Pak. j. life soc. Sci. (2024), 22(1): 2030-2043 E-ISSN: 2221-7630;P-ISSN: 1727-4915

**Pakistan Journal of Life and Social Sciences**

www.pjlss.edu.pk

https://doi.org/10.57239/PJLSS-2024-22.1.00148

RESEARCH ARTICLE

# Effect Of Azotobacter, Compost And Humic Acid On Growth And Mineral Content Of Lettuce (Lactuca Sativa Cv. Alfajr) Grown In Plastic House

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***ARTICLE INFO***

Received: May 28, 2024

Accepted: June 26, 2024

***Keywords***

Azotobacter

Compost

Humic acid

Lettuce

Organic productivity

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**ABSTRACT**

A two-seasonal experiment was conducted in a plastic house at the protected cultivation department in Zakho Technical Institute, Dohuk Polytechnic University, Kurdistan region/ Iraq for autumn seasons (2021-2022) and (2022-2023) to examine the effect of azotobacter, compost and humic acid on growth, and mineral composition of lettuce cv. (Alfajr). The study encompassed azotobacter (with and without), soil addition of compost with three levels (0, 1, and 2) ton.donum$^{-1}$ and foliar spraying of humic acid at four concentrations (0, 2, 4.and 8) ml.L$^{-1}$ and their combinations compared to control. The experiment was designed according to randomized complete block design (RCBD) with three replicates. The results displayed that the individual dose and dual interactions of all studied factors significantly improved foliage attributes [head length (cm), leaf area (cm$^2$), chlorophyll content (SPAD)] and leaf mineral content of N, P, and K in both seasons with the superiority for the second season over the first season. The highest ever values of all investigated attributes were measured for plants given azotobacter and compost at (2) ton.donum$^{-1}$ plus humic acid at concentration (8) ml.L$^{-1}$ in comparison with control. The azotobacter with compost and humic acid is recommended for organic production of Alfajr cultivar of lettuce.

## INTRODUCTION

Lettuce (*Lactuca sativa* L.) is an important annual plant from the family Asteraceae and one of the most popular crops among leafy vegetables and salad crops around the world which is grown in cool season (Mohammed et al., 2019). It contains various valuable vitamins and minerals and has several benefits for health owed to anti-inflammatory properties, antioxidant characteristics, insomnia reduction, antioxidant, antimicrobial, anti-cancer features and suppressing anxiety. Lettuce also has adequate amount of calcium, potassium, vitamins (B-complex, C and K) that are active for health protection (Yap & Teo, 2019).

In the Mediterranean region, the overutilization of chemicals for maximum crop production has brought about many disasters for soil structure and properties (Liang et al., 2013; Meena *et al.,* 2016).

Therefore, natural substances were offered by many researchers as marked alternatives for detrimental chemical fertilizers (Pradeepkumar *et al.,* 2017). Azotobacter spp. is an effective plant growth-promoting bacterium (PGPB) which is identified as the most promising bacteria among all microorganisms. It is an aerobic, free-living, and N2-fixer bacterium that usually live in soil, water, and sediments. It is utilized as a bio-fertilizer to ameliorate plant performance and nutrient use efficiency (Sagar *et al.,* 2022). Azotobacter is capable of directly affecting plant growth through the manufacture of phytohormones, vitamins, prevention of ethylene synthesis, anti-stress action and improved nutrient uptake, inorganic phosphate solubility and mineralization of organic phosphate leading to enhance plant stand and productivity (Amanolahi Baharvand *et al.,* 2020; Tan *et al.,* 2022). The efficiency of bio-fertilizer has been confirmed in many field studies. Chatterjee (2015) revealed that inoculating lettuce with azotobacter and Phosphate Solubilizing Bacteria combined with FYM (10 t $ha^{-1}$) and vermicompost (2.5 t $ha^{-1}$) gave the maximum foliage and yield as matched to control. Razmjooei *et al.,* (2022) displayed that treating lettuce plants with azotobacter significantly increased growth and yield traits of crop in comparison with control. Sahin *et al.,* (2015) observed that inoculating lettuce plants with two species of plant-growth promoting bacteria significantly elevated the element content under low irrigation circumstances.

Compost derived from plant, animal and food waste is an organic fertilizer enriched with enough nutritional elements required for plant outgrowth and productivity. The application of compost in agricultural production not only eliminates waste, but also improves plant outgrowth and harvest as the well qualitative organic compost must be enriched with essential nourishing elements and compounds for plant to sustain and yield. Furthermore, the compost also limits the utilization of hazardous synthesized fertilizers that cause serious issues to plant and to the environment (Khan *et al.,* 2019). Hernández *et al.,* (2016) proved that that manure and sewage sludge composts showed Excellency and increased growth and mineral content of lettuce crop whereas the nitrate levels was importantly decreased by such compost. Demir & Kiran (2020) confirmed that the addition of vermicompost at 5% significantly increased vegetative and harvest of lettuce plant as well as enhancing mineral content of macro and micro-elements under salt stress conditions. Cera (2022) indicated that a higher yield was created by plants received 100% compost pared with those delivered 100% chicken manures.

Humic acid is a natural substance produced from decomposition of plant and animal residues (Hayes and Swift, 2020). Humic acid fraction consists of approximately 60% organic carbon (C), which contributes in the soil microbial activity (Sible *et al.,* 2021). Humic acid is enriched with numerous essential nutrients such as N and S and can effectively raise up the soil physical and biochemical functioning through improving its architecture, texture, water holding capacity, and microbial population (Nardi *et al.,* 2017, 2021; Fuentes et al., 2018; Shah et al., 2018). The beneficial effect of humic acid on vegetation comes from enhancing plant hormones requested for outgrowth like auxin and cytokinin, which ameliorate resistance for stresses, metabolism of nutrients, and photosynthesis (Canellas *et al.,* 2020; Laskosky *et al.,* 2020; Nardi *et al.,* 2021; van Tol de Castroet *et al.,* 2021). Abou-El-Hassan and El-Shinawy (2015) showed that giving red cabbage plants 100 and 150% compost + humic acid + EM resulted in the highest foliage and yield attributes and maximized plant content of macro and micronutrients. Ekbiç & Köse (2022) found that vegetative growth and yield was obtained from dosing lettuce plants with 1000 kg/ha humus and 30 l/ha humic acid which surpassed the remained treatments. Mohammed & Kanimarani (2020) demonstrated that the utmost growth and harvest traits were ascribed to treating lettuce plants with 3 g. $L^{-1}$ of humic acid. The aim of this study is to investigate the effect of azotobacter, humic acid and compost on outgrowth and yield of lettuce cv. (Al-Fajr) to produce organic healthy crop with cheaper costs and lesser environmental pollution by use of natural inputs instead of synthesized chemicals.

## MATERIALS AND METHODS

The greenhouse research was undertaken in a plastic house (500 $m^2$). The seeds of lettuce cultivar (Al-Fajr) were planted in plate pods on September 15st 2020 a seed per each pod. The young lettuce plants were transplanted on October at distance of (20) cm between plants and spacing of (60 cm) between terraces. The experiment consisted of the effect of the soil inoculation with Azotobater and without, soil addition of compost at three levels (0, 2, and 4 $ton.donum^{-1}$) and application of humic acid at four concentrations (0, 2, 4, and 8) $ml.L^{-1}$ and their interactions compared to control. The compost was added to the holes a day before transplanting. The humic acid was sprayed to the foliage three times at ten days' intervals. The first spray was executed on November 1st and the second one was carried out on November 10th and the last one was performed on November 20th. The experiment was designed according to factorial randomized complete block design (RCBD). The study comprised of 24 treatments (2x3x4) each treatment was replicated three times. So, the numbers of experimental units were (72). The data analysis was done using (SAS 2010) program and means comparison was performed by Duncan's multiple range test at 5% level of confidence. The head length was measured with measuring bar. The leaf area was by the leaf area meter and the chlorophyll content was measured using chlorophyll Meter (SPAD-502, Konica Minolta). The nitrogen content was determined by Microkjeldahl instrument (A.O.A.C., 1980) that cited by Black (1965). The phosphorus content was measured referring with Spectrophotometer instrument (Matt, 1970) whereas potassium content was estimated using the Flame photometer instrument (A.O.A.C., 1970 and Al -Sahaf, 1989).

### RESULTS AND DISCUSSION

### Head length (cm)

Data listed in the table (1) displays the head length of lettuce in response to the application of azotobacter, compost , humic acid and their various interactions both growth seasons. The soil inoculation with azotobacter produced significantly longer heads (36.13) cm and (38.17) cm for both seasons, respectively as compared to control. The compost treatment significantly influenced head length with the highest mean values (36.67) cm and (38.47) cm being recorded for plants provided with compost at level of (2) $ton.donum^{-1}$ for both season successively relative to control. Similarly, the foliar addition of humic significantly enhanced head length with the highest averages means (36.16) cm and (37.79) cm was belonged to plants fed with humic acid at (8) $ml.L^{-1}$ in both succeeded seasons.

The results also showed that the dual interferences between all factors importantly increased head length of lettuce. In case of azotobacter * compost interaction, the maximum means value (37.52) cm, in the first season, and (39.78) cm, in the second season, was owed to plants received azotobacter and compost at 2 ton.donum-1. Plants inoculated with bio-fertilizer through soil and treated with humic acid at (8) $ml.L^{-1}$ possessed statistically better head lengths (37.47) cm and (39.36) than control and other doses for both growing seasons. In term of compost * humic acid interaction, the best mean values (38.03) cm and (39.92) cm were recorded due to dosing with humic at (8) $ml.L^{-1}$ that exceeded control and other different treatments for both seasons, respectively.

Concerning the peak mean value, plants inoculated with azotobacter via compost at (2) ton.donum-1 plus given humic acid at (8) $ml.L^{-1}$ owned the longest heads (39.42) cm and (41.50) cm in comparison with the shortest heads (32.017) cm recorded for control pared with (33.33) cm which belonged to plants given no azotobacter or composed and foliar fed with humic at dose of (2) $ml.L^{-1}$ as illustrated in the table (1).

**Leaf Area (cm2)**

The leaf area per plant for lettuce was significantly influenced by soil inoculation with azotobacter and soil addition of compost and foliar spraying of humic acid along with their interferences in the two growth season. The azotobacter alone created the maximum leaf areas (257.78) $cm^2$ and (281.23) $cm^2$ for both growing cropping seasons, respectively surpassing control. The individual dose of compost at rate of (2) $ton.donum^{-1}$ led to production of the peak average mean values (229.56) $cm^2$ and (253.86) $cm^2$ relative to control. Plants treated with humic acid at (8) $ml.L^{-1}$ owned statistically larger leaf areas (224.15) $cm^2$ and (248.29) $cm^2$ per plant in both seasons as compared to those delivered no concentration of humic acid.

Concerning the dual interactions, the highest averages leaf area (381.34) $cm^2$ and (305.52) $cm^2$ were owed to soil inoculation with bio-fertilizer and fertilizing with (2) $ton.donum^{-1}$ of compost for both seasons when encountered with no azotobacter and compost. In case of azotobacter * humic acid, plants soil-inoculated with azotobacter and treated with humic acid at (8) ml. $L^{-1}$ have had largest leaf areas 274.10) $cm^2$ and (299.99) $cm^2$ relative to control and other doses. Similarly, the best average leaf area (246.52) $cm^2$, for the first season, pared with the maximal leaf area (271.64) $cm^2$, for the second season, were favored to humic at (8) $ml.L^{-1}$ and compost at (2) $ton.donum^{-1}$ as compared to control.

The complex interaction between the three factors caused a profound increase in the leaf area of lettuce. The highest ever average values (307.36) $cm^2$ and (334.40) $cm^2$ were possessed by plants inoculated with azotobacter and treated with humic at (8) $ml.L^{-1}$ plus compost at (2) $ton.donum^{-1}$ against the least ever average value (164.87) $cm^2$ and (188.20) $cm^2$ being measured for plant given no treatment in both growth seasons, respectively (see: table 2).

**Chlorophyll Content (SPAD)**

The analyzed data in the table (3) revealed a significant increment in the chlorophyll content (SPAD) due to the inoculation with bio-fertilizer and soil application of compost, humic acid and their combinations in both growing seasons. The individual efficacy of each factor made a profound improvement in green pigment of lettuce and the azotobacter significantly affected chlorophyll content producing a mean values of (50.07) and (53.64) that was statistically differ from that of control. Relating compost impact, the maximum chlorophyll contents (50.49) and (53.64) were recorded when planted soil was applied with compost at (2) $ton.donum^{-1}$ against control in both seasons, successively. The sole action of humic acid at concentration (8) $ml.L^{-1}$ was also significant on the green pigment creating a mean values of (50.68) and (53.69), respectively in comparison with other treatments.

The dual interactions between factors significantly enhanced chlorophyll content during both seasons. An important averages (52.86) and (56.82) were produced when azotobacter was interacted with compost at (2) $ton.donum^{-1}$ relative to control. Humic acid at (8) $ml.L^{-1}$ combined with azotobacter resulted in the greatest average contents (52.97) and (56.22) as compared other treatments for both succeeded seasons. The last dual interaction (compost * humic acid) was effective in giving the best averages (54.23) and (57.59) when plants have had humic at (8) $ml.L^{-1}$ and compost at (2) $ton.donum^{-1}$ as matched to control. Furthermore, the biggest average chlorophyll contents (59.15 and 63.18) were belonged to lettuce plants soil-inoculated with azotobacter and soil-provided with compost at level of (2) $ton.donum^{-1}$, humic acid at (8) $ml.L^{-1}$ at both growing seasons whereas the least ever average contents (44.67 and 48.30) were measured in leaves of plants that did not receive any actual dose of the three factors.

**Nitrogen (N) Content (%)**

Table (4) displays different averages of nitrogen content in leaves of lettuce as affected by application of azotobacter, compost and humic acid, along with their interferences during both growing seasons. Azotobacter inoculation created the highest nitrogen contents (1.40 and 1.49) % over control. The compost application resulted in a remarkable improvement in the nitrogen contents (1.44 and 1.48) %, respectively when plants fertilized with compost at (2) ton.donum$^{-1}$ as compared to control. In state of humic, the largest contents (1.40) and 1.44) % were measured in leaves of plants treated with the humic acid at (8) ml.L$^{-1}$ in both cropping seasons.

Concerning binary interactions, significant average percentage (1.52 and 1.60) % were obtained from azotobacter with compost level at (2) ton.donum$^{-1}$ in comparison with control in the two growing seasons. The same important impact was created from combination between azotobacter and humic with the highest average percentages (1.45 and 1.56) % being recorded for plants treated with humic acid at concentration (8) ml.L$^{-1}$ and azotobacter surpassing other doses respective to both seasons. In case of compost * humic acid interaction, the leaf of plants applied with humic acid at (8) ml.L$^{-1}$ plus compost at (2) ton.donum$^{-1}$ contained the greatest amounts of nitrogen (1.53 and 1.57) % over control plants.

The triple interaction showed Excellency in ameliorating the nitrogen content with the maximum average values (1.61 and 1.70) % being estimated in leaves of plants dosed with the complex treatment; azotobacter + compost at (2) ton.donum$^{-1}$ + humic at (8) ml.L$^{-1}$ but the lowest average contents (1.17 and 1.20) % were measured in plants dosed with no humic or compost without inoculation with azotobacter as obvious in the table (4).

**Phosphorus (P) Content (%)**

Results showed in the table (5) demonstrate the phosphorus (P) content in leaves of lettuce in term of application of the three factors (bio-fertilizer, humic acid, and compost) and their combinations for both growing seasons. The azotobacter inoculated to the soil has resulted in the best mean (0.44) % for the first growing season followed by the maximum mean (0.52) % of P for the second season when compared to control. Respective to compost sole effect, the highest mean values of P (0.50 and 0.61) % were estimated in plants dosed with compost at level of (2) ton.donum$^{-1}$ in both growing seasons, respectively. The humic acid individually at dosages of (8) ml.L$^{-1}$ showed supremacy producing mean value of (0.47) % in the first season and mean value of (0.55) % for the second season surpassing control and other doses.

Regarding the double effects, plants treated with azotobacter and compost at (2 ton.donum$^{-1}$) possessed the biggest average contents (0. 55 and 0. 66) % relative to control. In case of azotobacter * humic effect, plants treated with azotobacter and humic acid at (8) ml.L$^{-1}$ owned the premium average P content (0.51) % for the first season pared with the next maximum average(0.60) % for the second season against the least content belonged to control. The binary interaction between compost and humic was significant on P content with the maximal averages (0.57and 0.68) % being measured in plants treated with humic at (8) ml.L$^{-1}$ and compost at (2) ton.donum$^{-1}$ over control. For the complicated interaction, the largest ever contents (0. 67and 0.76) % were determined for plants have had azotobacter and humic acid at (8) ml.L$^{-1}$ plus composting at (2) ton.donum$^{-1.}$ Moreover, the least average contents (0.28 and 0.20) % were referred to plants given no treatment as seen in the table (5).

**Potassium (K) Content (%)**

The percentage of potassium in lettuce at both cropping seasons was importantly excelled by application of studied factors and their interactions. The azotobacter alone inoculated to the soil created greatest mean (3.64) % for first season followed by highest mean (3.94) % content of K for the second season. The soil application of compost at (2) ton.donum$^{-1}$ resulted in the notable improve in K content (3.63 and 3.96) % in both cropping seasons as encountered with control. Related to humic acid impact, the maximum K contents (3.57 and 3.83) % was measured in plants given humic at concentration (8) ml.L$^{-1}$ as compared to other treatments.

About the azotobacter * compost effect, the premium K contents (3.93 and 4.27) % were measured for plants with inoculated azotobacter and soil-composted at (2) ton.donum$^{-1}$ over control in the two seasons, respectively. Regarding the azotobacter * humic interference, the largest amounts of K (3.82 and 4.25) % were recorded in plants treated with azotobacter and humic acid at (8) ml.L$^{-1}$ exceeding control and the rest of treatments. Significant enhancement was observed in K content of lettuce ascribed to interaction between humic and compost in comparison with control with the highest averages (3.89 and 4.38) % recorded for plants treated with humic at (8) ml.L$^{-1}$ with (2) ton.donum$^{-1}$ of compost.

The triple effect of the factors also importantly ameliorated the K percentage in lettuce with the maximum averages (4.34 and 4.98) % being estimated for plants applied with azotobacter and humic acid at concentration (8) ml.L$^{-1}$ plus (2) ton.donum$^{-1}$of compost fertilizer as matched to the fewest averages (2.67 and 3.76) % recorded for control treatments as conspicuous in the table (6).

It's evident from obtained results that the soil-inoculated azotobacter, soil addition of compost and foliar spraying of humic acid significantly and their combinations enhanced vegetative, harvest and mineral content of lettuce cv. (Al-Fajr) especially the triple one as matched to control in both growing seasons with the superiority for the second season. The increase in leaf area and chlorophyll content could attribute to the potential nitrogen fixation and excreting out of ammonia by azotobacter that improved the photosynthesis efficiency leading to the production of more carbohydrates giving more leaf area and green pigments and greater number of leaves and thus longer stems (Beovides-García *et al.,* 2022). In this context, our results are in line with that of Razmjooei *et al.,* (2022) who indicated that providing lettuce plants with azotobacter significantly elevated growth components of crop over control.

The ameliorated vegetative traits could also favor to the beneficial effect of humic acid and compost on soil fertility, soil physical, chemical and biological properties and nutrient obtainability and uptake by plants with better root system. These properties encompassed the aggregation and relative proportion of soil particles, the capability of soil to hold water, cation exchange capacity (CEC), pH, organic carbon in the soil, enzymes functioning, cycling of macronutrients and their obtainability (Ampong *et al.,* 2022). On the other hand, Application of organic-based substances to soil have favored its fertility, mainly due to their role in supplying necessary nutrients and their efficiency in impacting the physical characteristics of the soil. In farming systems, organic residues were the sole choice for providing soil with many nutrients, especially nitrogen (Dhankar, 2019). In such case, our findings agree with those of Ekbiç & Köse (2022) who confirmed that vegetative growth and yield was earned from applying lettuce plants with 1000 kg/ha humus and 30 l/ha humic acid as compared to control and those published by Cera (2022) who revealed that lettuce plants given 100% compost owned the maximum foliage and harvest attributes.

The increase in mineral traits of lettuce might be ascribed to the azotobacter contribution of fixing the atmospheric gaseous nitrogen into mineralized ionic one available for plant. These micro-organisms can make association with the plants and assist altering the organic form into inorganic form of nutrient elements (Kaushal, & Kukreja, 2020). Moreover, Addition of bio-fertilizers leads to enhancement in uptake of nutrients and water, root formation and proliferation, foliage development and nitrogen fixation which motivates synthesis of growth stimulating material such as vitamin-B complex, Indole acetic acid (IAA) and Gibberellins etc. Hence, they unleash growth stimulating substances and vitamins and assist in maintaining soil fertility (Pratap, 2012). Humic acid promotes many active operations that enhances plant out growth and supporting root growth, particularly vertically, thereby magnifying the roots ability for better absorb of water and nourishing nutrients. It elevates root respiration and the creation of root hairs and enhances the production of chlorophyll, sugars and amino acids leading to better productivity (Pettit and Robert, 2003; AL-Taey *et al.,* 2019).

The increase in mineral composition of lettuce by compost application may refer to the fact that compost have main positive effects on soil features, especially in poor fertile soil. it enhances the content of organic matter and then gives necessary macro and micro nutrients for plant development (Sanchez-Moneru *et al.,* 2004; Tejada *et al.,* 2009). Our study results concerning the triple effect are in agreement with that of Abou-El-Hassan and El-Shinawy (2015) on red cabbage who illustrated that dosing plants with 100 and 150% compost + humic acid + EM gave highest best vegetative and yield parameters and ameliorated plant content of macro and micronutrients.

**Table 1: Effect of azotobacter, Compost, humic acid and their interactions on head length (cm) of lettuce**

| First season | | | | | | | |
|---|---|---|---|---|---|---|---|
| Azotobacter | Compost | Humic acid ml/L | | | | Azotobacter * Compost | Azotobacter |
| | | 0 | 2 | 4 | 8 | | |
| With | 0 | 33.33e-h | 35.00b-g | 35.33b-f | 36.33b-d | 35.00b | 36.13a |
| | 1 | 35.17b-f | 35.67b-e | 36.00 b-d | 36.67 b-d | 35.88b | |
| | 2 | 36.33 b-d | 37.00bc | 37.33ab | 39.42a | 37.52a | |
| Without | 0 | 32.17h | 32.07gh | 33.00f-h | 33.53e-h | 32.69c | 33.96b |
| | 1 | 32.67h | 33.33e-h | 33.15f-h | 34.33d-h | 33.37c | |
| | 2 | 34.67c-g | 35.67b-e | 36.33 b-d | 36.65 b-d | 35.83b | |
| Humic acid | | 34.06c | 34.79bc | 35.19b | 36.16a | Compost | |
| Azotobacter * Humic acid | With | 34.94b-d | 35.89bc | 36.22b | 37.47a | | |
| | without | 33.17e | 33.69de | 34.16de | 34.84cd | | |
| Compost * Humic acid | 0 | 32.75f | 33.54ef | 34.17d-f | 34.93c-e | 0 | 33.85c |
| | 1 | 33.92d-f | 34.50de | 34.58de | 35.50b-d | 1 | 34.62b |
| | 2 | 35.50b-d | 36.33bc | 36.83ab | 38.03a | 2 | 36.67a |
| **Second season** | | | | | | | |
| Azotobacter | Compost | Humic acid ml/L | | | | Azotobacter * Compost | Azotobacter |
| | | 0 | 2 | 4 | 8 | | |
| With | 0 | 34.67f-j | 36.65b-j | 37.17b- | 38.33a-d | 36.70b | 38.17a |
| | 1 | 38.08b-f | 37.58b-g | 38.17a-e | 38.25a-e | 38.02b | |
| | 2 | 38.67a-d | 39.27a-c | 39.67ab | 41.50a | 39.78a | |
| Without | 0 | 33.33j | 34.33g-j | 34.00h-j | 34.67f-j | 34.08c | 35.28b |
| | 1 | 33.67ij | 34.83e-j | 34.17g-j | 35.67d-j | 34.58c | |
| | 2 | 36.00c-j | 37.00b-i | 37.33b-h | 38.33a-d | 37.17b | |
| Humic acid | | 35.74b | 36.61ab | 36.75ab | 37.79a | Compost | |
| Azotobacter * Humic acid | With | 37.14b-d | 37.83a-c | 38.33ab | 39.36a | | |
| | without | 34.33f | 35.39d-f | 35.17ef | 36.22c-e | | |
| Compost * Humic acid | 0 | 34.00e | 35.49de | 35.58de | 36.50b-d | 0 | 35.39b |
| | 1 | 35.88c-e | 36.21b-e | 36.17b-e | 36.96b-d | 1 | 36.30b |
| | 2 | 37.33b-d | 38.13a-c | 38.50ab | 39.92a | 2 | 38.47a |

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

**Table 2: Effect of azotobacter, compost, humic acid and their interactions on leaf area (cm2) of lettuce crop**

| First Season | | | | | | | |
|---|---|---|---|---|---|---|---|
| Azotobacter | Compost ton/donm | Humic acid ml L-1 | | | | Azotobacter * Compost | Azotobacter |
| | | 0 | 2 | 4 | 8 | | |
| with | 0 | 207.90 f | 226.31 ef | 228.78 e | 239.09 de | 225.52 c | 257.78 a |
| | 1 | 255.95 cd | 257.58 cd | 276.53 bc | 275.85 bc | 266.48 b | |
| | 2 | 255.16 cd | 270.58 c | 292.26 ab | 307.36 a | 281.34 a | |
| without | 0 | 164.87 g | 165.63 g | 166.96 g | 166.13 g | 165.90 e | 170.26 b |
| | 1 | 165.88 g | 164.92 g | 166.81 g | 170.80 g | 167.10 e | |
| | 2 | 173.04 g | 176.28 g | 176.12 g | 185.69 g | 177.78 d | |
| Humic acid | | 203.80 c | 210.22 bc | 217.91 ab | 224.15 a | Compost | |
| Azotobacter * Humic acid | With | 239.67 c | 251.49 b | 265.86 a | 274.10 a | | |
| | without | 167.93 d | 168.94 d | 169.96 d | 174.21 d | | |
| Compost * Humic acid | 0 | 186.38 g | 195.97 fg | 197.87 e-g | 202.61 d-f | 0 | 195.71 c |
| | 1 | 210.92 c-e | 211.25 c-e | 221.67 bc | 223.32 bc | 1 | 216.79 b |
| | 2 | 214.10 cd | 223.43 bc | 234.19 ab | 246.52 a | 2 | 229.56 a |
| Second season | | | | | | | |
| Azotobacter | Compost | Humic acid ml/L | | | | Azotobacter * Compost | Azotobacter |
| | | 0 | 2 | 4 | 8 | | |
| With | 0 | 231.38g | 249.54fg | 251.89f | 266.33ef | 249.78c | 281.23a |
| | 1 | 276.17de | 281.02c-e | 297.13b-d | 299.25bc | 288.39b | |
| | 2 | 278.60de | 293.70cd | 315.38ab | 334.40a | 305.52a | |
| Without | 0 | 188.20h | 189.20h | 190.13h | 189.77h | 189.33e | 193.72b |
| | 1 | 189.06h | 188.27h | 190.10h | 191.12h | 189.64e | |
| | 2 | 197.28h | 199.65h | 202.99h | 208.88h | 202.20d | |
| Humic acid | | 226.78c | 233.56bc | 241.27ab | 248.29a | Compost | |
| Azotobacter * Humic acid | With | 262.05d | 274.75c | 288.13b | 299.99a | | |
| | without | 191.51e | 192.37e | 194.41e | 196.59e | | |
| Compost * Humic acid | 0 | 209.79g | 219.37fg | 221.01e-g | 228.05d-f | 0 | 219.55c |
| | 1 | 232.61c-f | 234.64c-e | 243.61c | 245.19bc | 1 | 239.01b |
| | 2 | 237.94cd | 246.68bc | 259.18ab | 271.64a | 2 | 253.86a |

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

**Table 3: Effect of azotobacter, compost, humic acid and their interactions on chlorophyll content of lettuce crop**

| First season | | | | | | | |
|---|---|---|---|---|---|---|---|
| Azotobacter | Compost ton/donm | Humic acid ml L-1 | | | | Azotobacter * Compost | Azotobacter |
| | | 0 | 2 | 4 | 8 | | |
| with | 0 | 46.72 c-e | 46.85 c-e | 47.97 c-e | 49.23 b-d | 47.69 c | 50.07 a |
| | 1 | 47.07 c-e | 48.60 c-e | 52.47 b | 50.52 bc | 49.66 b | |
| | 2 | 49.40 b-d | 50.37 bc | 52.52 b | 59.15 a | 52.86 a | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| without | 0 | 44.67 e | 44.70 e | 45.60 de | 47.40 c-e | 45.59 d | 47.11 b |
| | 1 | 47.35 c-e | 47.02 c-e | 47.68 c-e | 48.45 c-e | 47.63 c | |
| | 2 | 47.28 c-e | 47.35 c-e | 48.57 c-e | 49.30 b-d | 48.13 bc | |
| Humic acid | | 47.08 c | 47.48 c | 49.13 b | 50.68 a | Compost | |
| Azotobacter * Humic acid | With | 47.73 cd | 48.61 c | 50.98 b | 52.97 a | | |
| | without | 46.43 d | 46.36 d | 47.28 cd | 48.38 cd | | |
| Compost * Humic acid | 0 | 45.69 f | 45.78 f | 46.78 ef | 48.32 b-f | 0 | 46.64 c |
| | 1 | 47.21 d-f | 47.81 c-f | 50.08 bc | 49.48 b-d | 1 | 48.64 b |
| | 2 | 48.34 b-f | 48.86 b-e | 50.54 b | 54.23 a | 2 | 50.49 a |
| Second season | | | | | | | |
| Azotobacter | Compost | Humic acid ml/L | | | | Azotobacter * Compost | Azotobacter |
| | | 0 | 2 | 4 | 8 | | |
| With | 0 | 49.42d-f | 51.12 b-f | 50.66 b-f | 52.18b-f | 50.84c | 53.64a |
| | 1 | 53.08b-d | 51.21 b-f | 55.49bc | 53.32b-d | 53.25b | |
| | 2 | 54.78b-d | 53.18b-d | 56.12b | 63.18a | 56.82a | |
| without | 0 | 48.30ef | 47.42f | 48.34ef | 50.15c-f | 48.55c | 49.84b |
| | 1 | 50.03c-f | 50.05c-f | 50.63 b-f | 51.30 b-f | 50.50c | |
| | 2 | 49.94c-f | 48.87ef | 51.05 b-f | 52.00 b-f | 50.47c | |
| Humic acid | | 50.93b | 50.29b | 52.05ab | 53.69a | Compost | |
| Azotobacter * Humic acid | With | 52.43bc | 51.81b-d | 54.09ab | 56.22a | | |
| | without | 49.42de | 48.78e | 50.01c-e | 51.15c-e | | |
| Compost * Humic acid | 0 | 48.86d | 49.27cd | 49.50cd | 51.16 b-d | 0 | 49.70c |
| | 1 | 51.56 b-d | 50.59 bd | 53.06bc | 52.31b-d | 1 | 51.88b |
| | 2 | 52.36 b-d | 51.03 bd | 53.59b | 57.59a | 2 | 53.64a |

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

**Table 4: Effect of azotobacter, compost, humic acid and their interactions on nitrogen content (%) of lettuce crop**

| First season | | | | | | | |
|---|---|---|---|---|---|---|---|
| Azotobacter | Compost | Humic acid ml/L | | | | Azotobacter * Compost | Azotobacter |
| | | 0 | 2 | 4 | 8 | | |
| With | 0 | 1.22h-j | 1.25g-j | 1.33e-h | 1.31e-i | 1.28d | 1.40a |
| | 1 | 1.34d-g | 1.44b-d | 1.47bc | 1.44b-d | 1.42b | |
| | 2 | 1.45bc | 1.50b | 1.51ab | 1.61a | 1.52a | |
| Without | 0 | 1.17j | 1.20ij | 1.25g-j | 1.22h-j | 1.21e | 1.29b |
| | 1 | 1.26g-j | 1.27f-j | 1.28e-j | 1.37c-f | 1.30d | |
| | 2 | 1.25g-j | 1.32e-h | 1.39c-e | 1.46bc | 1.35c | |
| Humic acid | | 1.28c | 1.33b | 1.37a | 1.40a | Compost | |
| Azotobacter * Humic acid | With | 1.34c | 1.39ab | 1.44a | 1.45a | | |
| | without | 1.23e | 1.26de | 1.31cd | 1.35bc | | |
| Compost * Humic acid | 0 | 1.20g | 1.22fg | 1.29d-f | 1.27e-g | 0 | 1.24c |
| | 1 | 1.30de | 1.36cd | 1.38c | 1.41bc | 1 | 1.36b |
| | 2 | 1.35cd | 1.41bc | 1.45b | 1.53a | 2 | 1.44a |
| **Second season** | | | | | | | |
| Azotobacter | Compost | Humic acid ml/L | | | | Azotobacter * Compost | Azotobacter |
| | | 0 | 2 | 4 | 8 | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| With | 0 | 1.31e-g | 1.33e-i | 1.36ef | 1.40d-f | 1.35c | 1.49a |
| | 1 | 1.49b-d | 1.54bc | 1.52b-d | 1.58b | 1.53b | |
| | 2 | 1.54bc | 1.58b | 1.56bc | 1.70a | 1.60a | |
| Without | 0 | 1.20i | 1.22hi | 1.27g-i | 1.20i | 1.21d | 1.30b |
| | 1 | 1.30f-i | 1.31f-i | 1.34e-h | 1.32f-i | 1.32c | |
| | 2 | 1.29f-i | 1.34e-h | 1.40d-f | 1.45c-e | 1.37c | |
| Humic acid | | 1.36b | 1.39b | 1.41ab | 1.44a | Compost | |
| Azotobacter * Humic acid | With | 1.45b | 1.49b | 1.48b | 1.56a | | |
| | without | 1.27c | 1.29c | 1.34c | 1.30c | | |
| Compost * Humic acid | 0 | 1.26c | 1.28c | 1.33c | 1.31c | 0 | 1.29c |
| | 1 | 1.40b | 1.43b | 1.43b | 1.45b | 1 | 1.43b |
| | 2 | 1.42b | 1.46b | 1.48b | 1.57a | 2 | 1.48a |

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

**Table 5: Effect of azotobacter, compost, humic acid and their interactions on phosphorus content (%) of lettuce crop**

| First season | | | | | | | |
|---|---|---|---|---|---|---|---|
| Azotobacter | Compost | Humic acid ml/L | | | | Azotobacter * Compost | Azotobacter |
| | | 0 | 2 | 4 | 8 | | |
| With | 0 | 0.30h-j | 0.36e-j | 0.37e-j | 0.35e-j | 0.35d | 0.44a |
| | 1 | 0.38e-j | 0.33g-j | 0.42d-g | 0.52bc | 0.41bc | |
| | 2 | 0.44c-e | 0.55b | 0.55b | 0.67a | 0.55a | |
| Without | 0 | 0.28j | 0.33g-j | 0.30ij | 0.34f-j | 0.31d | 0.38b |
| | 1 | 0.36e-j | 0.39d-i | 0.38e-j | 0.45c-e | 0.39c | |
| | 2 | 0.40d-h | 0.44c-e | 0.48b-d | 0.48b-d | 0.45b | |
| Humic acid | | 0.36c | 0.40b | 0.41b | 0.47a | Compost | |
| Azotobacter * Humic acid | With | 0.37cd | 0.41bc | 0.45b | 0.51a | | |
| | without | 0.35d | 0.39cd | 0.38cd | 0.42bc | | |
| Compost * Humic acid | 0 | 0.29e | 0.35de | 0.33de | 0.35de | 0 | 0.33c |
| | 1 | 0.37cd | 0.36cd | 0.40cd | 0.48b | 1 | 0.40b |
| | 2 | 0.42c | 0.49b | 0.52ab | 0.57a | 2 | 0.50a |
| **Second season** | | | | | | | |
| Azotobacter | Compost | Humic acid ml/L | | | | Azotobacter * Compost | Azotobacter |
| | | 0 | 2 | 4 | 8 | | |
| With | 0 | 0.31lm | 0.47f-i | 0.35kl | 0.34kl | 0.37d | 0.52a |
| | 1 | 0.45g-j | 0.40i-l | 0.53d-g | 0.72ab | 0.52b | |
| | 2 | 0.53d-g | 0.64bc | 0.73ab | 0.76a | 0.66a | |
| Without | 0 | 0.20n | 0.24mn | 0.32lm | 0.39i-l | 0.28e | 0.42b |
| | 1 | 0.33kl | 0.42h-k | 0.36j-l | 0.54d-f | 0.41c | |
| | 2 | 0.49e-h | 0.54d-g | 0.58c-e | 0.59cd | 0.55b | |
| Humic acid | | 0.38c | 0.45b | 0.48b | 0.55a | Compost | |
| Azotobacter * Humic acid | With | 0.43c | 0.50b | 0.53b | 0.60a | | |
| | without | 0.34d | 0.40c | 0.42c | 0.51b | | |
| Compost * Humic acid | 0 | 0.25g | 0.35ef | 0.33f | 0.36ef | 0 | 0.33c |
| | 1 | 0.39d-f | 0.41de | 0.44d | 0.63ab | 1 | 0.47b |
| | 2 | 0.51c | 0.59b | 0.65ab | 0.68a | 2 | 0.61a |

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

**Table 6: Effect of azotobacter, compost, humic acid and their interactions on potassium content (%) of lettuce crop**

| First season | | | | | | | |
|---|---|---|---|---|---|---|---|
| Azotobacter | Compost ton/donm | Humic acid ml L-1 | | | | Azotobacter * Compost | Azotobacter |
| | | 0 | 2 | 4 | 8 | | |
| with | 0 | 3.47 c-f | 3.32 e-g | 3.74 bc | 3.60 b-e | 3.53 b | 3.64 a |
| | 1 | 3.22 e-g | 3.48 c-f | 3.63 b-e | 3.53 c-f | 3.47 b | |
| | 2 | 3.85 b | 3.69 b-d | 3.85 b | 4.34 a | 3.93 a | |
| without | 0 | 2.76 i | 2.91 hi | 2.91 hi | 3.09 gh | 2.92 e | 3.14 b |
| | 1 | 2.78 i | 3.27 fg | 3.27 fg | 3.39 d-g | 3.18 d | |
| | 2 | 3.32 e-g | 3.32 e-g | 3.22 fg | 3.44 c-f | 3.33 c | |
| Humic acid | | 3.23 c | 3.33 bc | 3.44 b | 3.57 a | Compost | |
| Azotobacter * Humic acid | With | 3.51 b | 3.50 b | 3.74 a | 3.82 a | | |
| | without | 2.95 e | 3.17 cd | 3.13 d | 3.31 c | | |
| Compost * Humic acid | 0 | 3.12 d | 3.12 d | 3.33 c | 3.35 c | 0 | 3.23 c |
| | 1 | 3.00 d | 3.38 bc | 3.45 bc | 3.46 bc | 1 | 3.32 b |
| | 2 | 3.59 b | 3.51 bc | 3.53 bc | 3.89 a | 2 | 3.63 a |
| **Second season** | | | | | | | |
| Azotobacter | Compost | Humic acid ml/L | | | | Azotobacter * Compost | Azotobacter |
| | | 0 | 2 | 4 | 8 | | |
| with | 0 | 3.66b-f | 3.65b-f | 3.74b-f | 3.86b-d | 3.73b | 3.94a |
| | 1 | 3.55b-f | 3.81b-f | 3.96b-d | 3.90b-d | 3.81b | |
| | 2 | 3.85b-d | 4.02bc | 4.23b | 4.98a | 4.27a | |
| without | 0 | 3.76b-f | 3.24d-f | 3.24d-f | 3.09f | 3.34c | 3.45b |
| | 1 | 3.11ef | 3.27c-f | 3.60b-f | 3.39c-f | 3.34c | |
| | 2 | 3.65b-f | 3.65b-f | 3.55b-f | 3.78b-f | 3.66bc | |
| Humic acid | | 3.60a | 3.61a | 3.72a | 3.83a | Compost | |
| Azotobacter * Humic acid | With | 3.69b-d | 3.83bc | 3.98ab | 4.25a | | |
| | Without | 3.51cd | 3.39d | 3.46cd | 3.42d | | |
| Compost * Humic acid | 0 | 3.71bc | 3.45bc | 3.49bc | 3.48bc | 0 | 3.53b |
| | 1 | 3.33c | 3.54bc | 3.78bc | 3.64bc | 1 | 3.57b |
| | 2 | 3.75bc | 3.84bc | 3.89b | 4.38a | 2 | 3.96a |

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

## CONCLUSION

The use of natural products in cropping system is getting a notable attention during the recent year due to their efficacy and safety as they give crops with premium growth, yield and quality without polluting the environment. In our study, the azotobacter as bio-fertilizer, compost as organic fertilizer and humic acid as bio-stimulant either individually or in dual and triple combinations significantly enhanced lettuce growth and mineral nutrient content in comparison with control. Therefore, it is recommended that these three natural substances should be utilized for organic vegetable production in Iraqi Kurdistan Region with more scientific studies must be implemented with these products on other vegetables. The lettuce cultivar (Al-Fajr) is also advised for greenhouse production.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge the staff of the University of Duhok for their technical and general support.

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