Academic Journal of Nawroz University (AJNU), Vol.13, No.2, 2024

https://doi.org/10.25007/ajnu.v13n2a1551

Effect of Biofertilizer, Plant Growth Activator, and Plant Distance on Growth of Garlic (Allium sativum L.)

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ABSTRACT: The use of organic fertilizers instead of chemical (inorganic) ones with proper cultural practices is proposed to have a great impact on crop outgrowth and yield. A field experiment was carried out in the vegetable research field at the protected cultivation department, Zakho technical institute, Dohuk polytechnic University, Iraq to inspect the impact of azotobacter, plant growth activator with three levels (0 ml. L^{-1}, 1 ml. L^{-1}, and 2 ml. L^{-1}) and three plant spacing (5 cm, 10 cm, and 15 cm) on growth and leaf mineral composition of local garlic. The treatments were organized in a split-split randomized complete block design (split- split RCBD) with three replicates. The obtained results demonstrated that the single treatment of azotobacter surpassed activator and plant density and significantly ameliorated all vegetation parameters [plant height (cm), chlorophyll content (SPAD), leaf length and width (cm), number of leaves, neck diameter (cm), and dry matter percentage of vegetative growth (%)] as well as improving leaf mineral composition of N, P, and K as compared to control. Single effect of plant spacing at (15 cm) importantly improved leaf length, dry matter percentage of vegetative growth and the leaf potassium (K) content. The individual dose (ml. L^{-1}) of growth activator significantly enhanced chlorophyll content, neck diameter, dry matter percentage of vegetative growth and leaf content of phosphorus (P). Furthermore, the dual and triple combinations of all factors significantly increased vegetative and leaf mineral parameters of local garlic. The azotobacter either individually or along with growth activator and plant density is recommended for maximum growth of local garlic.

KEY WORDS: Azotobacter, plant growth activator, planting density, local garlic, organic production.

1. INTRODUCTION

This Garlic (Allium sativum L.) is considered as one of the most significant crop planted by human being since ancient times. It is a prominent member of Alliaceae family having compounded bulbs that contain several bulbets with varied volumes named as cloves that are curtained by white scale leaves (Das et al., 2020). It is an essential component of human diet around the world favored to its taste and health-stimulation values (Matłok et al. 2014). Garlic possesses precious nutritive and health values referred to its maximal content of indispensable substances that are vital from biological aspect, which their levels are commonly dependent on guaranteeing proper circumstances for crop outgrowth and development at span of vegetation (Matysiak et al. 2015).

In modern farming systems, the utilization of natural fertilizers in place of inorganic chemical formulas is the key factor to assure the production of vegetables with excellent qualitative features (Petrovic et al., 2019). Biofertilizers are recognized as natural preparations enriched with living cells of microorganisms utilized as efficient inputs sources in organic farming. They elevate the exploitation of plant nourishing elements and motivate outgrowth via synthesized growth factors (Choudhary et al., 2015). Biofertilizers are capable of uprising the obtainability of nitrogen and phosphorus through ameliorating the atmospheric nitrogen bio-fixation or via dissolution of the insoluble phosphates driving to a better fertile soil with premium microbial activities (Alori et al., 2017; Kalayu, 2019). Rady et al. (2018) showed that giving garlic plant the seaweed extract plus biofertilizer significantly enhanced harvest parameters like Bulbs dry matter (%), clove number/bulb, bulb fresh weight (g/plant), diameter of bulb (cm), and total yield of bulb (ton/fed) as well as enhancing mineral composition of bulbs. Meena et al. (2019) displayed that providing garlic crop with numerous biofertilizers importantly improved vegetation and harvest attributes relative to control. Bhushan et al. (2020) unveiled that the best foliage and yield traits of garlic was obtained from a complex dosage of Azotobactor 8 kg/ha + PSB 8 kg/ha+ VAM 8 kg/ha (T12) surpassing control and other dosages. Kaur et al. (2021) recorded the highest plant height, leaf length and stem diameter for garlic plants treated with Azotobacter + PSB.

For perfect management and extra quality, the planting density is a critical factor in garlic production. Though chemical constituents and sensory features of palatability, aroma and taste is firstly dependent upon genetic factors, management services such as planting density is also reliable upon in this context (Randle, 1997). The uptake and utilization of solar radiation, water and nutrients by plants is markedly altered by planting density influencing the canopy or rooting architecture (Hammer et al., 2009; Du et al., 2021). Increased planting density makes the intercepted photosynthetically active radiation better resulting in ameliorated leaf area index and ultimately enhance plant biomass and yield (Teixeira et al., 2014; Hernández et al., 2020). Temperini et al. (2010) illustrated that increasing the planting density had importantly improved garlic bulb harvest and its mineral composition. Nagina et al. (2017) displayed that the maximum foliage attributes were obtainable from plant spacing of 5 cm and 11 cm. Kumar et al. (2018), on garlic, demonstrated that the excellent vegetation and yield components were earned from planting at space of 15 cm × 10 cm. Lima et al. (2019) findings on the impact of plant spacing on garlic revealed that the maximal harvest and its quality were due to cultivating plants at density of 12.5 and 15.0 cm exceeding control and other planting densities.

Recently, many commercial preparations known as plant growth activators have been efficiently used in agriculture. These preparations mainly enriched with amino acids, seaweed extracts and other growth promoting substances. The physiological processes of plant are directly and indirectly impacted by amino acids (Khalel and Sultan, 2019). Amino acids are contributed in enzymes sovereign the photosynthesis process. Amino acids also make chelation with microelements, when given with microelements, the uptake and translocation of these microelements become facilitated within the plant (Ibrahim, 2010). Seaweed extracts, on the other hand, are significant biostimulants obtained from marine algae. They contain numerous beneficial compounds including sugars, N-enriched molecules (e.g., betaine), plant hormones and various nourishing nutrients that positively influence plant outgrowth (Thirumaran et al., 2009; Khan et al. 2009; Craigie 2011). Majkowska-Gadomska et al. (2019) reported that the addition of amino acids to garlic resulted in perfect values of vegetation and yield parameters comparing to control. Yousif (2018) showed that the application of humic acid and seaweed extracts significantly improved foliage and harvest traits of garlic. Mikulewicz et al. (2019) revealed that the harvest quantitative and qualitative traits of garlic was positively influenced by two amino acid derived biostimulants. Amal et al. (2016) reported a significant increment in the vegetation, yield and mineral content of garlic owing to foliar spraying of moringa leaf extract and two amino acids. The aim of this study is to find out the impact of biofertilizer, planting density and plant growth activators on outgrowth and yield of local garlic to ameliorate garlic productivity and to produce safe food (organic product) with low costs without contaminating the environment.

2. MATERIALS AND METHODS

2.1 Materials

The experiment was implemented in the vegetable research field at the protected cultivation department, Zakho technical institute, Dohuk polytechnic University, during winter season of 2022. The cloves of local garlic were used as plant material.

The land of the open field was plowed then softened. After that, the animal manure was added and incorporated into the soil with a second plow for improving soil fertility then the land was amended. The field land was divided into ten lines based on the distribution system of the drip irrigation.

The cloves of local garlic (Allium Sativum L..) were provided with the biofertilizer and planted in the field on (September 15th 2021) at a distance of 30 cm between line to line and (5,10,15) cm from plant to plant along the perforated hose line of drip irrigation system. The field was drip irrigated prior to planting. The cultural practices were done in each experimental unit such as weed cultivation and softening of soil around plants plus continuous monitoring of diseases and pests. In the study, biofertilizer (Biofertin), three plant spacing (5,10,15) cm and Growth activator were provided to the local garlic. The plants were harvested on March 15th.

2.2 Methods

The experiment comprised of clove treatment with the biofertilizer, foliar spraying of the growth activator at three concentrations (0 ml. L^{-1}, 1 ml. $L^{-1}$and 2 ml. L^{-1}) and three plant spacing (5,10,15) cm and their interactions. The growth activator was provided a month post planting at three times. The first spray was carried out on October 15th 2021 with ten days' interval between each spray. The second spray was done on October 25th and the last one on November 5th.

2.3 Vegetative growth parameters

Five plants from internal lines were determined for measuring vegetation parameters. The stature (height) of plant (cm) was determined with measuring bar commencing from the contact point between the stem and the soil surface till the upper growing point of plant stem. The chlorophyll content (SPAD) was estimated by the Chlorophyll Meter (SPAD-502, Konica Minolta). The leaf length (cm) and leaf width were measured employing the measuring bar. The leaf number was enumerated at the season termination. The neck diameter (cm) was evaluated with Vernier equipment. The dry matter percentage of vegetation was calculated after weighing the fresh weigh of vegetation and putting the plants in electric oven at (70) C0 for (48) hours to list down the dry weigh then the percentage was recorded based on the beneath equation:

$$\textbf{Dry matter}\ (\%) = \frac{\text{dry weight of vegetative growth}}{\text{fresh weigh of vegetative growth}} \times 100$$

2.4 Mineral contents in leaves

The leaf content of nitrogen was estimated following the modified method of Kjeldahl and the analysis was attained by using the Microkjeldahl equipment (A.O.A.C., 1980). The phosphorus content was estimated with colorimetric method by using spectrophotometer equipment (Matt, 1970) and the leaf potassium content was determined flame method using Flame photometer instrument (A.O.A.C., 1970 and Al -Sahaf, 1989).

2.5 Statistical Analysis

The treatments were organized following split-split randomized complete block design (split- split RCBD). The study encompassed (18) treatments (2 × 3 × 3 = 18) each treatment was replicated three times and 10 plant were representatives for each treatment. The data were analyzed depending upon (SAS, 2010) program.

3. RESULTS AND DISCUSSION

3.1 Plant Height (cm)

Data list down in the table (1) displays the impact of azotobacter, plant spacing, growth activator and their interactions on plant stature (height) of garlic. In term of single doses, only the azotobacter significantly improved plant height resulting in a height value of 93.49 cm relative to control dose (without biofertilizer) while the odd doses of the other two factors did not importantly enhance such parameter. The dual interference between azotobacter and plant spacing significantly affected plant

stature with the highest average (94.23 cm) being measured for plants treated with azotobacter and

planting at a distance of 10 cm over other treatments. In contrast, the (Activator * distance) did not markedly influence plant height relative to control whereas the same was not true for (azotobacter * activator) interaction, which prominently elevated plant height with the maximal average (94.90 cm) determined for plants received azotobacter and growth activator at level of (0 ml. L^{-1}) in comparison with control. In case of triple interaction, plants treated with azotobacter, give no activator, and planted at a distance of 5 cm were the tallest one (95.67 cm) exceeding other treatments.

Table 1. Effect of biofertilizer, plant growth activator, and plant spacing on plant height (cm) of garlic

Azotobacter	Activator	Distances cm			Azotobacter *Activator	Azotobacter
		5	10	15		
With	0	95.67 a	95.03 a	94.00 a	94.90 a	93.49 a
	1	95.00 a	92.00 a	90.78 a	92.59 a	
	2	91.23 a	95.65 a	92.00 a	92.96 a	
Without	0	76.30 c	80.40 bc	80.00 bc	78.90 b	79.98 b
	1	81.17 bc	83.60 b	82.00 bc	82.26 b	
	2	80.00 bc	78.28 bc	78.03 bc	78.77 b	
Distances		86.56 a	87.49 a	86.14 a	Activator	
Azotobacter * Distance	With	93.97 a	94.23 a	92.26 a		
	without	79.16 b	80.76 b	80.01 b		
Distance * Activator	0	85.98 a	87.72 a	87.00 a	0	86.90 a
	1	88.08 a	87.80 a	86.39 a	1	87.43 a
	2	85.62 a	86.97 a	85.02 a	2	85.87 a

3.2 Chlorophyll Content (SPAD)

According to results illustrated in the table (2), the plant spacing exhibited no efficacy on the chlorophyll content of garlic but the azotobacter have had a variant impact on such parameter granting the highest average (66.89) over control. Similarly, 1 ml. L^{-1} of growth activator gave the excellent average value (63.14) in comparison with control (59.55). The double dose of azotobacter and distance was efficient on chlorophyll content when plants received biofertilizer and spaced at 5 and 10 cm producing averages of 66.76 and 66.79, respectively. Relevance to azotobacter * activator interaction, the biggest average of chlorophyll content (63.88) was recorded in leaves of plants treated with azotobacter and delivered growth activator at 1 ml. L^{-1}. The interference between azotobacter and activators also created a premium average (70.19) when garlic plants provided with azotobater and sprayed with 1 ml. L^{-1} of growth activator. triple interaction between the three factors also significantly improved chlorophyll content and the highest average (70.47) was recorded in leaves of plants treated with azotobacter and foliar applied with activator at 1 ml. L^{-1} and planted as density of 15 cm against control and other treatments.

Table 2. Effect of biofertilizer, plant growth activator, and plant spacing on chlorophyll content (SPAD) of garlic

Azotobacter	Activator	Distances cm			Azotobacter *Activator	Azotobacter
		5	10	15		
With	0	63.63 abc	63.83 abc	64.73 ab	64.07 b	66.89 a
	1	69.93 a	70.17 a	70.47 a	70.19 a	
	2	66.70 a	66.37 a	66.17 a	66.41 b	
Without	0	58.30 bcd	53.37 d	53.43 d	55.03 c	55.42 b
	1	57.83 cd	53.60 d	56.87 cd	56.10 c	

	2	55.93 d	57.73 cd	51.70 d	55.12 c	
Distances		62.06 a	60.84 a	60.56 a	Activator	
Azotobacter * Distance	With	66.76 a	66.79 a	67.12 a		
	without	57.36 b	54.90 b	54.00 b		
Azotobacter * Activator	0	60.97 ab	58.60 b	59.08 ab	0	59.55 b
	1	63.88 a	61.88 ab	63.67 a	1	63.14 a
	2	61.32 ab	62.05 ab	58.93 ab	2	60.77 ab

3.3 Leaf Length (cm)

Results in the table (3) shows that the leaf length of garlic was ranged from (52.28 cm) to (63.42 cm). The azotobacter alone created a significant amelioration in the leaf length recording an average of (61.56 cm) as compared to control. The same notable impact of plant spacing was confirmed with the peak average (59.49 cm) being estimated for garlic plants spaced at 10 cm. No evident increment in leaf length has been measured due to the foliar spraying with growth activator. Concerning binary interactions, the greatest average of leaf length (62.49 cm) was owned by plants treated with azotobacter and planted at (10 cm) spacing relative to plants received no azotobacter and planted at (15 cm) spacing which possessed the least average value (53.39 cm). The binary interaction between activator and distance was effective at distance of 10 cm plus 1, and 2 ml/L of activator giving averages leaf length of (60.35 cm and 60.29 cm), respectively. The combination between azotobacter and activator also importantly increased leaf length (62.56 cm) over control (53.01 cm). Regarding the complex interference, the highest average leaf length (63.42 cm) was belonged to plants treated with biofertilizer and spaced at (15 cm) without foliar sprays of growth activator whereas the minimum average (51.28 cm) was enumerated for leaves of plants have had no azotobacter and activator (see: table 3).

Table 3. Effect of biofertilizer, plant growth activator, and plant spacing on leaf length (cm) of garlic

Azotobacter	Activator	Distances cm			Azotobacter *Activator	Azotobacter
		5	10	15		
With	0	62.65 abc	61.62 a-d	63.42 a	62.56a	61.56 a
	1	62.07 abc	63.17 ab	59.90 a-e	61.71 a	
	2	56.65 d-h	62.70 abc	61.83 a-d	60.39 a	
Without	0	51.28 h	54.07 fgh	53.68 fgh	53.01 b	54.65 b
	1	55.75 e-h	57.53 c-g	54.17 fgh	55.82 b	
	2	55.20 e-h	57.88 b-f	52.32 gh	55.13 b	
Distances		57.27 b	59.49 a	57.55 b	Activator	
Azotobacter * Distance	With	60.46 a	62.49 a	61.72 a		
	without	54.08 bc	56.49 b	53.39 c		
Distance * Activator	0	56.97 ab	57.84 ab	58.55 ab	0	57.79 a
	1	58.91 ab	60.35 a	57.03 ab	1	58.76 a
	2	55.93 b	60.29 a	57.08 ab	2	57.76 a

3.4 Leaf Width (cm)

Data of table (4) indicated that the single treatment of growth activator and plant spacing made no observable improvement in the leaf width but the azotbacter alone significantly augmented this parameter producing an average value (4.00 cm) as compared to control (3.19 cm). In differentiate, the interference between azotobacter and density caused a statistical amelioration in leaf width (4.08 cm, and 4.06 cm) at azotobacter provision plus distances of (10 and 5) cm, respectively. In case of distance * activator interaction, there had been no marked differences between treatments in respective to leaf width. For azotobacter * activator effect, the maximum average value (4.10 cm) were measured for plants given azotobacter and sprayed with 2 ml. L^{-1} of activator. Furthermore, the complicated effect of all factors significantly influenced leaf width and the best average (4.35 cm) was recorded for garlic plants treated with azotobacter, sprayed with activator at level of 2 ml/L and planted at distance of 10 cm when compared to plants without azotobacter plus 1 ml. L^{-1} of activator and planted at distance of 5 cm which owned the lowest average (2.85 cm) as shown in the table (4).

Table 4. Effect of biofertilizer, plant growth activator, and plant spacing on leaf width (cm) of garlic

Azotobacter	Activator	Distances cm			Azotobacter *Activator	Azotobacter
		5	10	15		
With	0	3.78 bc	3.88 abc	3.85 bc	3.84 a	4.00 a
	1	4.25 ab	4.02 ab	3.92 abc	4.06 a	
	2	4.15 ab	4.35 a	3.80 bc	4.10 a	
Without	0	3.15 de	3.13 de	3.27 de	3.18 b	3.19 b
	1	2.85 e	3.23 de	3.52 cd	3.20 b	
	2	3.15 de	3.22 de	3.23 de	3.20 b	
Distances		3.56 a	3.64 a	3.60 a	Activator	
Azotobacter * Distance	With	4.06 a	4.08 a	3.86 a		
	without	3.05 c	3.19 bc	3.34 b		
Distance * Activator	0	3.47 a	3.51 a	3.56 a	0	3.51 a
	1	3.55 a	3.63 a	3.72 a	1	3.63 a
	2	3.65 a	3.78 a	3.52 a	2	3.65 a

3.5 Number of leaves. plant-1

The table (5) demonstrates the various variables of leaf number of garlic in response to plant spacing and application of biofertilizer and growth activator. The individual impact of plant spacing and growth activator did not significantly increase number of leaves. plant-1 whereas the azotobacter led to production of the superior average (17.82) over control (15.44). In term of double interferences, a profound amelioration was proved with zotobacter and distance combination and the maximum average (18.33) was enumerated for plants given biofertilizer and plants at spacing of 10 cm against the least average (14.83) that calculated for plants received no azotobacter and planted at 10 cm. relating the dual interaction between activator and distance, plants did not have activator and planted at spacing of 10 cm possessed the largest average number of leaves (17.58). The same importantly positive impact of azotobacter interaction with activator was confirmed with the highest average leaf number (18.28) being enumerated for plants delivered no activator and treated with azotobacter. The combined impact of all factors was statistically significant on leaf number when plants given azotobacter without activator and planted at density of 15 cm leading to the determination of the peak average (18.83) as compared to the least average (13.67) calculated for plants applied with no biofertilizer and growth activator and planted at 5 cm as obvious in the table (5).

Table 5. Effect of biofertilizer, plant growth activator, and plant spacing on number of leaves. plant-1 of garlic

Azotobacter	Activator	Distances cm			Azotobacter *Activator	Azotobacter
		5	10	15		
With	0	17.33 ab	18.67 ab	18.83 a	18.28 a	17.82 a
	1	17.00 ab	18.67 ab	17.83 ab	17.83 a	
	2	17.70 ab	17.67 ab	16.67 abc	17.34 a	
Without	0	13.67 c	16.50 a-d	16.17 a-d	15.44 b	15.44 b
	1	16.83 abc	14.00 bc	16.17 a-d	15.67 b	
	2	16.00 a-d	14.00 bc	15.67 bcd	15.22 b	
Distances		16.42 a	16.58 a	16.89 a	Activator	
Azotobacter * Distance	With	17.34 ab	18.33 a	17.78 a		
	without	15.50 c	14.83 c	16.00 bc		
Distance * Activator	0	15.50 b	17.58 a	17.50 ab	0	16.86 a
	1	16.92 ab	16.33 ab	17.00 ab	1	16.75 a
	2	16.85 ab	15.83 ab	16.17 ab	2	16.28 a

3.6 Neck Diameter (cm)

The neck diameter of garlic displayed a positive response to the independent application of azotobacter and growth activator but did not significantly influence by plant density. The biggest average neck diameter (2.81 cm) was determined for plants applied with azotobacter pared with the second significant value (2.64 cm) that was measured for plants foliar fed with 1 ml. L^{-1} of activator when compared to control. The combined effect of factors markedly induced neck diameter. The maximal average neck diameter (2.83 cm) was estimated for plants cultivated at spacing of 5 cm and gave azotobacter as compared with those received no azotobacter and planted at increased spacing 15 cm which owned the least average (2.12 cm). The interaction between distance at 5 cm and activator at 1 ml. L^{-1} resulted in the best average neck diameter (2.73 cm) whereas in status of azotobacter * activator interference, the thickest neck (2.94 cm) was measured due to the application of azotobacter and activator al level of 1 ml. L^{-1}. the combination of all factors significantly improved neck diameter and the maximum average (3.23 cm) was recorded with the complex dose: distance of 5 cm + azotobacter + activator at 1 ml. L^{-1} relative to the least value (1.90 cm) generated for no azotobacter and activator at level of 2 ml. L^{-1} plus increased distance (15 cm) as revealed in the table (6).

Table 6. Effect of biofertilizer, plant growth activator, and plant spacing on neck diameter (cm) of garlic

Azotobacter	Activator	Distances cm			Azotobacter *Activator	Azotobacter
		5	10	15		
With	0	2.48 b-f	2.55 b-e	2.68 a-d	2.57 b	2.81 a
	1	3.23 a	2.92 ab	2.68 a-d	2.94 a	
	2	2.78 abc	2.93 ab	2.98 ab	2.90 a	
Without	0	1.95 fg	2.17 d-g	2.13 d-g	2.08 c	2.15 b

	1	2.22d-g	2.43 b-g	2.33 c-g	2.33 bc	
	2	2.25 c-g	2.00 efg	1.90 f	2.05 c	
Distances		2.49 a	2.50 a	2.45 a	Activator	
Azotobacter * Distance	With	2.83 a	2.80 a	2.78 a		
	without	2.14 b	2.20 b	2.12 b		
Distance * Activator	0	2.22 b	2.36 ab	2.41 ab	0	2.33 b
	1	2.73 a	2.68 ab	2.51 ab	1	2.64 a
	2	2.52 ab	2.47 ab	2.44 ab	2	2.48 ab

3.7 Dry Matter of Vegetative Growth (%)

The data shown in the table (7) represents the dry matter of vegetative growth of garlic in response to the treatment of azotobacter and activator along with plant spacing. The individual dose of azotobacter made the statistically mean variant percentage of dry matter (14.90 %) surpassing control (13.18 %). Increasing the plant spacing to 15 cm created the highest mean value (14.29 %) in comparison with the minimal spacing (5 cm). Mean the foliar feeding of activator significantly ameliorated dry matter of vegetative growth and plants sprayed with activator at 1 ml. L^{-1} have had the premium mean value (14.37 %) relative to control (13.64 %). Significant differences were observed in dry matter percentage of vegetative growth respective to the dual action of factors. Planting garlic at spacing of 15 cm and giving them azotobacter produced the peak dry matter percentage (15.42 %) over other treatments. In state of interaction of spacing with activator, the excellent average value (14.90 %) was belonged to plants delivered activator at dose of 1 ml. L^{-1} and planted at spacing of 10 ml. L^{-1}. Similar significant average percentage (15.26 %) was obtained from azotobacter in combination with 1 ml. L^{-1} of growth activator that exceeded control (12.90 %). On the other hand, the most prominent average percentage of dry matter (16.23 %) was measured for foliage of plants applied with azotobacetr and foliar sprayed with activator at 1 ml. L^{-1} plus planting at space of 10 cm as seen in the table (7).

Table 7. Effect of biofertilizer, plant growth activator, and plant spacing on dry matter of vegetative growth (%) of garlic

Azotobacter	Activator	Distances cm			Azotobacter *Activator	Azotobacter
		5	10	15		
With	0	12.33 e	14.73 abc	16.10 a	14.39 b	14.90 a
	1	15.20 ab	16.23 a	14.33 bcd	15.26 a	
	2	14.47 bcd	15.30 ab	15.43 ab	15.07 ab	
Without	0	12.97 de	12.63 e	13.10 de	12.90 c	13.18 b
	1	13.33 cde	13.57 cde	13.57 cde	13.49 c	
	2	13.03 de	13.23 cde	13.20 cde	13.16 c	
Distances		13.56 b	14.28 a	14.29 a	Activator	
Azotobacter * Distance	With	14.00 b	15.42 a	15.29 a		
	without	13.11 c	13.14 c	13.29 bc		
Distance * Activator	0	12.65 c	13.68 b	14.60 ab	0	13.64 b
	1	14.27 ab	14.90 a	13.95 ab	1	14.37 a
	2	13.75 b	14.27 ab	14.32 ab	2	14.11 ab

3.8 Mineral Content in Leaves

Nitrogen (N) content (%)

The data analysis of nitrogen content in leaves of garlic revealed that the parameter was importantly improved with sole treatment of azotobacter but did not significantly influence with individually foliar spraying of activator and plant spacing. Plants treated with the biofertilizer contained the highest nitrogen (3.19 %) exceeding control (2.86 %). Concerning the binary doses, the azotobacter in interference with spacing of 15 cm making the maximum mean average (3.25 %) which pared that obtained from treating plants with azotobacter without activator (0 ml. L^{-1}). No significant variations were earned from distance * activator interaction in term of nitrogen content of leaf. Relating the triple interaction of the three factors, the biggest average nitrogen content (3.48 %) was measured in leaves of plants planted at a distance of 5 cm and given azotobacter without any dose of activator against the lowest average (2.48 %) that estimated for plants have had activator at 1 ml. L^{-1} and planted at 10 cm with no azotobacter as demonstrated in the table (8).

Table 8. Effect of biofertilizer, plant growth activator, and plant spacing on nitrogen (N) content (%) in leaves of garlic

Azotobacter	Activator	Distances cm			Azotobacter *Activator	Azotobacter
		5	10	15		
With	0	3.48 a	3.15 abc	3.12 abc	3.25 a	3.19 a
	1	3.04 abc	3.17 abc	3.29 abc	3.17 a	
	2	3.01 a-d	3.11 abc	3.34 ab	3.15 a	
Without	0	2.83 bcd	2.95 a-d	2.78 cd	2.85 b	2.86 b
	1	2.86 bcd	2.48 d	2.87 bcd	2.74 b	
	2	2.77 cd	3.22 abc	2.94 a-d	2.98 ab	
Distances		3.00 a	3.01 a	3.06 a	Activator	
Azotobacter * Distance	With	3.17 a	3.15 ab	3.25 a		
	without	2.82 c	2.88 bc	2.86 bc		
Distance * Activator	0	3.15 a	3.05 a	2.95 a	0	3.05 a
	1	2.95 a	2.83 a	3.08 a	1	2.95 a
	2	2.89 a	3.17 a	3.14 a	2	3.07 a

Phosphorus (P) content (%)

The results of the table (9) depict the impact of azotobacter, activator and plant spacing on leaf content of phosphorus element. The odd dose of azotobacter caused a significant increment in the leaf content of P (0.53 %) comparing to control (0.41 %). The sole dose of activator at (2 ml. L^{-1}) gave the most prominent average of P (0.53 %) over control (0.41 %). In contrast, the plant density did not remarkably increase the leaf content of P. the same was not observed from double doses, plants that received azotobacter and planted at space of 5 cm contained the largest average percentage (0.56 %). Relevance to Distance * Activator effect, it was significant on P content when plants spaced at 5, and 10 cm and sprayed with 2 ml. L^{-1} of activator resulting in 0.56% and 0.55% of P, respectively. The efficacy of azotobacter with 2 ml. L^{-1} of activator brought about the peak average percentage of P (0.59 %) as compared to control (0.36 %). On the other side, the biggest ever percentage of P (0.62 %) was as a result of the complex dose: azotobacter + activator at 2 ml/L + plant spacing at 5 cm encountered with the lowest average (0.32 %) as a consequence of the complex dose: no azotobacter + 0 ml/L of activator + spacing of 15 cm (note: table 9).

Table 9. Effect of biofertilizer, plant growth activator, and plant spacing on phosphorus (P) content (%) in leaves of garlic

Azotobacter	Activator	Distances cm			Azotobacter *Activator	Azotobacter
		5	10	15		
With	0	0.52 bcd	0.55 abc	0.45 de	0.50 b	0.53 a
	1	0.54 abc	0.44 de	0.48 cd	0.49 b	
	2	0.62 a	0.59 ab	0.55 abc	0.59 a	
Without	0	0.37 ef	0.37 ef	0.32 f	0.36 c	0.41 b
	1	0.34 f	0.37 ef	0.47 cd	0.39 c	
	2	0.49 cd	0.51 bcd	0.43 de	0.47 b	
Distances		0.48 a	0.47 a	0.45 a	Activator	
Azotobacter * Distance	With	0.56 a	0.53 ab	0.49 b		
	without	0.40 c	0.42 c	0.41 c		
Distance * Activator	0	0.44 bcd	0.46 bc	0.39 d	0	0.43 b
	1	0.44 bcd	0.41 cd	0.48 b	1	0.44 b
	2	0.56 a	0.55 a	0.49 b	2	0.53 a

Potassium (K) Content (%)

The data given in the table (10) unveiled that the odd impact of azotobacter and plant spacing was statistically significant on leaf content of potassium while sole doses of activator did not exhibit effectiveness on leaf content of K. The azotobacter treatment was superior to control and produced the premium average percentage of K (3.30 %) pared with a closer profound average value (3.11 %) created from planting garlic at a distance of 15 cm. The intellectuality between factors made significant variations in leaf content of K. A high K content (3.42 %) was obtainable from treating plants with azotobacter and spacing them at 15 cm which dominated other treatments. The spacing of 15 cm in combination with activator at level of 2 ml. L^{-1} showed supremacy and led to production of the greatest average percentage (3.20 %) of K in leaves of garlic. Furthermore, the azotobacter * activator interaction has made the best average percentage of K (3.32 %) when plants treated with azotobacter and dosed with 1 ml. L^{-1} of growth activator in comparison with control. In status of triple impact, the peak average percentage (3.63 %) of K was measured in leaves of plants given azotobacter and activator at dose of 2 ml. L^{-1} along with planting at a spacing of 15 cm relative to the fewest average percentage (2.56 %) that was contained in leaves of plants treated with no azotobacter and/or activator and planted at a spacing of 10 cm as clarified in the table (10).

Table 10. Effect of biofertilizer, plant growth activator, and plant spacing on potassium (K) content (%) in leaves of garlic

Azotobacter	Activator	Distances cm			Azotobacter *Activator	Azotobacter
		5	10	15		
With	0	3.36 bc	3.28 bcd	3.23 bcd	3.29 a	3.30 a

	1	3.32 bcd	3.23 bcd	3.40 b	3.32 a	
	2	3.09 def	3.11 cde	3.63 a	3.28 a	
Without	0	2.88 efg	2.56 hi	2.88 efg	2.77 b	2.76 b
	1	2.86 fgh	2.69 ghi	2.78 ghi	2.78 b	
	2	2.61 hi	2.85 fgh	2.77 ghi	2.74 b	
Distances		3.02 b	2.96 b	3.11 a	Activator	
Azotobacter * Distance	With	3.26 b	3.21 b	3.42 a		
	without	2.78 c	2.70 c	2.81 c		
Distance * Activator	0	3.12 ab	2.92 bcd	3.05 abc	0	3.03 a
	1	3.09 abc	2.96 bcd	3.09 abc	1	3.05 a
	2	2.85 d	2.98 bcd	3.20 a	2	3.01 a

According to the findings of the field experiment, the application of azotobacter alone significantly ameliorated all foliage parameters and leaf mineral content (N, P, and K) whereas the individual effect of plant spacing was efficient on leaf length, dry matter percentage of vegetative growth and the leaf potassium (K) content. The individual dose (1 ml. L^{-1}) of growth activator significantly increased chlorophyll content, neck diameter, dry matter percentage of vegetative growth and leaf content of phosphorus (P). In contrast, dual and triple interactions of the three factors significantly improved vegetation parameters of garlic. The beneficial impact of biofertilizer, solely or interacted with other two factors, on vegetative components and leaf mineral content of garlic could be due to the fact that biofertilizer is enriched with microbial agents such as azotobacter that are very urgent for soil fertility and when added, they decompose organic material into organic matter, hence acting like a nutrient pool in the soil making nourishing elements to be obtainable for plant roots as well as fixing nitrogen and functioning as regulators of the proper advance of plant outgrowth and productivity (Bhat et al., 2015). The same results were confirmed by Rady et al. (2018) and Bhushan et al. (2020) on garlic who illustrated that the best foliage leaf mineral composition of garlic was obtained from treating plants with Azotobactor as compared to control.

The improvement in garlic vegetation and leaf mineral content ascribed to dual and triple doses may favor to the activator and its well content of amino acids, seaweed extracts and other growth promoting substances. Amino acids can have a great impact on plant physiological processes. They are able to optimize the nutrient absorption, transportation and metabolism and contribute to biosynthesis of vitamins as well as they can bio-stimulate outgrowth, induce stress endurance and participates in the production of chelate fertilizers (Sharma and Dietz 2006; Souri and Hatamian 2019). Amal et al. (2016) confirmed significant amelioration in the vegetation and mineral content of garlic with foliar spraying of moringa leaf extract and two amino acids. Seaweed extracts, on the other side, are natural substances containing numerous effective molecules like complex polysaccharides, plant outgrowth organizers, betaine-like compounds, different vitamins and numerous macro and micronutrients that help the plant to grow optimally with better qualitative yield (Battacharyya et al., 2015). Yousif (2018) revealed that the addition of humic acid and seaweed extracts significantly enhanced foliage traits of garlic. The combined effect of factors on garlic foliage and leaf mineral nutrients may also refer to the plant spacing. Increasing plants spacing positively affects plant outgrowth and progression. The proper utilization of solar energy and better nutrient uptake and obtainability is directly correlated with plant spacing which in turn affect canopy and root structure (Hammer et al., 2009; Du et al., 2021). Whenever the spacing is enhanced, the intercepted photosynthetically active radiation is improved leading to induction of leaf area index and ultimately raises up plant biomass (Hernández et al., 2020). Kumar et al. (2018) displayed that the best vegetation and yield components were obtained from planting at space of 15 cm × 10 cm.

4. CONCLUSION

In recent years, the rapid increment in the human population along with the continuous deterioration in the soil architecture and environmental health attributed to the irrational application of hazardous chemical fertilizers and pesticides have brought about serious threats for food security all over the world. For such reason, intensive efforts have been done to find out natural safe inputs pared with proper cultural practices for optimum crop outgrowth and productivity. Our results displayed that the azotobacter as biofertilizer possessed a significant positive impact on foliage and leaf mineral content of garlic either individually or in combination with growth activator and plant spacing. The influence of odd doses of growth activator significantly improved some vegetative growth parameters while exhibited more efficacy in dual and triple interactions. Meanwhile, the plant spacing solely also importantly affected some vegetation parameters but shower effectiveness and variance in binary and triple interferences. Therefore, it is recommended that the azotobacter along with growth activator and increased planting spacing (15 cm) should be utilized for maximum outgrowth and harvest of local garlic and more field studies must be implemented on other allium crops to examine the effect of these organic inputs added with other organic fertilizers to achieve more sustained organic crop production.

REFERENCES

A. O. A. C., 1970. Official Method of Analysis 11th edition Washington D.C. Association of official analysis chemist. P. 105.

A. O. A. C., 1980. Official Method of Analysis 11th edition Washington D.C. Association of official analysis chemist. P. 1015.

1. Alori ET, Glick BR, Babalola OO 2017. Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Frontiers in Microbiology; 8: 971.
2. AL-Sahaf, F. H., 1989. Practical Plant Nutrition. Ministry of Higher Education and Scientific Research. Bagdad univ. Iraq. (In arabic).
3. Amal, Z. Hegazi; Shimaa Kh.H. Hasan and Nadia A.M. El-Said 2016. Response of Garlic Plants to Foliar Application of Moringa Leaves Extract, Glutamine and Cysteine. J. Plant Production, Mansoura Univ., Vol. 7(1): 1 – 6.
4. Battacharyya, D., M. Z. Babgohari, P. Rathor and B. Prithiviraj 2015. Seaweed extracts as biostimulants in hortic. Sci. Hortic., http://dx. doi.org /10.1016/j.scienta.2015.09.012.
5. Bhat, T.A., L. Ahmad, M.A. Ganai, S. Ul-Haq and O.A. Khan (2015). Nitrogen fixing biofertilizers ; mechanism and growth promotion: A Review. Journal of Pure and Applied Microbiology, 9(2), 1675-1690.
6. Bhushan, C., Anil K. Y., Hemant K. Gangwar, B. K., Sunil K. K. and Nitin V. (2020). Effect of Bio-fertilizers on Growth and Yield of Garlic (Allium sativum Linn.). Int.J.Curr. Microbiol. App. Sci (2020) 9(11): 739-743.
7. Choudhary, M. K., Kavita, A. H., and Singh, B. 2015. Effect of Biofertilizers and Micronutrients on Quality Attributes of Garlic. Annals of Biology, 31(2), 275-278.
8. Craigie JS 2011) Seaweed extract stimuli in plant science and agriculture. J Appl Phycol 23:371–393.
9. Das, S., F. H. Rahman1, S. Mukherjee and K. Nag 2020. Response of Biofertilizers and Primary Nutrients on Growth and Yield of Garlic (Allium sativum L.) in New Alluvial Soil of West Bengal. CJAST, 39(10): 1-7.
10. Du, X., Wang, Z., Lei, W., and Kong, L. (2021). Increased planting density combined with reduced nitrogen rate to achieve high yield in maize. Sci. Rep. 11:358.
11. halel, A.M.S., Sultan, F.I. 2019. Influence of foliar spray and soil application of three aminoacids compounds in growth and yield of onion (Allium cepa L.). Plant Arch., 19(1), 531–534.
12. Hammer, G. L., Dong, Z., McLean, G., Doherty, A., Messina, C., Schussler, J., et al. (2009). Can changes in canopy and or root system architecture explain historical maize yield trends in the U.S. corn belt. Crop Sci. Soc. Am. 49, 299–312. doi: 10.2135/cropsci2008.03.0152.
13. Hernández, M. D., Alfonso, C., Cerrudo, A., Cambareri, M., Della Maggiora, A., Barbieri, P., et al. (2020). Eco-physiological processes underlying maize water use efficiency response to plant density under contrasting water regimes. Field Crops Res. 2020:254.
14. Ibrahim, M. E., M. A. Bekheta, A. El-Moursi and N.A. Gafar (2010). Improvement of growth and seed yield quality of Vicia fab a L. plants as affected by application of some bioregulators. Aust. J. Basic and Appl. Sci., 1(4): 657-666.
15. Kalayu G (2019). Phosphate solubilizing microorganisms: Promising approach as biofertilizers. International Journal of Agronomy ;1-7.
16. Khalel, A.M.S., Sultan, F.I. (2019). Influence of foliar spray and soil application of three aminoacids compounds in growth and yield of onion (Allium cepa L.). Plant Arch., 19(1), 531–534.
17. Kaur, Palampreet, Sachin Kishor & Sandeep Kumar Diwakar 2021. Influence of Various Biofertilizers and Mulching on Growth, Yield and Yield Attributing Traits of Garlic (Allium Sativum L.) Cv. PG-18.
18. Khan W, Rayirath UP, Subramanian S, Jithesh MN, Rayorath P, Hodges DM, Critchley AT, Craigie JS, Norrie J, Prithiviraj B (2009) Seaweed extracts as biostimulants of plant growth and development. J Plant Growth Regul 28:386–399.
19. Kumar, P., Sushil K. and Sukhwinder S. A. (2018). Effect of spacing and nitrogenous fertilizer on growth and yield parameters of garlic (Allium sativum L.). International Journal of Chemical Studies; 6(4): 356-359.
20. Lima, M.F., Lopes, W.D., Negreiros, M.Z., Grangeiro, L., Sousa, H.C., & Silva, O.M. 2019. GARLIC QUALITY AS A FUNCTION OF SEED CLOVE HEALTH AND SIZE AND SPACING BETWEEN PLANTS. Revista Caatinga, 32, 966-975.
21. Majkowska-Gadomska, J., Mikulewicz, E., Jadwisieńczak, K., Francke, A., and Młyńska, K. 2019. The influence of amino acid biostimulators on the size and quality of garlic (Allium sativum L.). Acta Agroph., 26(4), pp.31-38. https://doi.org/10.31545/aagr/116644

22. Matłok N., Gorzelany J., Bilek M., Pieniążek R., Kuźniar P., Kaniuczak J., 2014. Estimation of the content of fructose, glucose and saccharose in selected onion cultivars cultivated at three breeding and seed-production farms (in Polish). Zesz. Probl. Post. Nauk Rol., 576, 79-87.
23. Matt, J. 1970. Calorimetric Determination of Phosphorus in Soil and Plant Material with Ascorbic Acid. Soil. Sci. 109: 219-220.
24. Matysiak M., Gaweł-Bęben K., Rybczyńska K., Gmiński J., Surma S., 2015. Comparison of selected biological properties of garlic (Allium sativum L.) cultivated in
25. Meena, F. R., Meena, M.L., Singh, R., Ram Vilas, Meena, M.L., Mauriya, S.K., & Kumar, L. 2019. Effect of Organic Manures and Biofertilizers on Growth, Yield and Quality of Garlic (Allium sativum L.), Ind. J. Pure App. Biosci. 7(5), 405-409. doi: http://dx.doi.org/10.18782/2320- 7051.7776.
26. Mikulewicz, E., Majkowska-Gadomska, J., Jadwisieńczak, K. K., and Francke, A. 2019. Effect of selected biostimulants on the yield and quality of the common onion (Allium cepa L.). Acta Agroph., 26(1), pp.57-65. https://doi.org/10.31545/aagr/109050
27. Nagina, M., H. Muhammad, J.A. Muhammad, K. Nadra, H. Nasir and H., Babar 2017. Effect of planting density on growth, yield and quality of Garlic at Rawalakot, Azad Kashmir. Inter. J. Agron. Agric. Res., 10 (1): 42-51.
28. Petrovic B., Kopta T. and Pokluda R. 2019. Effect of biofertilizers on yield and morphological parameters of onion cultivars. Folia Horticulturae, Vol.31 (Issue 1), pp. 51-59. https://doi.org/10.2478/fhort-2019-0002
29. Rady, H.M.; Dina, S. EL-Mesirry and Nashwa, I. Aboel –Fadel 2018. Quality Improvement and Seed Yield of Two Garlic Cultivars (Allium Sativam L.) By Seaweed Extract and Mycorrhizae. Alex. J. Agric. Sci. Vol. 63, No.1, pp. 41-51.
30. Randle, W. M (1997). Onion flavor chemistry and factors influencing flavor intensity. ACM Symposium Series, v. 660, n. 1, p. 41-42.
31. Sharma S.S., Dietz K.J., The significance of amino acids and amino acid-derived molecules in plant responses and adaptation o heavy metal stress. Journal of Experimental Botany, 2006. 57(4), 711-726.
32. Souri M.K., Hatamian M., Aminochelates in plant nutrition: a review. Journal of Plant Nutrition, 2019, 42(1), 67-78.
33. Teixeira, E. I., George, M., Herreman, T., Brown, H., Fletcher, A., Chakwizira, E., et al. (2014). The impact of water and nitrogen limitation on maize biomass and resource-use efficiencies for radiation, water and nitrogen. Field Crops Res. 168, 109–118. doi: 10.1016/j.fcr.2014.08.002.
34. Temperini, O.; Ciancolini, A.; Parrano, L.: Lacobacci, L. and Colla, G. 2010. Influence of planting density on productivity and marketable quality in garlic (Allium sativum L.). Halus Hortus, 17 (2): 56 – 58.
35. Thirumaran, G., Arumugam, M., Arumugam, R., Anantharaman, P (2009): Effect of seaweed liquid fertilizer on growth and pigment concentration of Abelmoschus esculentus (l) medikus. Am Eurasian J Agron 2:57–66.
36. Yousif, K. H. 2018. Effect of Humic Acid and Seaweed Extract on Growth, Yield and Nutrient Content of Garlic (Allium sativum L.). Journal of Universityof Duhok., Vol.21, No.1(Agri. and Vet. Sciences), Pp8-18.