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Testing the Efficiency of Different Isolates of *Azotobacter*, *Azospirillum* and *Pseudomonas* for Some Traits Related to Plant Growth Promoters on Varieties of Onion

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ABSTRACT

The ability of rhizobacterial isolates (*Azotobacter, Azospirillum* and *Pseudomonas*) isolated at random from rhizosphere of different onion varieties to exhibit some plant growth promoting properties was evaluated *in vitro* conditions. After purification the isolates were tested towards their ability to solubilize phosphate and production of auxins. Additionally, field trials were conducted at Mallawi Agricultural Research Station, Minia Governorate, Egypt, during two successive winter seasons (2016/2017 and 2017/2018) to study the impacts of rhizobacterial inoculation on some chemical composition and yield characters of three onion varieties (Giza 6 Mohassan, Giza 20 and Giza Red) under two levels of mineral N- fertilizer. The experiments were laid out in a split-split plot design with three replicates of each treatment and the combined statistical analysis of variables was worked out for both tested seasons. The results of the laboratory experiments showed that the highest values of auxin production for *Azotobacter*, *Azospirillum* and *Pseudomonas* were recorded for isolates number (*Azt* 1), (*Azo* 1) and (*Ps* 1) as they produced 0.53, 0.45 and 0.46 µg/ml, respectively, with the variety Giza 6 Mohassan. While, isolates number (*Azt* 1), (*Azo* 1) with Giza 6 Mohassan variety and (*Ps* 6) with Giza red variety were the most efficient in dissolving phosphate. Results of field trials showed that Giza 6 Mohassan variety inoculated with *Pseudomonas* and amended with 75% from the recommended-N surpassed the other treatments in chemical composition and all yield characters. The promotive action of such treatment exerted highest values of bulb diameter (6.10 cm), onion yield (6.48 ton fed^{-1}), T.S.S (12.21%), N (1.950%), P (0.429%) and K (0.947%).

Keywords: *Onion, Azotobacter*, *Azospirillum*, *Pseudomonas*, Auxins, Phosphate solubilization.

Introduction

Onion (*Allium cepa*) is considered as one of the most important strategic commodities for the populations all over the world. Onion in Egypt represent export commodity besides its use as food and medical product that increase its value and lead to enhance the efforts to improve its productivity with less costs and in safe methods of using plant growth promoting rhizo-microorganisms (PGPR) in order to protect the environment from pollution. (Kloepper, 2003) discussed the problems associated with early research work on deleterious rhizobacteria, resulting from the use of soil-less systems lacking competition from native soil and rhizosphere bacteria, and from the use of a very high number of bacteria to inoculate plants, that can reach log 11.8 per seedling. These experimental conditions would not be encountered in nature, and the concept and nature of deleterious rhizobacteria can be questioned.

The IAA synthesized by PGPRs influenced the root hair development, respiration rate, metabolism and root proliferation which in turn resulted in better mineral uptake of the inoculated plants. IAA formation via indole-3-pyruvic acid and indole-3-acetic aldehyde is found in a majority of bacteria like*, Agrobacterium* and *Pseudomonas;* certain representatives of *Bradyrhizobium, Rhizobium, Azospirillum*, *Klebsiella, and Enterobacter*. Most *Rhizobium* species have been shown to produce IAA (Matiru and Dakora, 2004).

Plant growth promoting rhizobacteria (PGPR) was bacteria able to promote plant growth and serve as biofertilizers and biopesticides in agricultural crops. The PGPR may promote the plant growth through different direct and indirect mechanisms such as: fixation of nitrogen, solubilization of soil phosphates, production of hormones (auxin and gibberellin), production of siderophores (an iron

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chelator), increasing the uptake of water and minerals, increasing the enzymatic activity of the plant (Pérez-Montaño *et al*., 2014).

There is a currently considerable interest in the role of rhizobacteria in plant growth promotion and biological control of soil borne pathogens. They comprise a range of species with huge taxonomic diversity, especially within Firmicutes and Proteobacteria. These include, *Pseudomonas fluorescens* (Sahu *et al.,* 2018) *Azospirillum brasilense* (García *et al.,* 2017),*Azotobacter salinestris* (Chennappa *et al.,* 2018). A large number of microorganisms, including bacteria, fungi, actinomycetes and algae exhibit phosphate solubilization and mineralization ability. Soil bacteria that have been reported to mobilize poorly available phosphorus via solubilization and mineralization include *Pseudomonas spp., Agrobacterium spp.,* and *Bacillus circulans* (Babalola and Glick, 2012). Other phosphorus solubilizing and mineralizing bacteria include various strains of *Azotobacter* (Kumar *et al*., 2014). In this concern, Galal (2016) confirmed the importance of inoculation by plant growth promoting rhizobacteria to obtain the best performance in growth and yield of onion as well as higher values of auxins production for *Azotobacter, Pseudomonas* and *Azospirillum* was recorded, respectively. Highest values of phosphate solubilization for *Pseudomonas, Azotobacter*, and A*zospirillum*, respectively. However, there are several PGPR, whose plants growth-promoting activities have been demonstrated, including production of iron-sequestering siderophores and antimicrobial compounds that hinder colonization of hosts by phytopathogens (Verma *et al.,* 2010).

The present investigation aimed at examining some rhizobacterial isolates for an array of traits that are related to plant growth promoting rhizobacteria (PGPR) effects in the laboratory. The study was also concerned with the impacts of rhizobacterial inoculation on some chemical composition and yield characters of three onion varieties.

Materials and Methods

Eighteen isolates of *Azotobacter*, *Azospirillum* and *Pseudomonas* isolated at random from rhizosphere of different onion varieties (Giza 6 Mohassan, Giza 20 and Giza Red) were examined *in vitro* for their ability to promote plant growth through determination their efficiency to solubilize phosphate and production of auxins. Additionally, two field experiments were conducted at Mallawi Agricultural Research Station, Minia Governorate, Egypt, during two successive winter seasons of 2016/2017 and 2017/2018 to study the impacts of rhizobacterial inoculation on some chemical composition and yield characters of three onion varieties under two levels of mineral N- fertilizer.

1. Isolation, purification and maintenance of *Azotobacter*, *Azospirillum* and *Pseudomonas* isolates:

Isolation program was conducted to isolation *Azotobacter*, *Azospirillum* and *Pseudomonas* colonizing rhizosphere soil. Six isolates of *Azotobacter*, *Azospirillum* and *Pseudomonas* were picked up from the suitable media used. All *Azotobacter*, *Azospirillum* and *Pseudomonas* isolates were purified by successive streak on deficient modified Ashby, Dobereiner and King media, respectively, using the techniques adopted by (Abdel-Malek and Ishac, 1968) for *Azotobacter*, (Dobereiner and Day, 1976) for *Azospirillum* and (King *et al.,* 1954) for *Pseudomonas.* Purified isolates maintained in the same media were confirmed by microscopic examination for Gram stained young cells (18-24 hours old). Purified isolates were stored at 4°C.

2. Testing the efficiency of different isolates for some traits related to onion plant growth promoters:

To screen these microorganisms for traits related to onion plant growth promoters, eighteen isolates of *Azotobacter*, *Azospirillum* and *Pseudomonas* were isolated at random from rhizosphere of different onion varieties. After purification the previous isolates were tested under *in vitro* conditions towards their efficiency for auxins production and phosphate solubilization.

2.1. Screening the isolates for auxins production:

For rapid determination of auxins of *Pseudomonas, Azotobacter* and *Azospirillum* the calorimetric Salkowski reaction was performed according to Tang and Bonner (1947). One ml of bacterial supernatant was added to 4 ml of Salkowski reagent (2.025 g $FeCl_3$ + 300 ml H_2SO_4 + 500 ml H_2O). The mixture incubated in darkness for 15-30 min. and rosy color means the presence of auxins, which measured by spectrophotometer at 530 nm.

2.2. Screening the isolates for Phosphate solubilization

The Bunt and Rovira medium previously mentioned modified by (Abdel-Hafez, 1966) was used. The K_2HPO_4 in the medium was replaced by KCL. To each flask, containing 90 ml of the medium free from phosphate 5 ml of a 10 % K_2HPO_4 sterile solution followed by 10 ml of a 10 % $CaCl_2$ sterile solution was added and thoroughly mixed with the agar. This was carried out immediately before pouring into the betri dishes. This method was found to form fine precipitates of insoluble calcium phosphate in the medium. The pH was readjusted to 6.8 by sterile standard NaOH solution. The standard plate method was used for counting phosphate dissolving bacteria which were readily detected by clear zones around colonies after incubation at 30°C for 48 hr. (Bunt and Rovira, 1955).

3. Field experiments

Soil samples were collected from the top 20 cm layer in the experimental field, air-dried and sieved through a 2 mm screen. The main physical and chemical properties were carried out according to the methods described by Piper (1950) and Jackson (1973) and the obtained data were recorded in Table (1).

Table 1: Some physical and chemical properties of the experimental soil

Property		Values
Particle size distribution (%):		
Sand		9.96
Silt		30.73
Clay		59.31
Texture grade		Clay loam
$CaCO_3$		0.90
Saturation percent (S.P %)		41.00
pH (soil paste)		8.12
E.C (dS m^{-1}, at 25°C)		0.58
Soluble cations and anions (meq L^{-1}):		
Ca^{++}		1.60
Mg^{++}		2.30
Na^{+}		1.50
K^{+}		0.24
$CO^{=}_3$		0.00
HCO^{-}_3		2.88
Cl^{-}		2.12
$SO^{=}_4$		0.64
Total-N	(%)	0.045
Total soluble- N	(mg kg^{-1})	45.40
Available- P	(mg kg^{-1})	13.80
Available-K	(mg kg^{-1})	315.20

The experimental design was arranged in a split-split plot design with three replicates of each treatment with a plot area 10.5 m^2 (1/400 feddan). The main plots were assigned to three varieties of onion, while the sub-plots were allocated to N- fertilization treatments (namely, 75 and 100% of the recommended N-fertilizer) and the bacterial inoculation treatments (*Azotobacter, Azospirillum, Pseudomonas*, mixed inoculant and control) were randomly allotted in sub-sub plots.

All plots received calcium superphosphate (15.5% P_2O_5) during soil preparation at the rate of 150 kg fed^{-1} and potassium sulphate (48% K_2O) was applied after 15 and 45 days of sowing at the rate of 50 kg fed^{-1}. The recommended-N treatment (100% N) received 90 kg N fed^{-1}, while 75% recommended-N treatment received 67.5 kg N fed^{-1} as ammonium nitrate (33.5% N). Each N-level was splitted to two equal doses after 15 and 30 days from sowing. Optimum cultural practices were followed as recommended throughout the growing season.

Three different onion varieties (Giza 6 Mohassan, Giza 20 and Giza Red) that have been used in the study were obtained from the Onion Research Department, Field Crops Research Institute, ARC, Giza, Egypt. Onion seedlings were soaked in liquid cultures of each rhizobacteria or in mixed inoculum treatment (in equal portions from the cultures of three rhizobacteria) for 1 hour just before sowing. Also, soil inoculation was done at a rate of ten liter/fed as a soil drench after 15 days from sowing.

4. Chemical analysis

The oven dried plant materials were wet digested using a mixture of pure $HClO_4$ and H_2SO_4 at a ratio of 1:1, according to Jackson (1973). Total nitrogen was determined using the micro-Kjeldahel method, phosphorus was determined Spectrophotometrically using ammonium molybdate and stannus chloride reagents, while potassium was determined using Flamephotometer.

5. Statistical analysis:

Data collected were subjected to Analysis of Variance (ANOVA) for obtained data in the average two growing seasons were performed. The measured variables were analyzed using MSTATC. Differences among treatments were evaluated by LSD test at 5%, according to procedures outlined by (Elias and Karim, 1984) and (Gomez and Gomez, 1984).

Results and Discussion

1. The ability of the tested rhizobacterial isolates to exhibit some PGP-properties under *in vitro* condition

The ability of eighteen isolates of *Azotobacter*, *Azospirillum* and *Pseudomonas* for traits related to onion plant growth promoters were tested under *in vitro* conditions towards their efficiency for auxins production and phosphate solubilization. In general, all of the tested isolates were apparently able to produce auxins, however, most of isolates showed efficiency in dissolving phosphates, while some isolates gave negative results in this regard.

2. The efficiency of isolates in the production of auxins

The results in Table (2) show that the ability to produce auxins are the common feature of all the tested isolates, however, the auxin production by *Azotobacter* isolates depend on onion variety. The highest values of auxin production were obtained by variety Giza 6 Mohassan followed by Giza 20 then variety Giza red. It was also clear that differences were noticed between isolates in their activities in producing auxins in the same onion variety. In the quantitative measurements, the highest amount of auxins was produced by isolate No. (*Azt* 1) in variety Giza 6 Mohassan followed by isolate No. (*Azt* 5) for Giza 20, while the lowest values of auxins were produced for isolate No. (*Azt* 3) in variety Giza red, as they produced 0.53, 0.50 and 0.28 µg/ ml, respectively.

Table 2: Production of auxins by *Azotobacter* isolates

Auxins producing bacteria *Azotobacter* (µg/ ml)					
Isolate No.	**Auxins µg/ml**	**Onion variety**	**Isolate No.**	**Auxins** µg/ml	**Onion variety**
***Azt* .1**	0.53	Giza 6 Mohassan	*Azt* 4	0.40	Giza 20
***Azt* 2**	0.48	Giza 6 Mohassan	*Azt* 5	0.50	Giza 20
***Azt* 3**	0.28	Giza Red	*Azt* 6	0.33	Giza Red

Concerning the ability of *Azospirillum* isolates to produce auxins, data in Table (3) demonstrate that the auxin production by *Azospirillum* isolates depended on onion variety. As with isolates of *Azotobacter*, the highest values of the auxins were produced with variety Giza 6 Mohassan followed by Giza 20 then variety Giza red. There were also clear differences between *Azospirillum* isolates in their activities of producing auxins in onion plants. The highest values of auxins were produced by isolates number (*Azo* 1) and (*Azo* 2) in variety Giza 6 Mohassan, while the lowest values of auxins were produced for isolate No (*Azo* 6) in variety Giza red attaining values of 0.45, 0.44 and 0.22 µg/ ml, respectively.

Data in Table (4) presented that the values originated from quantitative assay reflected the ability of all tested *Pseudomonas* isolates to produce auxins. The results, again, clearly showed that *Pseudomonas* isolates depending on the onion variety, where the highest values of the auxins were produced with variety Giza 6 Mohassan followed by Giza 20 then variety Giza Red. Clear differences were also found between *Pseudomonas* isolates and the highest values of auxins were produced by isolate No. (*Ps* 1) with onion variety Giza 6 Mohassan then isolate No (*Ps* 2) in the same variety, as they produced 0.46 and 0.42 µg/ml, respectively. While the lowest values of auxins were produced for isolate No (*Ps* 3) in variety Giza red (0.26 µg/ ml).

Table 3: Production of auxins by *Azospirillum* isolates

Auxins producing bacteria *Azospirillum* (µg/ ml)					
Isolate No.	**Auxins µg/ml**	**Onion variety**	**Isolate No.**	**Auxins µg/ml**	**Onion variety**
***Azo* .1**	0.45	Giza 6 Mohassan	*Azo* 4	0.39	Giza 20
***Azo* 2**	0.41	Giza 6 Mohassan	*Azo* 5	0.36	Giza 20
***Azo* 3**	0.35	Giza Red	*Azo* 6	0.32	Giza Red

Table 4: Production of auxins by *Pseudomonas* isolates

Auxins producing bacteria *Pseudomonas* (µg/ ml)					
Isolate No.	**Auxins** µg/ml	**Onion variety**	**Isolate No.**	**Auxins** µg/ml	**Onion variety**
***Ps* .1**	0.46	Giza 6 Mohassan	*Ps* 4	0.39	Giza 20
***Ps* 2**	0.42	Giza 6 Mohassan	*Ps* 5	0.37	Giza 20
***Ps* 3**	0.26	Giza Red	*Ps* 6	0.29	Giza Red

Indeed, a high proportion of rhizobacteria including species of *Azotobacter, Azospirillum, Bacillus, Pseudomonas* and *Serratia* are able to produce plant growth auxins, which acts to stimulate root growth and provides it with more branching and larger surface area. Many investigators consider the auxins production by plant growth promoting rhizobacteria (PGPRs), as a vital mechanism to clarify the plant promotion (Pan *et al.,* 1999; Verma *et al.,* 2010; Rizk *et al.,* 2011 and Galal, 2016).

3. Efficiency of isolates in the phosphate solubilization:

Six isolates of *Azotobacter* have been tested for their efficiency in the phosphate solubilization where four isolates of them were found to have a positive effect in this regard and the other two isolates gave negative results (Table 5). Generally, the ability of *Azotobacter* isolates to dissolve phosphate is depending on the type of onion variety. The most outstanding varieties of onion with positive effects in dissolving phosphate were Giza 6 Mohassan variety with two *Azotobacter* isolates (Azt 1 and 2) and Giza Red variety with another two isolates (Azt 3 and 6). While, *Azotobacter* isolates (Azt 4 and 5) with onion variety Giza 20 gave a negative results.

Table 5: Phosphate solubilization by *Azotobacter* isolates

Phosphate solubilization (PS) *Azotobacter*					
Isolate No.	**PSB**	**Onion variety**	**Isolate No.**	**PSB**	**Onion variety**
***Azt* .1**	+++	Giza 6 Mohassan	*Azt* 4	-	Giza 20
***Azt* 2**	++	Giza 6 Mohassan	*Azt* 5	-	Giza 20
***Azt* 3**	++	Giza Red	*Azt* 6	++	Giza Red

Data in Table (6) represent the ability of *Azospirillum* isolated from the rhizosphere of three different onion varieties on dissolving phosphate. Again, the process of dissolving phosphorus was found to be affected by onion variety. From the six *Azospirillum* isolates investigated in this study to indicate their impact in dissolving phosphorus, three isolates were found to have positive effects and three isolates had negative effects. The onion varieties that have a high positive effect were Giza 6 Mohassan with one isolate (*Azo* 1) and Giza Red variety with two isolates (*Azo* 3 and 6). On the other hand, the negative impacts were clear with one isolate (*Azo* 2) on onion variety Giza 6 Mohassan and another two isolates (*Azo* 4 and 5) with Giza 20 variety.

Table 6: Phosphate solubilization by *Azospirillum* isolates

Phosphate solubilization (PS) *Azospirillum*					
IsolateNo.	**PSB**	**Onion variety**	**Isolate No.**	**PSB**	**Onion variety**
***Azo* .1**	+++	Giza 6 Mohassan	*Azo* 4	-	Giza 20
***Azo* 2**	-	Giza 6 Mohassan	*Azo* 5	-	Giza 20
***Azo* 3**	++	Giza Red	*Azo* 6	++	Giza Red

Results of *in vitro* examination for the ability of *Pseudomonas* isolates in solubilizing phosphate are shown in Table (7). Data confirmed again the phosphorus dissolving process is depending on the type of onion variety. Six isolates of *Pseudomonas* have been investigated for their impact on phosphate solubilization and it was found that four isolates out of them succeed to solubilize the phosphorus, while the other two isolates failed to solubilize phosphate. The most onion varieties had positive effects were Giza red with two isolates (*Ps* 3 and 6) and Giza 6 Mohassan variety with two isolates (*Ps* 1 and 2), while two isolates (*Ps* 4 and 5) had a negative effect with Giza 20 variety.

In view of the above results, it could be concluded that the capability of the rhizobacterial isolates to solubilize the precipitated phosphorus *in vitro* depends on its efficiency to produce inorganic and organic acids and/or CO_2. This finding was emphasized by other investigators (Trivedi and Sa, 2008; Kumar *et al*., 2012 and Galal, 2016) who reported that there are a large number of bacteria including, species of *Pseudomonas, Azospirillum* and *Azotobacter* enhanced plant growth by their different plant growth promoting activities including organic acids production and phosphate solubilization. Also, Verma *et al*. (2010), Badawi *et al.* (2011) and Rizk *et al.* (2011) illustrated that the ability of PGPRs to solubilize some insoluble or sparingly soluble minerals via three possible mechanisms: acidification of the medium, production of chelating metabolites and redox activity.

Table 7: Phosphate solubilization by *Pseudomonas* isolates

Phosphate solubilization (PS) *Pseudomonas*					
Isolate No.	**PSB**	**Onion variety**	**Isolate No.**	**PSB**	**Onion variety**
***Ps* .1**	++	Giza 6 Mohassan	*Ps* 4	-	Giza 20
***Ps* 2**	+	Giza 6 Mohassan	*Ps* 5	-	Giza 20
***Ps* 3**	+	Giza Red	*Ps* 6	+++	Giza Red

4. Onion yield measurements

Data presented in Table (8) show the main effect of rhizobacterial inoculation (*Azotobacter, Azospirillum, Pseudomonas,* mixed inoculant and control) on some yield characters, namely, bulb diameter, onion yield and total soluble solids (T.S.S) of three onion varieties (Giza 6 Mohassan, Giza 20 and Giza Red) under two levels of N-fertilizer namely, 75 and 100% from the recommended-N dose. The obtained data showed that all yield parameters were significantly affected by different treatments under study.

Regarding the influence of onion varieties, data in Table (8) elicited that Giza 6 Mohassan variety significantly surpassed the other tested onion varieties (Giza 20 and Giza Red) in bulb diameter (cm), onion yield (ton/ fed) and T.S.S% (the average two growing seasons). Giza 6 Mohassan variety recorded the highest values of bulb diameter (4.70 cm), onion yield (4.77 ton/fed) and T.S.S. (10.24%) compared to other onion varieties. The superiority of Giza 6 Mohassan variety over Giza 20 and Giza Red varieties could be attributed to differences in the genetic make-up which reflect on growth and yield habits.

Table 8: Average bulb diameter, onion yield and T.S.S of different onion varieties affected by different rhizobacterial inoculation and N-fertilization treatments (average two growing seasons)

Treatments		**Bulb diameter (cm)**	**Yield (ton/fed)**	**T.S.S (%)**
Onion varieties	**Giza 20**	4.14	4.38	9.79
	Giza 6 Mohassan	4.70	4.77	10.24
	Giza Red)	4.54	4.51	9.85
LSD at 0.05		0.0075	0.0012	0.0047
N- fertilization	**75% (N)**	4.55	5.11	10.00
	100%(N)	4.42	4.00	9.91
LSD at 0.05		0.0055	0.006	0.0035
Rhizobacterial inoculation	***Azotobacter***	5.15	5.17	10.36
	Azospirillum	4.76	4.61	10.22
	Pseudomonas	5.48	5.96	10.40
	mixed inoculant	4.05	3.96	9.82
	Control	2.97	3.08	8.99
LSD at 0.05		0.0071	0.0010	0.0045

Control = Uninoculated treatment.

75 and 100% N = 67.5 and 90 kg N fed^{-1}, respectively**.**

Concerning the main effect of mineral nitrogen fertilizer treatments, data in Table (8) revealed that all yield characters significantly affected by N-fertilization treatments. Soil fertilized with 75% of the recommended-N (67.5 kg N/fed) recorded the highest values of bulb diameter (4.55 cm), onion yield (5.11 ton/fed) and T.S.S (10.00%). The increase in tested yield characters may be due to stimulation of cell division and internodes elongation resulting from nitrogen application and its function as the main constituent of protein, amino acids and many essential compounds in plant system (Desoky and El-Sayed, 2016).

In respect to rhizobacterial inoculation treatments, the results demonstrated in Table (8) revealed that the highest bulb diameter (cm), onion yield (ton/ fed) and T.S.S (%) were resulted from *Pseudomonas* inoculation treatment as they gave values of bulb diameter (5.48 cm), onion yield (5.96 ton/ fed) and T.S.S (10.40%) followed by *Azotobacter* treatment with values of bulb diameter (5.15 cm), onion yield (5.17 ton/fed) and T.S.S (10.36%). While, the uninoculated treatment gave the lowest values of bulb diameter (2.97 cm), onion yield (3.08 ton/fed) and T.S.S (8.99%) during the combined two growing seasons. The promotive effect of such inoculation treatments could be due to the biological role of such rhizobacteria in promoting plant growth, N_2-fixation performance as well as P mobilization, phytohormone production and saving the bio-protection against phytopathogens. Such promotion is confirmed by many investigators (Tilak *et al.,* 2005 and Verma *et al.,* 2010). In this concern, Fawaz (2011) reported that *Ps. flourscens* significantly reduced the percentage of infected plants with root disease. In addition, Hussein (2008) and Galal (2016) confirmed the importance of inoculation by rhizobacteria (*Azotobacter*, *Pseudomonas* and *Azospirillum*) to obtain the best performance in growth and yield of different varieties of onion plants.

Considering the interaction effect, data in Table (9) show the dual interaction effect between each two factors on bulb diameter, onion yield and T.S.S. Generally, all tested parameters were significantly affected by all the possible dual interactions. The interaction between three onion varieties and two N-fertilization rates showed that Giza 6 Mohassan variety under 75% N-fertilization treatment (67.5 kg N/fed) recorded the highest values of all tested parameters, namely, bulb diameter (4.8 cm), onion yield (5.10 ton fed^{-1}) and T.S.S (10.11%). On the other hand, the interaction effect between different onion varieties and rhizobacterial inoculation treatment showed that Giza 6 Mohassan variety inoculated with *Pseudomonas* recorded the highest values of bulb diameter (5.95 cm), onion yield (6.76 ton fed^{-1}) and T.S.S (11.17%) compared to other tested treatments. While, the di-interaction effect between N-fertilization levels and rhizobacterial inoculation treatments (Table 9) revealed that *Pseudomonas* inoculation treatment fertilized with 75% N surpassed the other combined treatments, which recorded the highest values of bulb diameter (5.70 cm), onion yield (6.83 ton fed^{-1}) and T.S.S (10.77%).

In fact, the effect of rhizobacterial inoculation or mineral nitrogen fertilization was more pronounced with Giza 6 Mohassan variety compared with other tested onion varieties. However, the promotive effect of the integrated rhizobacteria with mineral nitrogen fertilization may be explained on the basis of the beneficial effect of bacteria on the nutrient availability, vital enzymes, hormonal stimulating effect on plant growth or the increasing of photosynthetic activity (Desoky and El-Sayed, 2016). In this respect, Jayathilake *et al.* (2003) reported that the effects of integrated nutrient management using biofertilizers (*Azotobacter chroococcum* and *Azospirillum brasilense*) on the growth of onion (*Allium cepa*) in terms of plant height; number of leaves per plant; weight and quality significantly increased with the application of biofertilizers in combination with mineral nitrogen fertilization.

Concerning the tri-interaction effect among three onion varieties, two chemical N-fertilization rates and five rhizobacterial inoculation treatments, data in Table (10) show that all studied yield characters (bulb diameter, onion yield and T.S.S) were significantly responded to such interaction. Meanwhile, the highest values of such yield aspects were obtained by Giza 6 Mohassan variety in combination with 75% recommended-N (67.5 kg N/fed) and *Pseudomonas* inoculation, which recorded bulb diameter (6.10 cm), onion yield (6.48 ton/fed) and T.S.S (12.21%). While, the same variety fertilized with 75% recommended-N and inoculated with *Azotobacter* came in the second rank, which recorded bulb diameter (6.10 cm), onion yield (6.16 ton/ fed^{-1}) and T.S.S (10.68%). As general, uninoculated treatments combined with any level of nitrogen fertilization in three onion varieties gave the lowest values of all tested yield characters.

These results may be attributed to the nature of root exudates of onion plants (especially Giza 6 Mohassan variety), which act as suitable substrates for the associative rhizobacteria, that release plant promoting substances, mainly indole acetic acid, gibbrellines and cytokinines as well as synthesis of siderophores and disease suppression. These promotive effects of PGPRs combined with nitrogen fertilization could stimulate plant growth, absorption of nutrients and their efficiency, as well as the metabolism of photosynthates and the crop productivity. Such mechanisms have been reported by many investigators (Jayathilake *et al.,* 2003; Tilak *et al.,* 2005; Verma *et al.,* 2010 and Rizk *et al.*, 2011).

Table 9: Average bulb diameter, onion yield and T.S.S as affected by dual interactions between three onion varieties, mineral nitrogen fertilization levels and different rhizobacterial inoculation treatments (average two growing seasons)

Treatments		**Bulb diameter (cm)**	**Yield (ton/fed)**	**TSS (%)**
		Interactions A&B		
Giza 20	**75% (N)**	4.08	4.94	9.70
	100%(N)	4.20	3.83	9.88
Giza 6 Mohassan	**75% (N)**	4.80	5.10	10.11
	100%(N)	4.73	3.92	9.93
Giza Red	**75% (N)**	4.76	5.29	10.37
	100%(N)	4.32	4.20	9.76
LSD at 0.05		**0.0093**	**0.0013**	**0.0063**
		Interactions A&C		
Giza 20	***Azotobacter***	4.60	5.00	10.15
	Azospirillum	4.45	3.86	10.00
	Pseudomonas	4.75	5.42	10.30
	Mixed inoculant	4.10	3.74	9.74
	Control	2.80	2.56	8.75
Giza 6 Mohassan	***Azotobacter***	5.50	4.92	10.60
	Azospirillum	4.97	4.76	10.39
	Pseudomonas	5.95	6.76	11.17
	Mixed inoculant	4.35	4.16	9.93
	Control	3.25	3.30	9.11
Giza Red	***Azotobacter***	5.35	5.60	10.12
	Azospirillum	4.85	5.20	10.02
	Pseudomonas	5.75	5.70	10.20
	Mixed inoculant	3.70	3.98	9.80
	Control	2.85	3.38	9.11
LSD at 0.05		**0.0122**	**0.0015**	**0.0079**
		Interactions B&C		
75% (N)	***Azotobacter***	5.40	5.87	10.30
	Azospirillum	5.07	5.23	10.02
	Pseudomonas	5.70	6.83	10.77
	Mixed inoculant	4.17	4.28	9.89
	Control	2.40	3.35	8.94
100% (N)	***Azotobacter***	4.90	4.48	10.31
	Azospirillum	4.44	3.99	10.13
	Pseudomonas	5.26	5.09	10.42
	Mixed inoculant	3.93	3.64	9.76
	Control	3.53	2.81	9.04
LSD at 0.05		**0.0099**	**0.0015**	**0.0065**

Table 10: Average bulb diameter, onion yield and T.S.S as affected by tri-interaction effect among three onion varieties, two levels of N-fertilization and different rhizobacterial inoculation treatments (average two growing seasons)

Treatments			Bulb diameter (cm)	Yield (ton/fed)	TSS (%)
Interactions A&B&C					
Giza 20	75% (N)	*Azotobacter*	4.70	5.52	10.08
		Azospirillum	4.60	4.24	9.90
		Pseudomonas	4.90	5.73	10.21
		Mixed inoculant	4.2	4.04	9.70
		Control	2.00	3.04	8.60
	100% (N)	*Azotobacter*	4.50	4.48	10.21
		Azospirillum	4.30	3.48	10.11
		Pseudomonas	4.60	5.68	10.38
		Mixed inoculant	4.00	3.44	9.79
		Control	3.60	2.08	8.90
Giza 6 Mohassan	75% (N)	*Azotobacter*	6.10	6.16	10.68
		Azospirillum	5.10	5.68	10.12
		Pseudomonas	6.10	6.48	12.21
		Mixed inoculant	4.50	4.56	9.97
		Control	2.60	3.36	9.00
	100% (N)	*Azotobacter*	5.30	4.08	10.44
		Azospirillum	4.83	3.84	10.09
		Pseudomonas	5.40	4.68	10.76
		Mixed inoculant	4.20	3.70	9.88
		Control	3.90	3.24	9.22
Giza Red	75% (N)	*Azotobacter*	3.80	6.32	10.15
		Azospirillum	5.50	5.76	10.03
		Pseudomonas	5.80	5.76	10.28
		Mixed inoculant	3.60	4.24	10.00
		Control	2.60	3.64	9.21
	100% (N)	*Azotobacter*	4.90	4.88	10.09
		Azospirillum	4.20	4.64	10.00
		Pseudomonas	5.80	4.92	10.11
		Mixed inoculant	3.60	3.72	9.60
		Control	3.10	3.12	9.00
LSD at 0.05			**0.0173**	**0.0018**	**0.0113**

5. Chemical composition

Data in Table (11) shows the main effect of three different onion varieties, two rates of N-fertilization (75% and 100% of the recommended-N dose) and different rhizobacterial inoculation treatments on the N, P and K contents of onion bulb. Generally, results behaved in a similar manner as in onion yield parameters and confirmed that Giza 6 Mohassan variety gave the highest values of N (1.50%), P (0.391%) and K (0.800%) in onion bulbs compared to other varieties. Additionally, application of 75% recommended-N (67.5 kg N/fed) recorded the highest values of N (1.59 %), P (0.388 %) and K (0.792%). The results of rhizobacterial inoculation treatments (Table 11) confirmed again the superiority of *Pseudomonas* inoculation treatment as they gave the highest values of bulb N content (1.77%), P content (0.418%) and K content (0.868%) followed by *Azotobacter* treatment with values of N (1.74%), P (0.394%) and K (0.812%). While, uninoculated treatment gave the lowest values during the combined two growing seasons.

In fact, this improvement in the N, P and K percentages in onion bulb could be attributed to the ability of studied rhizobacteria to fix nitrogen in association or endophytic manners, P mobilization and/or certain growth promoting substances, which positively affect root development and consequently their function in the uptake of both water and nutrients. Also, application of N fertilizer had a positive effect on root growth and the absorption sites which enhance absorption of nutrients. Such results are in conformity with those of (Haque and Dave, 2005; Verma *et al.,* 2010 & Desoky and El-Sayed, 2016). In this concern, Galal (2016) reported that the highest N% in onion bulbs was recorded in treatment inoculated with *Azospirillum* followed by *Azotobacter*, while the highest P% was recorded in treatment inoculated with *Pseudomonas*.

Table 11: The main effect of three different onion varieties, two rates of N-fertilization and different rhizobacterial inoculation treatments on the N, P and K contents of onion bulb (average two growing seasons)

Treatments		N%	P%	K%
Onion varieties	Giza 20	1.46	0.381	0.769
	Giza 6 Mohassan	1.50	0.391	0.800
	Giza Red)	1.50	0.390	0. 794
LSD at 0.05		0.0032	0.0029	0.0031
N- fertilization	75% (N)	1.59	0.388	0.792
	100%(N)	1.40	0.386	0.783
LSD at 0.05		0.0021	0.0017	0.0087
Rhizobacterial inoculation	*Azotobacter*	1.74	0.394	0.812
	Azospirillum	1. 59	0.394	0.803
	Pseudomonas	1.77	0.418	0.868
	mixed inoculant	1.43	0.384	0.783
	Control	1.23	0.345	0.670
LSD at 0.05		0.0035	0.0022	0.0055

Control = Uninoculated treatment.
75 and 100% N = 67.5 and 90 kg N fed^{-1}, respectively.

The results in Table (12) showed that all the double interaction of the studied three factors significantly affected the contents of N, P and K of onion bulb and took the same trend as in onion yield parameters. Whereas, the di-interaction effect between three onion varieties (Giza 20, Giza 6 Mohassan and Giza Red) and two N-fertilization rates (75 and 100% recommended-N) showed that Giza 6 Mohassan variety under 75% N recorded the highest values of N (1.608 %), P (0.395 %) and K (0.825 %) contents of onion bulb in the average two growing seasons. Considering the interaction effect between three onion varieties and rhizobacterial inoculation treatments showed significant effect on N, P and K contents of onion bulb (Table 12). Data showed that Giza 6 Mohassan variety inoculated with *Pseudomonas* recorded the highest values of N (1.810%), P (0.422%) and K (0.897%) contents of onion bulb. Meanwhile, the uninoculated treatment (control) recorded the lowest values during the combined two growing seasons. The di-interaction effect between N-fertilization levels and rhizobacterial inoculation treatments on N, P and K contents of onion bulb are shown in Table (12). As mentioned before in onion yield parameters, *Pseudomonas* inoculation treatment fertilized with 75% N surpassed the other tested combination treatments, which recorded the highest values of N (1.927%), P (0.419%) and K (0.880%) contents of onion bulb.

The synergistic effect of such combination between mineral nitrogen fertilizers and rhizobacterial inoculation on enhancing the percentages of N, P and K of onion bulb may be related to improve root development via stimulating the proliferation of lateral root hairs, which resulted in better nutrient uptake capability and increase the supply of N, P and K. These results are in harmony with those obtained by (Jayathilake *et al.,* 2003 & Desoky and El-Sayed, 2016).

Data are presented in Table (13) show the tri-interaction effect among three onion varieties, two levels of nitrogen fertilization and five treatments of rhizobacterial inoculation on the percentages of N, P and K of onion bulbs. As mentioned before in studied onion yield characters, the highest values were obtained by Giza 6 Mohassan variety in combination with 75% recommended-N (67.5 kg N/fed) and *Pseudomonas* inoculation, which recorded N (1.950 %), P (0.429%) and K (0.947%) followed by the same variety amended with 75% N and inoculated with *Azotobacter*, which recorded N (1.940 %), P (0.422 %) and K (0.877%). While, uninoculated treatments combined with any level of nitrogen fertilization in three onion varieties gave the lowest values of N, P and K contents of onion bulb in the average two growing seasons.

The improvement in nutrient percentages in onion bulbs due to rhizobacterial inoculation may be elucidated by their ability to enhance the N_2-fixation performance, solubilization of minerals, synthesis of siderophores (ferric-specific ligands), synthesis of plant hormones (such as auxins or gibberellins) and disease suppression (Tilak *et al.,* 2005; Verma *et al.,* 2010 and Pérez-Montaño *et al.*, 2014).

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Additionally, the combined effect between rhizobacteria and nitrogen fertilization could enhance onion growth, absorption of nutrients and their efficiency; consequently, increase the percentages of N, P and K in plants. These results are in line with those obtained by Jayathilake *et al. (*2003), Haque and Dave (2005) & Desoky and El-Sayed, (2016).

From these results, there is considerable evidence that the plant growth promoting rhizobacteria (PGPRs) may be acting as a good practice for improving the quality and productivity of onion, as well as to provide plants with natural bio-protection against phytopathogens and reduce the reliance on agrochemicals, leading to healthier food under sustainable agricultural systems. However, these trials are in need to be repeated under different soil conditions to reach the level of recommendation.

Table 12: The di-interaction effect between three onion varieties, two rates of N-fertilization and different rhizobacterial inoculation treatments on the N, P and K contents of onion bulb (average two growing seasons)

Treatments		**N %**	**P %**	**K %**
		Interactions A&B		
Giza 20	**75% (N)**	1.563	0.380	0.766
	100%(N)	1.350	0.382	0.771
Giza 6 Mohassan	**75% (N)**	1.608	0.395	0.825
	100%(N)	1.452	0.387	0.775
Giza Red	**75% (N)**	1.604	0.389	0.785
	100%(N)	1.402	0.390	0.803
LSD at 0.05		0.0031	0.0022	0.0124
		Interactions A&C		
Giza 20	***Azotobacter***	1.525	0.392	0.802
	Azospirillum	1.427	0.385	0.787
	Pseudomonas	1.715	0.412	0.842
	Mixed inoculant	1.375	0.371	0.757
	Control	1.240	0.344	0.657
Giza 6 Mohassan	***Azotobacter***	1.630	0.390	0.807
	Azospirillum	1.525	0.403	0.817
	Pseudomonas	1.810	0.422	0.897
	Mixed inoculant	1.475	0.394	0.817
	Control	1.210	0.345	0.662
Giza Red	***Azotobacter***	1.605	0.400	0.827
	Azospirillum	1.470	0.395	0.807
	Pseudomonas	1.775	0.420	0.867
	Mixed inoculant	1.430	0.387	0.777
	Control	1.235	0.346	0.692
LSD at 0.05		0.0040	0.0024	0.0079
		Interactions B&C		
75% (N)	***Azotobacter***	1.670	0.393	0.803
	Azospirillum	1.561	0.392	0.806
	Pseudomonas	1.927	0.419	0.880
	Mixed inoculant	1.547	0.391	0.796
	Control	1.253	0.345	0.674
100% (N)	***Azotobacter***	1.503	0.395	0.820
	Azospirillum	1.387	0.397	0.801
	Pseudomonas	1.607	0.417	0.857
	Mixed inoculant	1.307	0.377	0.771
	Control	1.203	0.345	0.666
LSD at 0.05		0.0040	0.0024	0.0065

Table 13: The tri-interaction effect among three onion varieties, two rates of N-fertilization and different rhizobacterial inoculation treatments on the N, P and K contents of onion bulb (average two growing seasons)

Treatments			N%	P%	K%
		Interactions A&B&C			
Giza 20	**75% (N)**	***Azotobacter***	1.620	0.389	0.797
		Azospirillum	1.523	0.382	0.786
		Pseudomonas	1.890	0.409	0.837
		mixed inoculant	1.500	0.379	0.756
		Control	1.280	0.342	0.660
	100% (N)	***Azotobacter***	1.430	0.395	0.807
		Azospirillum	1.330	0.389	0.790
		Pseudomonas	1.540	0.415	0.847
		Mixed inoculant	1.250	0.363	0.760
		Control	1.200	0.346	0.653
Giza 6 Mohassan	**75% (N)**	***Azotobacter***	1.940	0.422	0.887
		Azospirillum	1.550	0.392	0.797
		Pseudomonas	1.950	0.429	0.947
		Mixed inoculant	1.550	0.386	0.767
		Control	1.250	0.349	0.687
	100% (N)	***Azotobacter***	1.490	0.401	0.837
		Azospirillum	1.390	0.397	0.847
		Pseudomonas	1.610	0.418	0.857
		Mixed inoculant	1.310	0.388	0.787
		Control	1.210	0.343	0.697
Giza Red	**75% (N)**	***Azotobacter***	1.670	0.391	0.797
		Azospirillum	1.610	0.401	0.837
		Pseudomonas	1.720	0.339	0.817
		Mixed inoculant	1.590	0.408	0.867
		Control	1.22	0.345	0.677
	100% (N)	***Azotobacter***	1.59	0.390	0.817
		Azospirillum	1.440	0.405	0.797
		Pseudomonas	1.670	0.415	0.847
		Mixed inoculant	1.360	0.381	0.767
		Control	1.200	0.346	0.647
LSD at 0.05			0.0068	0.0050	0.011

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