Journal of Soil Sciences and Agricultural Engineering

Journal homepage & Available online at: www.jssae.journals.ekb.eg

Using of Biofertilizers and Some Micronutrients in Improving Onion Productivity under Siwa Oasis Conditions

Mona M. El-shazly[1]; Amal E. Ahmed[1]; Heba A. K. Ibrahim[1*] and Sally A. Ismail[2]

[1]Soil Fertility and Microbiology Department, Desert Research Center
[2]Dept. Soil and Water Sci., Fac. Environ. Agric. Sci., Arish Univ., Egypt

Cross Mark

ABSTRACT

A field experiment was conducted during the two growing seasons (2019-2020), At Agricultural Experimental Station of the Desert Research Center at Khamisa Village (29.21º N and 25.40º E), Siwa Oasis, Matrouh Governorate, Egypt, to study the effect of biofertilizers (*Azotobacter chroococcum*, *Bacillus megatherium* and *Pseudomans fluorescence)* and foliar applications with copper, manganese, and iron at two concentrations of 0.2 and 0.4% (Cu, Mn, and Fe). The best results recorded with *A. chroococcum* combined with 0.4%iron for most parameters. The outcomes were as follows: Both biofertilizer inoculation and micronutrients foliar application gave a synergistic effect and enhanced the uptake of minerals and their metabolism in plants. The application significantly impacted the development, yield, and microbial community in the rhizosphere of inoculated plants. Compared to the control, the interaction treatments between biofertilizer inoculation and micronutrient foliar application had the most positive effects on onion plant growth indices, yield, and microbial activity in the rhizosphere.*A.chroococcum* increased plant height, leaves number, plant fresh and dry weight specifically with 4.0% iron, bulb diameter with Mn and Fe, yield ,total microbial count with Fe at 2.0 and 4.0% compared with *B. megaterium*, and *Ps.fluorescence*. Comparing with *Ps.fluorescence* and *A.chroococcum*, *B.megaterium* recorded the highest, bulb weight, bulb diameter with copper (Cu), total soluble solids (TSS), total microbial count with Cu and Mn, *Bacillus* count and phosphatase activity.

Keywords: Biofertilizers, Micronutrients, Onion, Azotobacter, Siwa Oasis

INTRODUCTION

Onion (*Allium cepa* L.) is one of the most dominant economically extended vegetable crops globally coming secondly after tomato (FAO, 2012), used all the year round, fresh and processed, it is sensitive to the length of photoperiod. The annual wide-ranging for onion production around 80 million tons, and the global exports about 7 million tons/year. According to FAO (2018), the substantial countries for onion production around the world, are China (stands first), India (second larger producer), USA, Turkey, Japan, Spain, Brazil, Poland and Egypt. Onion production depends on some factors like soil type and nutrient requirements (Dineshkumar *et al.*, 2020). Fertilization application rates are very important for onion production, since nitrogen or phosphorus over fertilization indirectly play a part in pest attack at field or in storing (Ware and McCollum, 1980), while lower fertilization lead to decreasing in the yield and quality.

Onion contains plenty amounts from sulfur compounds and allicins, which are responsible for the smell of onion. It is known to boost cardiovascular health, reduce high blood pressure and hold back cancer. Edible bulb rich in minerals (50%) and carbohydrates (11%), beside protein (1.2%), fiber (0.6%), nicotinic and riboflavin (Brinjh *et al.*, 2014).

Random use of chemical fertilizers like N, P and K that play a crucial role in crops growth, yield and quality can cause an opposite effects as reducing crops productivity and quality. Excess using of chemical fertilizers lead to decelerate soil fertility, soil biota activity and nutrients shortage (Singh *et al.*, 2017).

Onion has higher nutrient needs. Biofertilizers are low cost alternatives to chemical fertilizers, sustain soil fertility and productivity and enhance the content of minerals and total soluble solids of onion, as its chemical and physical properties (Gadelrabh and Elamin, 2013). Organic fertilizers enhance the N absorbance by a superficial fragile root system of onion, so more chlorophylls and carotenoids synthesized (Shedeed *et al.*, 2014).

Biofertilizers, or bioinoculants, are an alternative supplement to chemical fertilizers. They are applying as soil drench, root dip works by absorbing water and slowly releasing it to the plant root or as seed treatment. Using organic fertilizers lower the elevated costs of chemical fertilizers in agriculture. Bioinoculants increase speedily, establish a dense population in the rhizosphere, and facilitate nutrients supply (Wani *et al.*, 2013). Field application of plant growth promoting bacteria individually or in groups, increase the growth, yield of the cultivated crops and reduce chemical fertilizers (Adesemoye *et al.*, 2009).

Azotobacter plays an important role in soil fertility and plant nutrition, by producing plant growth promoting substances like auxins, cytokinins and gibberellins that regulate the promotion of cell division and elongation, stem elongation, root growth and seed germination (Wani *et al.*, 2013). That helps in crop production sustainability by maintaining soil productivity.

*** Corresponding author.**
E-mail address: hebaahmed286@hotmail.com
DOI: 10.21608/jssae.2023.223093.1169

Phosphate solubilizing microorganisms (PSMs) have the ability to dissolve insoluble inorganic and organic phosphorus compounds by producing organic acids such as citric and propionic acid that can release phosphate through ion exchange. Bacillus and Pseudomonas are most important useful bacteria in dissolving phosphate (Rawat *et al*., 2021; Armandeh *et al*., 2022).

Micronutrient required in minute quantities and involved in certain plant metabolic processes, playing a significant role in crops' growth and yield (Tohamy *et al*., 2009 and Alam *et al*., 2010). Micronutrient function as stimulus in most of plant metabolic activities, including macronutrients uptake and use, enzyme activities, cell division, respiration, nitrogen fixation as well as chlorophyll formation and photosynthesis and much more (Ballabh *et al*., 2013).

Using organic manures along with chemical fertilizers, These micronutrients, such as iron, copper, manganese, zinc, and boron, have an active role in the photosynthesis process and the synthesis of chlorophyll, so using them together with biofertilizers improved onion performance for growth and yield qualities as well as increasing chlorophyll content (K Pramanik and P Tripathy, 2017). While manganese application improved phosphorous and sulphur uptake, foliar application of zinc and boron improved nitrogen and potassium nutrient uptake, After planting, foliar spraying with trace element mixtures containing iron, zinc, and manganese had a favorable impact on growth and yield parameters(Biswas *et al*., 2020), Because zinc is involved in the stimulation of enzymes involved in glucose metabolism, the bulb yield in this study was higher. (Kaur *et al*., 2022). After planting, foliar spraying with trace element mixtures containing iron, zinc, and manganese had a favorable impact on growth and yield parameters(Biswas *et al*., 2020).

Gnanasundari *et al*., 2022 reported that, Microbial inoculants, also known as biofertilizers, would replace the extensive use of chemical fertilizers, improve farm productivity, activate the soil microbial community, and promote all aspects of plant growth. These microbial inoculants are a source of nutrients for plants that can be added to soil or added to seeds to help plants get the nutrients they need to grow.

This study was conducted to investigate the synergetic effect of biofertilizers with foliar application of some micronutrients on the onion crop productivity under Siwa Oasis , conditions.

MATERIALS AND METHODS

The field experiment was carried out during the two growing seasons (2019-2020), at Khamisa Village (29.21º N and 25.40º E), Siwa Oasis, Matrouh Governorate, Egypt, to study the effect of biofertilizers (*A.chroococcum*, *B.megatherium* and *Ps. fluorescence)* with foliar application of micronutrients copper, manganese, and iron (Cu, Mn, and Fe). Recommended Practice including rates of minerals and organic manure were adhered.

Soil analysis:

Physical and chemical analysis was conducted at Desert Research Center labs according to Page and Huyer., 1984 (Table, 1).

Table 1. Physico- chemical properties of the experimental soil.

Depth cm	pH (1:2.5)	E.C dS/m	OM	CaCO3	Sand	Silt	Clay	Texture	C.E.C C mol /kg
					%				
0-30	8.36	1.51	2.97	27.4	68.09	16.02	15.89	S.lL	12.13
30-60	8.44	1.65	2.15	30.6	60.48	21.16	18.36	S.LL	15.10
Soluble cations and anions in soil (me/L)									
Depth	Na^+		K^+	Ca^{++}	Mg^{++}	HCO_3^-1		Cl^-	SO_4^-
0-30	3.87		0.58	4.90	5.75	0.80		9.67	4.63
30-60	4.56		0.60	5.39	5.95	0.85		10.44	5.21
Available nutrients in soil (µg/g)									
Depth	N		P	K	Fe	Mn		Zn	Cu
0-30	43.4		10.4	81	4.47	3.03		0.89	0.37
30-60	41.1		8.81	87.5	5.54	3.47		1.05	0.41

Seeds

Seeds of onion (obtained from Agronomy Research Institute, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt)**.**

Bacteria isolation and purification

Soil samples collected from the rhizosphere of several growing plants in Siwa, and *Azotobacter chroocoocum* isolated Ashby's Nitrogen-free selective media, Modified Bunt and Rovira medium used for *Bacillus megatirum* isolation and King’s B for *Pseudomonas fluorescens*. The selective isolates purified and identified according to Krieg and Holt (Bergey's Manual, 1984). Moreover, used in the form of single and mixed inoculations at the rate of ~10^8 cfu/ml. لBiochemical activities was evaluated under *in vitro* conditions, through determination of their efficiency to solubilize phosphate (Nautiyal, 1999), produce of growth regulators (Rizzolo *et al*., 1993), carry out nitrogen fixation (Scholhorn & Burris, 1967), and produce antibiotics (Jarlier *et al*., 1996) .

Inoculum preparation

A liquid culture from each bacterial strain was diluted to 1% and applied for seed inoculation. Three replicates were used for each treatment in split plot design experiment.

Organic matter

Added as compost by rate (20 m^3/fed) as Recommended for sandy soil.

Micronutrients

Copper, manganese, and iron (Cu, Mn, and Fe) at two concentrations 0.2% and 0.4% were applied as foliar spray after 30, 45 and 60 days from transplanting (at rate 100L/fed.) Bacterial isolates (biofertilizers) were added to soil at 30, 45 and 60 days after transplanting

Field experiment

Onion seeds were planted in a nursery for 55 days. Then they were moved for planting, beside the irrigation line, which was 25 cm long and had a dripper in it. The experiment was laid out in a split plot design with four replications. The main plots were consisted of two concentration from each micronutrients Cu, Mn and Fe (0.2% and 0.4%). . The sub-plots included biofertilization treatments as without and with, respectively. Plots with the same size were created within the experimental area; each plot's area was ($2×3$ m^{2}). Minerals were added as follow: mono-calcium phosphate (15.5% P_2O_5) at rate 50 kg/fed., ammonium sulphate $(NH_4)_2SO_4$ (21.5% N) were applied at a rate of 60 kg N/fed., in two equal doses, the first 21 days after transplanting and the second 21 days following the first and potassium sulfate K_2So_4 (48%K_2O) as 100 Kg/fed.

Plant samples were taken 110 days after transplanting for estimating plant height, leaves No and both fresh and dry weight (g $plant^{-1}$). The economical yield expressed as bulbs weight (kg ha^{-1}) was measured at the harvest time. Morover, shoots, roots, bulbs were dried and ground to determine the macro, micronutrients and protein content according to the method described by Cottenie *et al.* (1982).

Chemical analysis of soil was carried out to determine total nitrogen according to Page and Huyer (1984), Nitrogen in the leaves was determined according to Bremner and Mulvaney, (1982). For yield characteristics: At harvesting (about 120 days from transplanting), total bulb yield was determined from each plot. Also, four plants from each plot were randomly taken to measure bulb diameter and bulb weight. Moreover, total soluble solids were measured by using Digital Refractometer. Protein content calculated by multiplying N% by 6.25. Seed oil content was determined according to A.O.A.C. (1980).

Microbial analysis

Nutrient agar was used for total microbial counts, modified Bunt and Rovira (1955) medium used for phosphate solubilizing bacteria (PSB) counts and Ashby's Nitrogen-free selective media for Azotobacter counts.

Enzymes activities

Dehydrogenase activity was measured according to (Casida *et al.*, 1964). nitrogenase activity was determined according to (Haahtela *et al.*, 1981) and for phosphatase activity, disodium phenyl phosphate served as enzyme substrate (Õhlinger, 1996).

Statistical analysis

Treatment means were compared using the new least significant difference (L.S.D.) test shown by Waller and Duncan, (1969), at the 5% level.

RESULTS AND DISCUSSION

Biochemical activities of selected isolates

Selected isolates, which showed the qualified activities from each bacterium, were tested for hormonal activities and antibiotic production beside total nitrogen and phosphate solubilization. All isolate showed phosphate solubilization activities, hormonal activities, antibiotic production activity, while only Azotobacter showed nitrogen activity by 143 ppm (Table 2). Bacillus gave the highest phosphate dissolving activity (2.8 cm clear zone) comparing with Pseudomonas and Azotobacter that was the lowest one (1.3 cm). Azotobacter gave the highest amount for Gibberellins and cytokinin by 2.3 and 23.4 µg/ml respectively, while Pseudomonas gave the highest Indole acetic acid (IAA) production by 2.44 µg/ml comparing with other tested isolates.

The highest antibiotic activity with all pathogenic tested isolates was with Pseudomonas, followed by Bacillus.

Table 2. Biochemical activities of selected isolates

Isolate	Total N (ppm)	Phosphate Solubilization Inhibition zone(cm)	Hormonal activity Quantitative (HPLC) / µg/ml			Antibiotic production Inhibition zone(cm)			
			IAA	GA_3	Cytokinin	*E. coli*	*Staph. typhimurium*	*F. oxysporum*	*R. solani*
Azotobacter	143	1.3	0.29	2.3	23.4	18	12	19	10
Bacillus	-	2.8	0.35	0.97	10.7	23	16	22	26
Pseudomonase	-	2.2	2.44	1.92	17.3	25	31	27	30

Effect of biofertilizers with foliar spray of micronutrients on the growth parameters of onion

Micronutrients can improve growth, quality, and yield properties in addition to the main sources of macronutrients. Additionally, they play a role in the absorption of important nutrients that support the activation of numerous plant metabolic processes. Applying fertilizers through soil or foliar spraying could make up for the lack of micronutrients. Iron, manganese, and copper are micronutrients required for plant growth and development. Zinc is essential for the synthesis of enzymes and for the activation of plant metabolism (Gnanasundari *et al*., 2022).

From Table (3), all treatments increased plant height, leaves number; plant fresh weight and plant dry weight comparing with control, But Azotobacter gave the highest record for all parameters comparing with Bacillus and Pseudomonas with all treatments and the highest numbers for all measured parameters came from the treatment of Azotobacter with 4.0% iron. It is clear that the combination of the micronutrients with all bacterial isolates is more effective than using the micronutrients alone.

According to the research (Attia *et al*., 2022) which claimed that using various biofertilizers increased indicators of growth in onions more than unruly plants, the promoting effect of biofertilizers on onions was consistent with this. Additionally, our results agreed with the work.

Table 3. Effect of biofertilizers with foliar spray of micronutrients on the growth parameters of onion

Micronutrients	Biofertilizers	Plant height (cm)	Leaves no.	Plant fresh wt. (gm)	Plant dry wt. (gm)
Cu 2.0%	Cont.	41.53	10.5	131	28.2
	Bacillus	46.31	11.3	139	36.7
	Azotobacter	47.52	11.6	142	38.1
	Pseudomonas	45.29	11.0	136	35.4
Cu 4.0%	Cont.	41.15	10.7	134	29.4
	Bacillus	47.10	10.8	142	36.9
	Azotobacter	48.81	11.9	145	39.4
	Pseudomonas	46.40	11.2	138	37.0
Mn 2.0%	Cont.	41.92	11.0	129	28.9
	Bacillus	44.93	11.0	135	37.2
	Azotobacter	46.28	11.3	139	39.3
	Pseudomonas	45.20	11.2	133	36.5
Mn 4.0%	Cont.	41.32	10.9	133	29.8
	Bacillus	46.20	11.4	139	38.5
	Azotobacter	47.93	11.5	144	40.2
	Pseudomonas	47.10	11.3	137	38.4
Fe 2.0%	Cont.	42.05	10.8	131	29.3
	Bacillus	48.50	11.6	141	37.8
	Azotobacter	49.25	12.0	145	40.2
	Pseudomonas	47.80	11.5	140	38.2
Fe 4.0%	Cont.	41.84	10.8	134	29.9
	Bacillus	48.90	12.2	143	38.6
	Azotobacter	50.51	12.5	148	41.5
	Pseudomonas	48.30	11.5	142	39.9
Significance	*	*	*	*	*
L.S.D. at 0.5%	Bio	0.0298	0.0907	0.0298	0.0697
	Micronutrient	0.0206	0.0139	0.0206	0.0834
	interaction	0.0411	0.02772	0.0411	0.1668

Effect of biofertilizers with foliar spray of micronutrients on the yield parameters in onion

From table (4), all treatments with all bacterial preparations increased the bulb weight, bulb diameter, yield and the total soluble solids (TSS),This is consistent with(Pramanik and Tripathy, 2017). Bacillus was the highest one increased the bulb weight with all micronutrients concentrations and the bulb diameter with copper (Cu) comparing with Azotobacter and Pseudomonas, while, Azotobacter with manganese (Mn) and iron (Fe) gave the highest bulb diameter comparing with Bacillus and pseudomonas, in addition of the highest yield for all treatments comparing with either Bacillus or Pseudomonas. Total soluble solids (TSS) gave the highest values with Bacillus and all micronutrients concentrations comparing with Azotobacter and Pseudomonas, that is agree with ,(Kaur *et al*., 2022).

Table 4. Effect of biofertilizers with foliar spray of micronutrients on the yield parameters of onion

Micronutrients	Biofertilizers	Bulb Wt Gm / bulb	Bulb diameter cm	yield T/ fed	TSS %
Cu 2.0%	Cont.	87.84	6.81	14.62	13.92
	Bacillus	93.72	7.05	15.28	14.71
	Azotobacter	92.40	7.10	15.47	14.44
	Pseudomonas	91.56	6.98	15.21	14.25
Cu 4.0%	Cont.	90.12	6.95	15.20	14.81
	Bacillus	95.52	7.18	15.56	16.49
	Azoto.	95.04	7.20	16.00	16.20
	Ps	93.60	7.03	15.50	15.84
Mn 2.0%	Cont.	89.76	6.93	14.95	14.25
	Bacillus	93.48	7.11	15.61	14.83
	Azotobacter	91.68	7.29	16.00	14.55
	Pseudomonas	91.32	7.24	15.40	14.62
Mu 4.0%	Cont.	90.00	7.02	15.20	14.96
	Bacillus	93.96	7.18	16.14	15.25
	Azotobacter	93.12	7.26	16.60	15.11
	Pseudomonas	93.48	7.15	16.30	15.21
Fe 2.0%	Cont.	90.72	7.05	16.00	14.56
	Bacillus	94.32	7.25	16.60	14.95
	Azotobacter	93.96	7.31	17.70	14.83
	Pseudomonas	93.84	7.16	16.75	14.70
Fe 4.0%	Cont.	91.32	7.14	16.20	15.06
	Bacillus	96.24	7.39	18.10	16.52
	Azotobacter	95.64	7.43	19.30	16.45
	Pseudomonas	94.20	7.33	16.66	16.13
Significance	*	*	*	*	*
L.S.D. at 0.5%	Bio	0.0578	0.0156	0.1001	0.0106
	Micronutrient	0.0483	0.0173	0.0797	0.0147
	interaction	0.1687	0.0346	0.1593	0.0294

Effect of biofertilizers with foliar spray of micronutrients on soil microbial count

From Table (5), all treatments increased the total microbial count, Azotobacter count, Bacillus count and Pseudomonas count in soil comparing with control, that is agree with (Gnanasundari *et al*., 2022). Bacillus treatments with Cu and Mn gave the highest total microbial count and the highest Bacillus count in soil with Cu, Mn and Fe comparing with Azotobacter and Pseudomonas. While Azotobacter gave the highest total microbial count with Fe at 2.0 and 4.0% and the highest Azotobacter count with all micronutrients comparing with Bacillus and Pseudomonas. Bacillus with all micronutrients gave the highest Bacillus count in soil comparing with Azotobacter and Pseudomonas. All micronutrients with Pseudomonas gave the highest Pseudomonas number in soil.

Table 5. Effect of biofertilizers with foliar spray of micronutrients on soil microbial count

Micronutrients	Biofertilizers	Total Count (10^5)	Azotobacter (10^4)	Bacillus (10^2)	Pseudomonas (10^2)
Cu 2.0%	Control	52	31	21	13
	Bacillus	67	36	34	21
	Azotobacter	64	44	29	18
	Pseudomonas	61	32	31	26
Cu 4.0%	Cont.	56	33	24	14
	Bacillus	72	39	39	25
	Azotobacter	70	51	32	22
	Pseudomonas	66	37	36	29
Mn 2.0%	Cont.	52	31	23	15
	Bacillus	65	38	37	29
	Azotobacter	62	4`9	32	22
	Pseudomonas	59	36	33	29
Mu 4.0%	Cont.	54	35	25	17
	Bacillus	69	42	41	30
	Azotobacter	68	53	35	24
	Pseudomonas	58	39	39	32
Fe 2.0%	Cont.	53	32	22	14
	Bacillus	68	37	38	28
	Azotobacter	71	46	35	25
	Pseudomonas	64	35	36	30
Fe 4.0%	Cont.	56	34	25	16
	Bacillus	73	45	45	32
	Azotobacter	79	55	38	29
	Pseudomonas	67	42	39	37
Significance	*	*	*	*	*
L.S.D. at 0.5%	Bio	0.2324	0.1521	0.2576	0.1119
	Micronutrient	0.3476	0.2739	0.3962	0.3329
	interaction	0.6952	0.5477	0.7923	0.6658

Total microbial counts (initial total microbial counts 51×10^5 cfu/g dry soil)
Azotobacter densities (initial Azotobacter density 29×10^4 cfu/g dry soil)
Total phosphate dissolving bacteria inoculum (initial PDB counts 18×10^2 cfu/g dry soil)
Total Pseudomonas density (initial Pseudomonas densities 12×10^2 cfu/g dry soil)

Effect of biofertilizers with foliar spray of micronutrients on the activity of enzymes.

From Table (6) all bacterial treatments with micronutrients increased the activity for nitrogenase, phosphatase and dehydrogenase enzymes comparing with control. Treatment with Azotobacter with all micronutrients increased the activities for nitrogenase and dehydrogenase enzymes comparing with Bacillus and Pseudomonas treatments. However, phosphatase activity increased the most with Bacillus and all tested micronutrients comparing with Azotobacter and Pseudomonas.

Effect of biofertilizers with foliar spray of micronutrients on the macronutrients and protein contents in plant

From Table (7), all bacterial treatments with all tested micronutrients increased the percentages of nitrogen, phosphorus, potassium and protein in onion plants comparing with control. Azotobacter was the bacteria that gave the highest percentages for all measured parameters in table (7) compared with Bacillus and Pseudomonas. Iron with Azotobacter was best micronutrient giving the highest values for all measured parameters comparing with Cu and Mn.

Bulbs with micronutrient applications to the leaves may have improved metabolic processes involved in forming the macronutrients and protein contents in plant. This might be because onion crops produce more carbohydrates as a result of photosynthesis. (Kaur *et al*., 2022).

Table 6. Effect of biofertilizers with foliar spray of micronutrients on the the activity of enzymes

Micronutrients	Biofertilizers	Nitrogenase (µmole C_2H_2)	Phosphatase (mg phenol/ g soil/24h)	Dehydrogenase (µg TPF/ g dry soil/24 hours)
Cu 2.0%	Control	29	0.17	11
	Bacillus	36	0.23	17
	Azotobacter	45	0. 21	26
	Pseudomonas	33	0.2	15
Cu 4.0%	Cont.	31	0.18	13
	Bacillus	40	0.28	24
	Azotobacter	51	0.22	38
	Pseudomonas	38	0.25	19
Mn 2.0%	Cont.	30	0.18	12
	Bacillus	39	0.24	21
	Azotobacter	48	0.2	33
	Pseudomonas	41	0.21	18
Mu 4.0%	Cont.	31	0.18	15
	Bacillus	43	0.29	30
	Azotobacter	54	0.22	41
	Pseudomonas	42	0.24	25
Fe 2.0%	Cont.	30	0.17	13
	Bacillus	42	0.27	27
	Azotobacter	52	0.25	39
	Pseudomonas	43	0.26	28
Fe 4.0%	Cont.	31	0.19	16
	Bacillus	49	032	31
	Azotobacter	58	0.28	46
	Pseudomonas	47	0.3	35

Table 7. Effect of biofertilizers with foliar spray of micronutrients on the macronutrients and protein contents in plant

Micronutrients	Biofertilizers	%N	%P	%K	%Protein
Cu 2.0%	Control	1.15	0.19	0.46	7.18
	Bacillus	1.18	0.21	0.49	7.38
	Azotobacter	1.26	0.24	0.51	7.87
	Pseudomonas	1.19	0.22	0.48	7.43
Cu 4.0%	Cont.	1.16	0.2	0.47	7.25
	Bacillus	1.22	0.23	0.53	7.6
	Azotobacter	1.31	0.28	0.58	8.18
	Pseudomonas	1.2	0.25	0.51	7.5
Mn 2.0%	Cont.	1.16	0.21	0.47	7.25
	Bacillus	1.24	0.29	0.52	7.75
	Azotobacter	1.36	0.35	0.56	8.5
	Pseudomonas	1.24	0.27	0.49	7.75
Mu 4.0%	Cont.	1.17	0.21	0.48	7.31
	Bacillus	1.28	0.3	0.56	8
	Azotobacter	1.39	0.39	0.61	8.68
	Pseudomonas	1.27	0.28	0.53	7.93
Fe 2.0%	Cont.	1.18	0.22	0.48	7.37
	Bacillus	1.29	0.34	0.59	8.06
	Azotobacter	1.41	0.39	0.64	8.81
	Pseudomonas	1.27	0.29	0.57	7.93
Fe 4.0%	Cont.	1.18	0.23	0.49	7.375
	Bacillus	1.36	0.37	0.65	8.5
	Azotobacter	1.47	0.39	0.69	9.1875
	Pseudomonas	1.34	0.33	0.64	8.375
Significance	*	*	*		*
L.S.D. at 0.5%	Bio	0.0206	0.0151	0.082	0.0162
	Micronutrient	0.0171	0.0165	0.0113	0.0221
	Interaction	0.0169	0.491	0.0789	0.0442

Effect of biofertilizers with foliar spray of micronutrients on the micronutrients contents in plant

From Table (8), all used bacteria with different rates of micronutrients increased copper, manganese, zinc and iron contents in onion plants comparing with control that agree with (Gnanasundari *et al*., 2022). Azotobacter with all micronutrients (Cu, Mn and Fe) increased Cu, Mn, Zn and Fe the most comparing with Bacillus and Pseudomonas, but the highest increasing was with Fe comparing with Cu and Mn. The highest values for Cu content came from Bacillus with iron at concentration of 4.0%.

Increased dry mass in the plant and the redistribution of micronutrients, which may have strengthened during the curing process, are the two factors that contribute to higher accumulation. When compared to the control, the treatment of micronutrients increased the amount of Fe and Cu that accumulated. In general, the largest micronutrient accumulation in onion was favored by the combined application of the three micronutrients with the highest dose of micronutrients (Bertino *et al*., 2022).

Table 8. Effect of biofertilizers with foliar spray of micronutrients on the micronutrients contents in onion plant

Micronutrients	Biofertilizers	Cu (mg Kg^{-1})	Mn (mg Kg^{-1})	Zn (mg Kg^{-1})	Fe (mg Kg^{-1})
Cu 2.0%	Control	9.52	22	28.2	214
	Bacillus	10.31	23.1	30.1	229
	Azotobacter	9.88	24.8	32.4	235
	Pseudomonas	10.26	22.9	29.5	223
Cu 4.0%	Cont.	9.64	23.4	29.1	218
	Bacillus	11.29	25.2	32	232
	Azotobacter	10.3	25.6	35	239
	Pseudomonas	10.66	24.8	31	228
Mn 2.0%	Cont.	9.59	22.4	28.7	216
	Bacillus	10.4	23.9	31.8	238
	Azotobacter	10.1	25.2	33.9	246
	Pseudomonas	10.33	23.5	31.2	235
Mu 4.0%	Cont.	9.7	23.2	29.6	219
	Bacillus	10.8	24.6	33.8	241
	Azotobacter	10.3	26.1	36.4	255
	Pseudomonas	10.6	24.2	32.8	239
Fe 2.0%	Cont.	9.8	22.8	29.2	214
	Bacillus	10.9	24.4	33.5	240
	Azotobacter	10.2	26.4	39.1	248
	Pseudomonas	10.6	24.1	36.4	233
Fe 4.0%	Cont.	10.1	23.5	30.1	218
	Bacillus	11.4	26.2	39.2	249
	Azotobacter	10.7	28.9	42.5	261
	Pseudomonas	11.2	25.7	38.8	240
Significance	*	*	*	*	*
L.S.D. at 0.5%	Bio	0.0262	0.0232	0.1036	0.1620
	Micronutrient	0.0530	0.0508	0.1391	0.3594
	interaction	0.0546	0.0951	0.2782	0.3936

Results From Table (2) to Table (8) indicate that application of treatments significantly affected the development, yield, and microbial community in the rhizosphere of inoculated plants compared to the control, the interaction treatments between biofertilizer inoculation and micronutrient foliar application had the most positive effects on onion plant growth, yield, and microbial activity in the rhizosphere. Biofertilizer inoculation with micronutrients foliar application gave a synergistic effect; enhance the uptake of minerals and their metabolism in plants. Integrated nutrient management increase the macro and micronutrients uptake (Subbaiah *et al*., 2014; Thangasamy and Lawande 2018) and Azotobacter population in soil (Jayathilake *et al*.2003). In Table, (3) and Table (4) growth and yield parameters improved significantly at 5% with biofertilizer treatments. Onion plant height, number of leaves /plant and fresh weight increased as mentioned by Sarhan and Bashandy, (2021). Azotobacter gave highest N, P, K and concentration in onion leaves and improved onion properties in terms of plant height (50.51cm), leaf number (12.5), bulb diameter (7.43 cm) and bulb weight (96.24 gm), Increase yfed) and TSS (16.52%). The result is in agreed with (Kaur and Singh (2019), Jayathilake *et al*. (2003) ; Akoun (2004), Mohammad and Hassanpour (2012). Organic fertilizers help in onions bulbs production (Petrovic *et al*., 2019; Vachan and Tripathi ,2017; Shah *et al*., 2019; El zemrany and Faiyad 2021). The reason for these results may be due to released nutrients and hormone increase soil fertility by increasing soil biota and enhances the plant growth and bulb yield. N, P and K content in onion leaves increased by using biofertilizer (Shaheen *et al*., 2007). Biofertilizers sometimes raised N-fixing process, so induced growth substances, and consequently improved the nutrient adsorption by plant roots (Wu *et al*., 2005).

CONCLUSION

The sustainability of environmental quality and food production is at risk due to agricultural land becoming more alkaline as a result of climate change and poor farming practices. Use of efficient microbial with various application techniques is one of the remedial solutions that have been recommended. These elements might make microbial a crucial component of a plan for agricultural production that aims to manage alkalinity. Therefore, we recommend using Biofertilization and some micronutrients to improve onion crops and other crops because of their economic and environmental benefits.

REFERENCES

Adesemoye, A.O; Torbert. H. A. and Kloepper, J.W. (2009). Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol 58, 921–929.

A.O. A. C. (1980) *"Association of official methods of analysis*". 13th ed., Washington, D.C.

Akoun, J. (2004). Effect plant density and manure on the yield and yield components the common of the onion (Allium cepa L.) var. Nsukka red. Hortic. Sci. J. 9, 43-48

Alam. M.N.; Abedin, M.J. and Azad, M.A.K. (2010). Effect of micronutrients on growth and yield of onion under calcareous soil environment. Journal of Agriculture Science. 1(3),56-61

Armandeh, M.; Mahmoudi, N. and Fallah Nosratabad, A.R. (2022). Screening and evaluation of phosphate-solubilizing bacteria isolated from aquaculture ponds in a step-by-step strategy as potential biofertilizer. J. Appl. Microbiol. 133,1581–1596.

Attia YA, Al-Khalaifah HS, Alqhtani AH, Abd El-Hamid HS, Alyileili SR, El-Hamid AEEA, Bovera F, El-Shafey AA. (2022). The impact of multi-enzyme fortification on growth performance, intestinal morphology, nutrient digestibility, and meat quality of broiler chickens fed a standard or low-density diet. Front Vet Sci. 24(9):1012462. doi: 10.3389/fvets.2022.1012462. Erratum in: Front Vet Sci. 2023 May 09; 10:1200260. PMID: 36504838; PMCID: PMC9731804.

Ballabh, K.; Rana, D.K.and Rawat, S.S. (2013). Effects of foliar application of micronutrients on growth, yield and quality of onion. Indian Journal of Horticulture. 70(2), 260-265.

Bertino, N.M.F.; Grangeiro, L.C.; Costa, J.P.N.D.; Costa, R.M.C.; Lacerda, R.R.D.A. and Gomes, V.E.D.V. (2022). Growth, nutrient accumulation and yield of onion as a function of micronutrient fertilization. Rev. Bras. Eng. Agríc. E Ambient. 26, 126–134. https://doi.org/10.1590/1807-1929/agriambi.v26n2p126-134

Biswas, P., Das, S., Bar, A., Maity, T.K., Mandal, A.R., 2020. Effect of Micronutrient Application on Vegetative Growth and Bulb Yield Attributes of Rabi Onion (Allium cepa L.). Int. J. Curr. Microbiol. Appl. Sci. 9, 556–565. https://doi.org/10.20546/ijcmas.2020.903.065

Biswas, P., Das, S., Bar, A., Maity, T.K., Mandal, A.R.(2020). Effect of Micronutrient Application on Vegetative Growth and Bulb Yield Attributes of Rabi Onion (Allium cepa L.). Int. J. Curr. Microbiol. Appl. Sci. 9, 556–565. https://doi.org/10.20546/ijcmas.2020.903.065

Bremner, J. M. and Mulvaney, C.S. (1982). Nitrogen-total. In A. L. Page, R. H. Miller, and D. R. Keeney (eds.), Methods of Soil Analysis, Part 2. Agronomy 9,595-624.

Brinjh, S.; Kumar, S.; Kumar, D. and Kumar, M.(2014). Effect of integrated nutrient management on growth, yield and quality in onion cv. Pusa Madhvi. Plant Archives. 14(1),557-9.

Bunt, J. S. and Rovira, A. D. (1955). Microbiological studies of some sub –ataractic soils. J. Soil Sci., 6,119.

Casida, Jr. LE.; Klein, D.A. and Santoro, T. (1964). Soil dehydrogenase activity. Soil science. 98(6),371-376.

Cottenie, A.; Verloo, M.; Kiekens, L; Velghe, G.and Camerlynck, R. (1982). "Chemical Analysis of Plants and Soils". Lab. Agroch. State Univ. Gent, Belgium. 63.

Dineshkumar, R., Subramanian, J., Arumugam, A., Ahamed Rasheeq, A., Sampathkumar, P. (2020). Exploring the microalgae biofertilizer effect on onion cultivation by field experiment. Waste and Biomass Valorization. 11, 77-87.

El Zemrany, H. M. and Faiyad, R. M. (2021). Maximizing use efficiency of mineral fertilizers using K fulvate and *Azotobacter chroococcum* DSM 2286 and their effect on wheat production and nutrients uptake. Egypt. J. Soil. Sci. (1),13-25

FAO. (2012). World onion production, Food and Agriculture Organization of the United Nations. Rome, Italy.

FAO. (2018). Food and Agriculture Organization of the United Nations. Rome, Italy p. 57.

Gadelrabh, H.M.; Elamin, S.M. (2013). Effect of different organic fertilizers on growth, yield and total soluble solid of the onion (Allium cepa L.) variety Baftaim-s. J. Agric. Vet. Sci. 14, 61–67.

Gnanasundari K, S.; Srivignesh, Krishna, K.R.; Kumar, M.;and Kumar, A.R.. (2022). Integrated Nutrient Management in Onion-A Review. Ecol. Environ. Conserv. 28, 182–192. https://doi.org/10.53550/EEC.2022.v28i07s.030

Haahtela, K.; Wartiovaara, T.; Sundman, V.and Skujiņš, J. (1981). Root-associated N2 fixation (acetylene reduction) by Enterobacteriaceae and Azospirillum strains in cold-climate spodosols. Applied and environmental microbiology. (1):203-206.

Jarlier, V., Fosse, T. and Philipon, A. (1996) Antibiotic susceptibility in aerobic Gram-negative bacilli isolated in intensive care units in 39 French teaching hospitals (ICU study). *Intensive Care Mod.* 22, 1057-1065.

Jayathilake, P.K.S., Reddy, I.P., Srihari, D., Reddy, K.R., Neeraja, G. (2003). Integrated nutrient management in onion (Allium cepa L.). Trop. Agr. Res. 15, 1-9

Kaur, A. and Singh, S. (2019). Role of various fertilizers and azotobacter (biofertilizer) on the performance of kharif onion (Allium cepa L.) cv. Agrifound Dark Red. Journal of Pharmacognosy and Phytochemistry. 8 (4S) : 146-151.

Kaur, M.; Dhillon, N.; Kaur, A. and Dhillon, S. (2022). Influence of foliar nutrients application on growth, yield and quality of onion (Allium cepa L.). The Pharma Innovation Journal; 11(8): 973-976.

Krieg N.R, and Holt J.G. (1984). Bergey's manual of systematic bacteriology. Yi Hsien Publishing Co.

Mohammad, F.M. and Hassanpour, A.M. (2012). Onion yield, quality and storability as affected with different soil moisture and nitrogen regimes. South Western J. Hortic. Biol. Environ. 3(2), 145-165.

Nautiyal, C.S. (1999) Inefficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiology Letters.* 170, 265- 270.

Öhlinger, R. (1996). Soil sampling and sample preparation. In Methods in soil biology 7-11. Berlin, Heidelberg: Springer Berlin Heidelberg.

Page, W.J. and Huyer, M.A. (1984). Derepression of the *Azotobacter vinelandii* siderophore system, using iron-containing minerals to limit iron repletion. Journal of bacteriology. 158(2), 496-502.

Petrovic, B.; Kopta, T.and Pokluda, R. (2019). Effect of biofertilizers on yield and morphological parameters of onion cultivars. Folia Horticulturae. 31(1): 51-59.

Pramanik, K., & Tripathy, P. (2017). Effect of micronutrients on growth and total yield of Onion (Allium cepa L.). *The Bioscan*, *12*(1), 322-326.

Rawat, P.; Das, S.; Shankhdhar, D.and Shankhdhar, S.C. (2021). Phosphate-Solubilizing Microorganisms: Mechanism and Their Role in Phosphate Solubilization and Uptake. J. Soil Sci. Plant Nutr. 21, 49–68.

Rizzolo, L. J. and Joshi, H. C. (1993) Apical orientation of the microtubule organizing center and associated γ-tubulin during the polarization of the retinal pigment epithelium *in vivo. Dev. Biol.*157, 147-156.

Sarhan, M.G. and Bashandy, S. (2021). Enhancing Onion Yield, Quality, Storability and Profitability by Using FYM, Copper and Bio-fertilizer. Egyptian Journal of Soil Science. 61(3), 323-35.

Shah, Kh. Na.; Chaudhary, I. J.; Ran, D. k.and Singh, V. (2019). Impact assessment of different organic manures on growth, morphology and yield of onion (*Allium cepa* L.) cultivar; Asian Journal Agricultural Research. 13 (1), 20-27.

Shaheen, A.M.; Rizk, F.A.and Singer, S.M. (2007). Growing onion plants without chemical fertilization. Research Journal of Agriculture and Biological Sciences, 3 (2), 95-104.

Scholhorn, R. and R.H. Burris (1967) Acetylene as a competitive inhibitor of N2 fixation. *Proc. Natl. Acad. Sci. USA* 58, 213-216.

Shedeed, S.I., El-Sayed, S.A.A. and Bash, D.A., (2014). Effectiveness of bio-fertilizers with organic matter on the growth, yield and nutrient content of onion (Allium cepa L.) plants. *European International Journal of Science and Technology*, *3*(9), pp.115-122.

Singh, Vikram.; Sharma, K.C.and Sharma, H.R. (2017). Effect of BioInoculants and Graded Level of Fertilizers on Nutrient Uptake in Garlic. Int. J. Curr. Microbiol. App. Sci. 6 (5), 1200- 1209.

Subbaiah, P. V.; Sairam, A. and Rao, P.C.S. (2014). Effect of Integrated Use of Organic and Inorganic Sources of Nutrients and Biofertilizers on Drymatter Production, N, P, K, S and Micronutrient Uptake in Maize–Onion Cropping System. The Andhra Agricultural Journal, 61(2), 330-334.

Thangasamy, A. and Lawande, K. E. (2018). Effect of Integrated Nutrient Management on Garlic Yield, Nutrient Uptake and Pungency. Agropedology. 28 (01), 8-13

Tohamy, W.A.; Khalid, A.K.; El-Abagy, H.M. and Abou-Hussein, S..D (2009). Essential oil, growth and yield of onion (*Allium cepa* L.) in response to application of some micronutrients, Australia Journal of Basic Applied Science. 3(1), 201-205

Vachan, R. and Tripathi, S.M. (2017) Study on the effect of biofertilizer with chemical fertilizer on plant growth, yield and economic of Rabi season onion (*Allium cepa* L.) CV. NHROF Red 2. Journal of Pharmacognosy and Phytochemistry, 6 (5), 1496-1499

Waller, R.A. and Duncan, D.B. (1969). A Bayes rule for the symmetric multiple comparisons problem. Journal of the American Statistical Association. 64 (328),1484-503.

Wani, S.A.; Chand, S. and Ali, T. (2013). Potential use of *Azotobacter chroococcum* in crop production: an overview. Curr Agric Res J. 1(1), 35-38.

Ware, G.W. and McCollum, J.P. (1980). Spinach. Producing Vegetable Crops. 3rd edn. Danville, IL: Interstate Printers and Publishers. 437-449.

Wu, S.C.; Cao Z.H. ; Li, Z.G. and Chung, K.C. (2005). Effect of biofertilizer containing N2-fixen, P and K solubilizers and A. M. fungi on maize growth: A greenhouse trail. Geoderma, 125, 155-166

استخدام التسميد الحيوي وبعض العناصر الصغرى في تحسين انتاجية البصل تحت ظروف واحة سيوة

منى مرسى الشاذلي[١] ، أمال السيد أحمد[١]، هبه أحمد خليل إبراهيم[١] و سالي أحمد إسماعيل[٢]

[١]قسم خصوبة وميكروبيولوجي الأراضي بمركز بحوث الصحراء
[٢]قسم علوم التربة والمياه كلية العلوم الزراعية والبيئية بجامعة العريش

الملخص

تم إجراء تجربة حقلية بمحطة بحوث مركز بحوث الصحراء بواحة سيوة على نبات البصل خلال موسمين (٢٠١٩-٢٠٢٠) لدراسة تأثير الأسمدة الحيوية (*Azotobacter choroccoum, Bacillus megatherium* and *Pseudomans fluorescence*) مع الرش الورقى للعناصر الصغرى من النحاس والحديد والمنجنيز وكانت النتائج كالتالى : كل المعاملات التي تمت مع بكتريا (*Azotobacter choroccoum, Bacillus megatherium* and *Pseudomans fluorescence*) مع العناصر الصغرى من الحديد والمنجنيز والنحاس أعطت نتائج إيجابية مقارنة بالكنترول. بكتريا Azotobacter أعطت نشاط نيتروجينى ١٤٣ جزء في المليون وأعطت أعلى قيمة من الجبريلين بمقدار ٢,٣ ميكروجرام / مللى وكذلك من السيتوكينين بمقدار ٢٣,٤ ميكروجرام / مللى وكذلك عملت على زيادة كل من طول النبات وعدد الأوراق والوزن الجاف والرطب للنبات خاصة مع تركيز ٤,٠% من الحديد وكذلك زادت من قطر لب البصل مع كل من المنجنيز والحديد كما زادت كل من المحصول والعدد الكلى للميكروبات في التربه مع تركيزات الحديد كما أدت المعاملات بالأزوتوباكتر الى زيادة عد ميكروبات الأزوتوباكتر بالتربة كذلك إنزيمات النيتروجينيز والهيدروجينيز و العناصر الكبرى (نيتروجين وفوسفور وبوتاسيوم) والصغرى (منجنيز وزنك وحديد) وذلك مقارنة ب Bacillus , Pseudomonas . بكتريا *Bacillus megatherium* أعطت أعلى نشاط في إذابة الفوسفات بمقدار ٢,٨ سم قطر هالة الأذابة ، كذلك عملت على زيادة قطر ووزن لب البصل عند إضافتها مع النحاس أو المنجنيز كما عملت على زيادة المواد الصلبة الكليه TSSوعدد خلايا بكتريا Bacillus في التربة وكذلك نشاط إنزيم الفوسفاتيز . أما بكتريا Pseudomonas فقد أعطت أعلى إنتاجية من الأندول أسيتيك أسيد بمقدار ٢,٤٤ ميكروجرام / مللى وكذلك أظهرت أعلى نشاط مضاد للميكروبات مع جميع تركيزات العناصر الصغرى المستخدمة في التجربه مقارنة ببكتريا *Bacillus megatherium* *Aaotobacter choroccoum*