the treatment mix + 75 % humic + NPK gave the highest activities of both nitrogenase and dehyrogenase enzymes after 75 days respectively.

Table 9: Effect of cultivars and nutrient treatments on enzymes, and total soluble solids content in lettuce leaves

Cultivars	Treatment	Enzymes: DeHydrogenase TPF/gm soil		Enzymes: Nitrogenase µmole C_2H_4 / gm soil		T.S.S. Content %	
		2012	2013	2012	2013	2012	2013
Dark Green	T1: 50% NPK + HBF	30.50	35.11	11.15	13.2	2.47	2.63
	T2: 50% NPK + HCF	30.43	36.0	10.81	12.56	2.47	2.50
	T3: 50% NPK + HBS	40.30	44.30	12.01	14.0	2.60	2.97
	T4: 50% NPK + HCS	35.63	42.21	26.13	28.3	2.73	2.93
	T5: 50% NPK + HBF+ HCS	45.23	48.54	35.95	37.23	2.87	2.77
	T6: 50% NPK + HCF+ HBS	41.10	45.12	18.34	22.33	3.03	3.40
	T7: 100 % NPK (control)	20.23	28.89	12.18	13.52	2.53	2.73
Big Bell	T1: 50% NPK + HBF	29.60	32.11	21.54	22.30	2.20	2.17
	T2: 50% NPK + HCF	25.80	29.31	14.63	15.20	2.27	1.87
	T3: 50% NPK + HBS	34.17	39.12	22.21	23.98	2.50	2.70
	T4: 50% NPK + HCS	39.83	45.32	35.20	38.16	2.47	2.40
	T5: 50% NPK + HBF+ HCS	53.60	66.10	44.88	51.2	2.30	2.33

	T6: 50% NPK + HCF+ HBS	38.53	49.89	16.97	18.30	2.53	2.77
	T7: 100 % NPK (control)	20.63	30.10	24.16	27.5	2.27	2.23
LSD at 0.5	T			25.18	35.15	21.01	24.22

HBF: Humic substances extracted from biogas as foliar, HCF: Humic substances extracted from compost as foliar, HBS: Humic substances extracted from biogas as soil treatment, HCS: Humic substances extracted from compost as soil treatment.

3.2.5. Total Soluble Solids (TSS) in lettuce heads: Potassium plays an important role increasing TSS and quality properties of fruits [77]. Although Dark Green plants attained a higher level of total soluble solids than Big-Bell plants in both seasons (Table 9), there were non-significant differences in TSS% between the two lettuce cultivars. In general plants treated with HBS or HCF combined with 50%NPK gave higher level of soluble solids in both seasons compared to control plants. These treatments could be considered as advantages of organic growing. On the other hand, the treatments: 50% NPK+HBS, 50% NPK+HCS, and 50% NPK+HCF+HBS, recorded highly significant TSS% compared to the control plants in both cultivars.

Table 10: Effect of some nutrient treatments on Total Nitrogen, Phosphorus, and Potassium of two lettuce cultivars during 2013

Cultivars	N%	P%	K%
Dark Green	3.62	0.55	2.65
Big Bell	2.41	0.57	3.61
LSD at 0.5			
Treatments			
T1: 50% NPK + HBF	2.55	0.60	3.47
T2: 50% NPK + HCF	1.37	0.41	2.59
T3: 50% NPK + HBS	1.64	0.51	2.66
T4: 50% NPK + HCS	2.24	0.51	3.60
T5: 50% NPK + HBF+HCS	2.54	0.63	3.14
T6: 50% NPK + HCF+ HBS	1.85	0.73	2.84
T7: 100 % NPK (control)	1.94	0.54	3.62
LSD at 0.5	0.73	0.15	0.54

HBF: Humic substances extracted from biogas as foliar, HCF: Humic substances extracted from compost as foliar, HBS: Humic substances extracted from biogas as soil treatment, HCS: Humic substances extracted from compost as soil treatment.

3.2.6. Total Nitrogen: Nitrogen plays a crucial role in the synthesis of amino acids and proteins, plant growth, chlorophyll formation, leaf photosynthesis, and yield of lettuce [7]. As shown in Table 10, the total N content of Big-Bell was significantly higher than Dark Green cultivar. The treatment T1 (50% NPK + HBF) recorded the best results in both cultivars. Foliar application with HBF (T1) and its combinations (HBF + HCS) showed high N % (2.55 and 3.5 % in Dark Green and Big-Bell cultivars respectively (**Erreur ! Référence non valide pour un signet.**). On the other hand, foliar application of compost (HCF), and biogas soil application (HBS), recorded the lowest N% in both lettuce cultivars. N is the main yield factor and considered as the characteristic constituent of functional plasma, an integral part of chlorophyll molecules, proteins, amino acids, nucleic acids (RNA and DNA), nucleotides, phosphotides, alkaloids, enzymes, coenzymes, hormones, and vitamins [57]. Wolkowski [78] reported that relatively high applications of composted wastes should be added to supply, crop N needs and produce yields similar to those found with recommended doses of commercial fertilizer. Bar-Tal [79] found that incorporating compost has a positive effect on crops only when additional N applications are carried out, and that the organic matter content and net N mineralization increases over time in soils treated with compost. These results are matched with [80] who found that the highest values of N content sesame in seeds was achieved by application of humic acid combined with the high rate of mineral N fertilizer.

3.2.7. Total Phosphorus: Regarding total P content in lettuce leaves, data in Table 10 showed that Big-Bell cultivar had significantly higher P content than "Dark Green". Moreover, treatment T6 (50% NPK+HCF+HBS) showed a significant increase in total phosphorus content in lettuce leaves (0.71 and 0.75 % in both cultivars) compared to the control and other treatments (**Erreur ! Référence non valide pour un signet.**). Organic fertilizers are used for their organic matter contribution and nutrients, mainly N and P [81]. The availability of nutrients in organic fertilizers does not depend on its total content of material, but on the dynamics of the process; thus, some elements can become more available because of pH, moisture, and aeration, or in composting for the temperature allowing the development of specialized organisms. Likewise, the earthworm's action can affect, in one way or another, the availability of an element [82].

Table 11: Effect of interaction between cultivars and nutrient treatments on total Nitrogen, Phosphorus, and Potassium in lettuce leaves during 2013

Treatment	TN%		TP%		TK%	
	Dark Green	Big Bell	Dark Green	Big Bell	Dark Green	Big Bell
T1: 50% NPK + HBF	2.50	2.60	0.61	0.58	2.73	4.20
T2: 50% NPK + HCF	1.20	1.53	0.38	0.43	2.00	3.17
T3: 50% NPK + HBS	1.17	2.10	0.48	0.53	1.85	3.47
T4: 50% NPK + HCS	2.00	2.47	0.48	0.53	3.63	3.57
T5: 50% NPK+ HBF+HCS	1.57	3.50	0.65	0.60	2.47	3.80
T6: 50% NPK+HCF+ HBS	1.43	2.27	0.71	0.75	2.37	3.30
T7: 100 % NPK (control)	1.47	2.40	0.51	0.57	3.50	3.73
LSD at 0.5	0.71		0.18		0.55	

HBF: Humic substances extracted from biogas as foliar, HCF: Humic substances extracted from compost as foliar, HBS: Humic substances extracted from biogas as soil treatment, HCS: Humic substances extracted from compost as soil treatment.

3.2.8. Total Potassium: There is a relationship between K concentration and nitrate accumulation [14]. Data presented in Table 10 recorded significant increases in K contents in Big-Bell cultivar compared to Dark Green plants. Moreover, leaves K content in T1and T4 were higher than other treatments (3.63 and 4.20 % in both Dark Green and Big-Bell cultivars respectively). Similar finding was gained by [80] who reported that the highest values of K contents in sesame seeds were achieved by soil application of humic acid combined with the high rate of mineral N fertilizer. The results also are in agreement with those reported by [4183] who found that, the combined application of compost with mineral fertilization recorded the highest K contents in Oregano plants. This effect could be attributed to the role of mineral fertilization in increasing the absorption and accumulation of potassium in the plant organs [8441]. Also the organic manure improved the soil microbial biomass and activity, as well as potassium dissolving bacteria, and consequently the available K for the plant [4185]. So, using combination of mineral fertilizer and organic manure unified these advantages [4183].

Conclusion

We recommend using humic substances extracted from compost and biogas manure as organic stimulators for lettuce plants because, it has beneficial effects on biological activities in soil and reflect that on enhancement of plant growth and yield. Also, it gave the lowest concentration of NO_3 in leaves. So, they consider friendly to the environment and they have no harmful effect on human health. The best treatments were T1 (50% NPK+HBF), T2 (50% NPK+HCF), T5 (50% NPK+ HBF+HCS) and T6 which they gave highly fresh yield and low levels of nitrate.

References

1. Midan S.A., Sorial M.E., Australian J. of Basic and Appl. Sci. 5(2011)127.
2. Lucier G., Jerardo A., Electronic Outlook Report from the Economic Research Service USDA (2005).
3. Hanafy A.A., Mishriky J.F., Khalil M.K., ICEHM, Cairo Univ., Egypt (2000)509.
4. Norman J.C., Tropical Vegetable Crops, United Kingdom (1992).
5. Masarirambi M.T., Dlamini P., Wahome P.K., Oseni T.O., American-Eurasian J. Agric. & Environ. Sci. 12(2012)399.
6. Ministry of Agriculture and Land Reclamation, Economic Affairs Sector. The Indicators Agriculture Statistics 2(2012)219.
7. Sukor A. M.Sc. thesis, Colorado State Univ., USA (2013).
8. Hosseny M.H., Ahmed M.M., Ass. Univ. Bull. Environ. Res. 12 (2009)79.
9. Al-Moshileh A.M., Errebhi M.A., Motawei M.I., Emir. J. Agric. Sci. 17 (2005) 01.
10. Durman P., Custic M., Agronomski Glasnik 52(1990) 361.
11. Paterson C.D., Rahn C.R., Acta Hortic. 428 (1996) 105.
12. Blom-Zandstra M., Ann. Appl. Biol. 155(1989) 553.
13. Kheir N.F., Hanafy A.A., Abou El-Hassan E.A., Harb, E.M., Bull. Fac. Agric. Cairo Univ., Egypt 42(1991)557.
14. Hanafy A.A., J. Agric. Sci. Mansoura Univ., Egypt 22(1997)697.
15. Keeney D.R., ln: Stevenson F.J. Nitrogen in Agriculture Soils. S series Agronomy. Madison, U.S.A. (1982) 605.
16. Al-Redhaiman K.N., Alexandria Sci. Exchange 24(2002) 372.
17. Custic M., Poliak M., Cosic T., Acta Hortic. 371(1994) 407.
18. Smith S.R., Hadley P., Plant and Soil 115 (1989)135.
19. Willer H., Organic Horticulture Productivity and Sustainability Symposium (2010).
20. Mariola L., Janina G.W., Katarzyna K., Bio-stimulators in modern agriculture-vegetable crops (2008).
21. QuiltyJ.R., Cattle S.R. Soil Res. 49(2011)1.
22. Lulakis M.D., Petsas S.I., Bioresource Tech. 54(1995)179.
23. Tejada M., Hermendez M.T., Carcia C., J. Environ. Qual. 35(2006)1010.
24. Habashy N.R., Amal W.A., Rafat N.Z., Soil Res. J. Agric. & Biol. Sci. 4(2008)452.
25. Russo R.O., Berlyn G., J. Sust. Agric. 1(1990)19.
26. Jackson M.L. Soil Chemical Analysis. Prentice-Hall of Englewood Cliiffs, New Jersey, USA (1973)925.
27. Page A.L., Miller R.H., Keeney D.R., Chemical and microbiological properties, 2nd ed., Difco Laboratories, USA (1982)623.
28. Difco manual. Dehydrated culture media and reagents for microbiology, 10th ed., Difco Laboratories, USA (1985) 487.
29. Allen U.N., Experiments in soil bacteriology, 1st ed. Burges pub. , USA (1959)117.
30. Difco manual. Microbiological laboratory procedures, 9th ed., Difco Laboratories, USA (1977) 855.
31. Taylor A.L., Sasser J.N., Cooperative publication of department of plant pathology, North Carolina State Univ. and the United States Agency for international development (1978)111.
32. Sanchez- Monedero M.A., Roid A., Cegarra J., Bernal M.P., Paredes C., Eur. J. Soil Sci. 53(2002)375.
33. Murphy J., Riley J.P., Anal. Chem. Acta 27(1962)31.
34. Chapman H.D., Pratt P.F., Univ. California, Div. Agric., Sci. Riverside, USA (1961)309.
35. Dragunova A.F., Nauch Trudy, Mosk. (1958). In Zh. Chonon Inst. Ser. Khinprioz-vod., Cited by Kononova (1966) 544.
36. Cottenie A., Verloo M., Kiekers L., Velghe G., Camrbynek R., Chemical analysis of plants and soils, Belgium (1982) 1.
37. FAO. Soil and plant analysis. Soils Bulletin 38(1980)250.
38. Watanabe F.S., Olsen S.R., Soil Sci. Soc. Amer. Proc. 29(1965)677.
39. Holty J.G., Potworowski H.S., Environ. Sci. Tech. 6(1972) 835.
40. Somasegarn P., Hoben H.J., Hand Book for Rhizobia, Springer Veriag, New York, USA (1994).
41. El–Shinawy M.Z., Abd–Elmoniem E.M. Abou–Hadid A.F., Acta Hortic. 435(1999)315.
42. Sunanda R.N., Mallareddy K., Karnataka J. of Agric. Sci. 20(2007)686.
43. Marschner H., Mineral nutrition of higher plants, Academic press limited, London (1995)889.
44. Paudel K.P., Sukprakarn S., Sidathani K., Osotsapar Y., Kasetsart J. 38(2004)31.
45. Zakaria A., Vimala P., FAO Expert Group Workshop on Preparation of Technical Guidelines on Organic Cultivation of Tropical and Subtropical Fruits. Intan Bukit Kiara, Kuala Lumpur (2002).

46. Roe N.E., Stofella P.J., Graetz D., J. Am. Soc. Hort. Sci. 122(1997)427.
47. Tittonell P.A., de Grazia J., Chiesa A., Agricultura Tropica and Subtropica 36(2003)82.
48. Afifi M.M., Ph.D. thesis, Fac. Agric., Cairo Univ., Egypt (2010)156.
49. Gonella M., Serio F., Conversa G., Santamaria P., Acta Hortic. 644(2004)61.
50. Gourley C.J., Allan, D.L., Russelle M.P., Plant Soil 158(1994)29.
51. Rubeiz I.G., Khansa M., Freiwat M.M., Soil Sci. Plant Anal. 29(1998)161.
52. Xu H.L., Wang R., Xu R.Y., Mridha M.A.U., Goyal S., Acta Hortic. 627(2005)25.
53. Takebe M., Satou N., Ishii K., Yoneyama T., Japanese J. Soil Sci. and Plant Nutri. 67(1996)147.
54. Magkos F., Arvaniti F., Zampelas A., Int. J. Food Sci. Nutri. 54(2003)357.
55. Owen P., http://www.calettuceresearchboard.org/Origin.html (2003).
56. Tabatabaie S.J., Malakouti M.J., Iranian J. Soil and Water Res.11 (1997)25.
57. Castellanos J.Z., Uvalle-Bueno J.X., Aguilar-Santelises A., 2nd ed. INIFAP, Chapingo, Mexico (2000).
58. Politycka B., Golcz A., Folia Hortic. Ann. 16(2004)23.
59. Boroujerdnia M., Ansari N.A., Middle Eastern and Russian J. Plant Sci. and Biotech.1(2007)47.
60. Maynard D.N., Barer A.V., Minotti P.L., Peck N.H., Adv. Agron. 28(1976)71.
61. Fontes P.C., Pereira P.R., Conde R.M., J. Plant Nutr. 20(1997)106.
62. Devienne-Barret F., Justes E., Machet J.M., Mary B., Ann. Bot. 86(2000)995.
63. Ahmed A.H., Mishriky J.F., Khalil M.K., Cairo Univ., Egypt (2000)509.
64. Shahein M.M., Abouelhassan S., Ragab A.A., Hortt. Sci. J. Suez canal Univ., Egypt 1(2013)77.
65. Williams C.M. Proc. Nutr. Soc. 61(2002)19.
66. Vogtmann H., Matthies K., Kehres B., Meier-Ploeger A., Compost Sci. Util. 1(1993)82.
67. Stopes C., Woodward L., Forde G., Vogtmann H., Biol. Agric. & Hortic. 5(1988)215.
68. Dapoigny L., De Tourdonnet S., Roger-Estrade J., Jeuffroy M.H., Fleury A., Agronomie 20(2000)843.
69. Lisiewska Z., Kmiecik W., Post. Nauk Roln. 3(1991)11.
70. Insam H., Soil Biol. Biochem. 22(1990)525.
71. Huck T., Porter A., Bushell N., Eng. J. Gen. Microbiol. 137 (1991)2321.
72. Trasar-Cepeda C., Camina F., Leiros C., Gil-Sotres F., Soil Biol. Biochem. 31(1999)483.
73. Abou-Aly H.E., Annals of Agric. Sci., Moshtohor, Egypt 43(2005)595.
74. Shafik D.A., M.Sc. thesis, Fac. Agric., Ain Shams Univ., Egypt (2011).
75. Dkhar M.S., Mishra R.R., Plant and Soil 70(1983)327.
76. Massoud O.N., Afifi M.M., El-Akshar Y.S., El-Sayed, G.A., J. Agric. and Biol. Sci. 9(2013)104.
77. Abdel-Hady S.A., M. Sc. thesis, Fac. Agric., Ain Shams Univ., Egypt (2001).
78. Wolkowski R.P., J. of Environ. Qual. 32(2003)1844.
79. Bar-Tal A., Yermiyahu U., Beraud J., Keinan M., Rosenberg R., Sohar D., J. Environ. Qual. 33(2004)1855.
80. Khaled A.S., Mona G.A.E., Zeinab M.K., J. Appl. Sci. Res. (2012)1568.
81. Fuente B., Bolan N., Naidu R., Mora M., Revista de la Ciencia del Sueloy Nutricion Vegetal 6(2006)64.
82. Melgarejo M.R., Ballesteros M.I. Bendeck M., Revista Colombiana de Quimica 26(1997)1.
83. EL-Hefnawy N.N., Shalaby A.S., Ghanem K.M. and Algharib A.M., 1st International Conf. On Environ. Studies and Res., Minufiya Univ., Egypt (2008)94.
84. Jacoub R.W., Ph.D. thesis, Fac. Agric., Cairo Univ., Egypt (1999).
85. Christoph E., Report on the consultancy stay on the occasion of the UNDP–RDA program: Developing and Promoting Support System and International Cooperation on Environment–friendly Agriculture, Korea. (2003).