Effect of Azotobacter, compost and Humic acid on yield and quality of lettuce (Lactuca Sativa CV. Alfajr) grown in plastic house

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Abstract

A field trial was executed in a plastic house during the autumn season (2021-2022) to investigate the effect of azotobacter (with100ml and without), compost fertilizer at three levels (0, 1, and 2) ton.donum^{-1} and humic acid at four concentrations (0, 2, 4.and 8) ml.L^{-1} and their interactions on yield and quality of lettuce cv. (Alfajr). The treatments were arranged in a randomized complete block design (RCBD) with three replicates. The data were analyzed using SAS program (2010). The obtained results revealed that the soil inoculation with azotobacter, soil addition of compost at level (2) ton.donum^{-1} and foliar spraying of humic acid at (8) ml.L^{-1} either singularly or combined significantly enhanced yield traits [fresh head weigh(1566.67) g, Marketable yield (8.05) ton. ha^{-1}, yield (8.70) kg.m^{-2}, yield per house(4351.85)kg, and total yield(76.59) ton. ha^{-1}] as well increasing the quality traits [total soluble solids (%TSS), ascorbic Acid (%) as compared to control. However, the maximum ever values of yield and its quality were measured for plants received the dose: azotobacter + compost at (2) ton.donum^{-1} + humic acid at (8) ml.L^{-1} whereas the lowest ever values were recorded for plants given no dose of all studied factors (control). The application of azotobacter, compost and humic acid is recommended for organic production of lettuce crop.

Key words: Azotobacter, compost, humic acid, lettuce, organic production.
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Introduction

Lettuce (*Lactuca sativa L*.) is an important annual leafy herb which belongs to the Astaraceae family grown in cool season. It is well-known as a popular salad crop for which the global largest production land is devoted. It occupies the rank 26th among vegetables and fruits in term of nutritive value and is considered as the 4th widely consumed crop [1]. It is a main part in human diet owed to its enrichment of vitamins, minerals and antioxidants. The lettuce offers extra benefits when eaten raw since it keeps more nutrients than thermally processed food [2].

Bio-fertilizers are economically and ecologically interested substances used as alternatives for chemical inputs to ameliorate qualitative and quantitative characters of vegetable crops. They are enriched with living cells of microbes that are potential to convert elements from unobtainable form to obtainable one via several biological processes [3].Azotobacter is categorized as N-fixing bacterium capable of improving plant growth and nutrient use efficiency [4]. It also enhances nutrient absorption, inorganic phosphate solubility and mineralization of organic phosphate which in turn increase plant performance and yield [5][6]. [7] observed that the least nitrate accumulation in lettuce (cv. 'Luna') and the highest yield was measured due to application of bio-fertilizer (PGPR) relative to chemical fertilizer. [8] showed that inoculating lettuce plants with azotobacter resulted in a significant increment in growth and yield traits of crop over control.

Compost is a type of organic fertilizer produced from plant, animal and food waste and contains adequate amount of nourishing nutrients needed by plant to grow and yield well. The utilization of compost as organic input makes soil more fertile, improves its physical, chemical and biological features [9][10]. [11] displayed that the highest yield attributes and the lowest nitrate content of lettuce was owed to the addition of compost fertilizer relative to inorganic fertilizer and vermicompost. [12] fertilzing soil with compost and compost extract obtained from agro-industrial wastes resulted in a significant amelioration in yield and quality of baby leaf red lettuce as compared to control. [13] revealed that the highest yield was recorded for lettuce plants given 100% compost pared with those provided with 100% chicken manures.

Humic acid is defined as natural substance made from breakdown of plant and animal remnants [14]. Humic substances such as humic acid and fulvic acid are the major components (65-70%) of soil organic matter. They are able to ameliorate plant growth and they perpetrate that via stimulating cell membrane permeability, photosynthesis, respiration, oxygen and phosphorus uptake boosting root cell outgrowth [15].[16] observed that the red cabbage plants received 100 and 150% compost + humic acid + EM possessed the maximum foliage and yield attributes. [17] displayed that the premium yield and quality was obtained from treating lettuce plants with T4 (1.5 ml/L soil application) of humic acid succeeded by those treated with T1 (2.5 ml/L foliar application). [18] demonstrated, in a field study, that the best harvest income and quality characteristics were measured for lettuce plants give humic acid at concentration (800) $mg.l^{-1}$ in comparison with control. The aim of this study was to inspect the effect of azotobacter, humic acid, compost and their interactions on yield and quality of lettuce cv. (Al-Fajr) to produce organic crop with better quality and lesser inputs by use of natural substances without using detrimental chemicals.

Material And Methods

The greenhouse study was carried out in a plastic house (500 m^2). The seeds of lettuce cultivar (Al-Fajr) were sown in plate pods on September 15th 2021. The seedlings were transplanted on October at spacing of (20) cm between plants and (60 cm) between terraces. The experiment comprised of the soil inoculation with100ml of *Azotobater* and without, compost soil-added at three rates (0, 2, and 4 $ton.donum^{-1}$) and humic acid with four levels (0, 2, 4, and 8) $ml.L^{-1}$ and their combinations matched to control. The compost was added a day prior to transplanting. The humic acid sprayed three times with ten days between each spray. The first spray was implemented on November 1st

and the second spray was carried out on November 10th whereas the third one was done on November 20th. The experiment was designed with factorial randomized complete block design (RCBD). The study encompassed 24 treatments (2x3x4) with three replicates each replication planting with twelve plants.

The experiment included (72) experimental units. The data were analyzed with (SAS 2010) program and means were compared by Duncan's multiple range test at 5% level of confidence. The fresh head weight was measured by scale. The marketable yield was recorded as the total head weight after the removal of undesirable leaves for consumption. The yield (kg $.m^{-2}$) was measured by counting the total yield of experimental unit subdivided by the number of plants in the experimental unit. The yield per house (kg) was measure as total yield of experimental unit (kg) subdivided by area of experimental unit multiplied by (500). The TSS was measured by Hand Refractometer device [19]. Ascorbic acid or vitamin C was determined by 2, 6 Dichloro phenol indophenols as described by [20].

Results

1. Fresh Head Weight (g)

Data analysis of the table (1) shows significant differences in the fresh head weight (g) of lettuce under inoculation of azotobacter and compost and foliar application of humic acid interactions. The triple effect of all factors, the heaviest head (1566.67) g was weighed for plants having azotobacter and compost at (2) ton.donum^{-1} with humic acid at concentration (8 ml.L^{-1}) over control (886.67) g without doses.

Table (1): Effect the interactions of Azotobacter, Compost, Humic Acid on fresh head weight (g) of lettuce crop

Azotobacter	Compost ton/donum	Humic acid ml L-1			
		0	2	4	8
with	0	1150.00 f	1266.67 e	1266.67 e	1300.00 de
	1	1166.67 f	1300.00 de	1366.67 b-d	1400.00 bc
	2	1333.33 c-e	1433.33 b	1373.33 b-d	1566.67 a
without	0	866.67 i	926.67 hi	920.00 hi	940.00 hi
	1	920.00 hi	923.33 hi	966.67 h	985.00 h
	2	980.00 h	959.67 hi	1010.00 gh	1083.33 fg

*Means with same letter for each interaction are not significantly different at 5% level based on Duncan's Multiple Rang Test.

2. Marketable yield

The data shown in table (2) depict the marketable yield under the application of bio-fertilizer (azotobacter), bio-stimulant (humic), and organic fertilizer (compost).

The peak average (8.05) kg.m^{-2}marketable yield was earned from treating lettuce plants with azotobacter and dosing them with soil amending with compost at level (2) ton.donum^{-1} plus humic at concentration (8) ml.L^{-1} whereas the minimum average (4.38k) kg.m^{-2} was calculated for control plants.

Table (2): Effect the interactions of Azotobacter, Compost, Humic Acid on Marketable yield (ton.ha-2) of lettuce crop

Azotobacter	Compost ton/donum	Humic acid ml/L			
		0	2	4	8
With	0	5.87fg	6.48e	6.51e	6.67de
	1	5.96f	6.69de	7.09b-d	7.29bc
	2	6.84c-e	7.39b	7.00b-e	8.05a
Without	0	4.38k	4.60i-k	4.51i-k	4.76i-k
	1	4.68ijk	4.49i-k	4.86i-k	4.90i-k
	2	5.01h-j	4.74i-k	5.11hi	5.44gh

*Means with same letter for each interaction are not significantly different at 5% level based on Duncan's Multiple Rang Test.

3. Yield (kg .m^{-2})

The lettuce yield (kg.m^{-2}) was significantly influenced by triple combinations between all factors resulted in the biggest yield (8.70) kg.m^{-2} when lettuce plants inoculated with azotobacter via soil and leaf-sprayed with humic acid at (8) ml.L^{-1} plus compost at level of (2) ton.donum^{-1} over the lowest average (4.81) kg.m^{-2} that belonged to control plants as observed in table (3).

Table (3): Effect the interactions of Azotobacter, Compost, Humic Acid on yield (kg.m-2) of lettuce crop

Azotobacter	Compost ton/donum	Humic acid ml L-1			
		0	2	4	8
with	0	6.39 f	7.04 e	7.04 e	7.22 de
	1	6.48 f	7.22 de	7.59 b-d	7.78 bc
	2	7.41 c-e	7.96 b	7.63 bc	8.70 a
without	0	4.81 i	5.15 hi	5.11 hi	5.22 hi
	1	5.11 hi	5.13 hi	5.37 h	5.47 h
	2	5.44 h	5.33 hi	5.61 gh	6.02 fg

*Means with same letter for each interaction are not significantly different at 5% level based on Duncan's Multiple Rang Test.

4. Yield per house (kg)

Results of table (4) represent the variable of yield per house of lettuce the highest ever average (4351.85) kg was estimated for plants provided with azotobacter plus soil amendment with compost at (2) ton.donum^{-1} and humic acid at (8) ml.L^{-1} doses as matched to the minimal average (2407.41) kg that was measured for control plants.

Table (4): Effect the interactions of Azotobacter, Compost, Humic Acid and on Yield per house (kg) of lettuce crop

Azotobacter	Compost ton/donum	Humic acid ml L-1			
		0	2	4	8
with	0	3194.44 f	3518.52 e	3518.52 e	3611.11 de
	1	3240.74 f	3611.11 de	3796.30 b-d	3888.89 bc
	2	3703.70 c-e	3981.48 b	3814.81 b-d	4351.85 a
without	0	2407.41 i	2574.07 hi	2555.56 hi	2611.11 hi
	1	2555.56 hi	2564.81 hi	2685.19 hi	2736.11 h
	2	2722.22 h	2665.74 hi	2805.56gh	3009.26 fg

*Means with same letter for each interaction are not significantly different at 5% level based on Duncan's Multiple Rang Test.

5. Total Yield (ton. ha^{-1})

The triple interaction of factors was markedly magnificent on total yield of lettuce. The greatest total yield (76.59) ton.ha^{-1} was yielded by plants having azotobacter via soil composting at (2) ton.donum^{-1} and provided with humic acid at (8) ml.L^{-1} whereas the least total yield (42.37) ton.ha^{-1} was recorded for plants given none of the three factors.

Table (5): Effect the interactions of Azotobacter, Compost, Humic Acid on total yield (ton.ha-1) of lettuce crop

Azotobacter	Compost ton/donum	Humic acid ml L-1			
		0	2	4	8
with	0	56.22 f	61.93 e	61.93 e	63.56 de
	1	57.04 f	63.56 de	66.81 b-d	68.44 bc
	2	65.19 c-e	70.07 b	67.14 b-d	76.59 a
without	0	42.37 i	45.30 hi	44.98 hi	45.96 hi
	1	44.98 hi	45.14 hi	47.26 h	48.16 h
	2	47.91 h	46.92 hi	49.38 gh	52.96 fg

*Means with same letter for each interaction are not significantly different at 5% level based on Duncan's Multiple Rang Test.

6. Total Soluble Solids (TSS %)

The results of the table (6) show the different variables of total soluble solids (TSS %) for lettuce responding to the application of bio-fertilizer, compost, and humic acid fertilizer the biggest recorded average value (6.33) %, this was measured due to soil-inoculating with azotobacter, dosing with humic at (8) $ml.L^{-1}$ and soil composting at (2) $ton.donum^{-1}$ against the lowest ever content (3.60) % ascribed to the blank control (without treatment).

Table (6): Effect of Azotobacter, Compost, Humic Acid and their interactions on total soluble solids (TSS %) of lettuce crop

Azotobacter	Compost ton/donum	Humic acid ml/L			
		0	2	4	8
With	0	4.67b-f	4.83 b-e	4.75 b-e	5.00 b-e
	1	4.77 b-e	5.08 b-e	5.33b-d	5.67ab
	2	5.12b-e	5.48a-c	5.67ab	6.33a
Without	0	3.60g	4.13e-g	4.41d-g	4.60c-g
	1	3.67fg	4.33d-g	4.67b-f	4.53c-g
	2	4.75 b-e	4.68b-f	4.82 b-e	5.07 b-e

*Means with same letter for each interaction are not significantly different at 5% level based on Duncan's Multiple Rang Test.

7. Ascorbic acid (%)

According to the study findings of vitamin C, the bio-fertilizer, humic acid bio-stimulant, and the organic compost fertilizer possessed a positive effect on this vitamin, the complex interference of factors, the peak average value (7.48) % of vitamin C was owed to azotobacter + (2 $ton.donum^{-1}$) compost + (8 $ml.L^{-1}$) humic while the fewest average (4.52 %) was referred to the control (without treatment) as seen in the table (7).

Table (7): Effect the interactions of Azotobacter, Compost, Humic Acid on ascorbic acid (%) of lettuce crop

Azotobacter	Compost ton/donum	Humic acid ml L-1			
		0	2	4	8
with	0	5.14 jk	5.17 i	5.33 g	5.44 g
	1	5.16 i	6.18 f	6.48 d	5.11 l
	2	6.92 b	6.92 b	6.92 b	7.48 a
without	0	4.52 o	4.63 m	4.84 m	5.13 kl
	1	4.62 n	4.83 m	4.85 m	5.18 i
	2	4.63 n	6.22 e	5.17 i	6.55 c

*Means with same letter for each interaction are not significantly different at 5% level based on Duncan's Multiple Rang Test.

Discussion

The obtained unveiled that the soil-inoculated azotobacter and soil addition of compost and humic acid significantly and their combinations, especially the triple one enhanced harvest quantitative and qualitative traits of lettuce cv. (Al-Fajr) in comparison with control. The increase in yield and quality could attribute to the efficiency of azotobacter on improving plant growth, soil microbial activity and soil fertility. This bio-fertilizer plays a major role in the nitrogen cycle in nature that links atmospheric nitrogen unobtainable to plants and triggers it in the form of ammonium ions accessible to plants in the soil with capability of fixing about 20kg N/ha per year. Azotobacter rises about 10-12 % harvest of the entire agriculture crop [21].

According to several studies, the Azotobacter spp. existed in soils had a positive reflection on plants by notably enhancing soil physical-chemical and microbiological properties. The same results were demonstrated by [22] who showed that giving cauliflower plants PSB + Azotobacter produced the best qualitative traits of head and resulted in the highest values of TSS (9.35 %), Vitamin C (79.55 mg/100 g), Nitrogen (0.37 %) and Crude protein (2.33 %) when compared with control. [23] also observed that the azotobacter inoculation

caused a significant enhance in growth and yield of lettuce relative to control.

The ameliorated yield qualitative and quantitative traits under soil application of compost at (2) ton. donum-^{1}may owe to the positive impact of compost on soil characteristics which include the aggregation of soil particles, the potency of soil to retain water, cation exchange capacity (CEC), pH, organic carbon in the soil, enzymes action, cycling of macronutrients and their obtainability [24]. Compost is depicted as a steady broken down organic matter which is resulted from bacterial decomposition process. Numerous aerobic microorganisms population break down and convert organic material into a variety of complex organic substances during composting operation [25][26]. Our findings are in line with that of [27] who found that the maximum yield characters and the least nitrate content of lettuce was measured due to application of compost fertilizer surpassing inorganic fertilizer and vermicompost and with those obtained by [28] who displayed that amending soil with compost and compost extract derived from agro-industrial wastes significantly improved in yield and quality of baby leaf red lettuce relative to control.

On the other hand, the humic acid at (8) ml.L^{-1} significantly increased yield and its quality of lettuce. Similar outcomes were illustrated by [17] who confirmed that providing lettuce plants with T4 (1.5 ml/L soil application) of humic acid and T1 (2.5 ml/L foliar application) gave the peak yield and quality. [18] showed that the highest harvest and quality traits were recorded for lettuce plants received humic acid at concentration (800) mg.l^{-1} as matched to control.

Conclusion

The organic production of important vegetables such as lettuce is getting more attention in recent years due to overuse of harmful inorganic fertilizers that deteriorates the soil and human being health add to that the high increase of palatability of organic produce by consumers. In this study, the inoculation with azotobacter and soil addition of compost at (2) ton.donum^{-1} and foliar spraying of humic acid at (8) ml.L^{-1} and their combinations resulted in maximum attributes of yield and its quality in comparison with control. Therefore, the use of these natural inputs instead of chemicals is advised for organic cropping of lettuce with further studies be implemented in that context.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication and/or funding of this manuscript.

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تأثير الآزوتوباكتر والسماد العضوي وحمض الهيوميك على انتاج ونوعية محصول الخس (LACTUCA SATIVA CV. ALFAJR) المزروع في البيوت البلاستيكية

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- تاريخ استلام البحث2023/11/26 وتاريخ قبوله 2023/12/31.

الملخص

تم اجراء التجربة في البيت البلاستيكي خلال الموسم الخريفي (2021–2022) لدراسة تأثير البكتريا الآزوتوباكتر (مع وبدون)، الكمبوست عند المستويات الثلاثة (0، 1، 2) طن. دونم–1 وحامض الهيوميك بأربعة تراكيز (0، 2، 4 و 8) مل.لتر–1 وتداخلاتها على الحاصل ونوعية نبات الخس (الفجر). تم ترتيب التعاملات وفق تصميم القطاعات العشوائية الكاملة (RCBD) بثلاث مكررات. تم تحليل البيانات باستخدام برنامج SAS (2010). أظهرت النتائج أن تغذية التربة بالآزوتوباكتر وإضافة السماد العضوي بمستوى (2) طن دونم–1 والرش الورقي بحامض الهيوميك بمعدل (8) مل.لتر–1 إما بشكل منفرد أو جماعي أدى إلى زيادة مميزات المحصول [الطازج، وزن الرأس (غم)، المحصول (كغم.م–2)، المحصول القابل للتسويق (طن. هكتار–1)، المحصول لكل بيت بلاستيكي (كغم)، المحصول الكلي (طن. هكتار–1)] بالإضافة إلى زيادة صفات الجودة [إجمالي المواد الصلبة الذائبة (TSS%)، حمض الأسكوربيك (%) مقارنةال control. أما أعلى القيم على الإطلاق للمحصول وجودته فقد تم قياسها للنباتات التي تلقت جرعة: آزوتوباكتر + كمبوست بمعدل (2) طن.دونم–1 + حامض الهيوميك بمعدل (8) مل.لتر 1 بينما سجلت أقل القيم على الإطلاق للنباتات التي لم تتلقى أي جرعة من جميع العوامل المدروسة (control). وعلية يوصى باستخدام الآزوتوباكتر والسماد وحمض الهيوميك للإنتاج العضوي لمحصول الخس.

الكلمات المفتاحية: الآزوتوباكتر، الأسمدة العضوية، حامض الهيوميك، الخس، الإنتاج العضوي.