Archives of Microbiology (2021) 203:169–181
https://doi.org/10.1007/s00203-020-01991-z

ORIGINAL PAPER

Maximizing growth and productivity of onion (*Allium cepa* L.) by *Spirulina platensis* extract and nitrogen-fixing endophyte *Pseudomonas stutzeri*

L. S. M. Geries[1] · Abdelgawad Y. Elsadany[2]

Received: 4 February 2020 / Revised: 13 July 2020 / Accepted: 20 July 2020 / Published online: 13 August 2020
© Springer-Verlag GmbH Germany, part of Springer Nature 2020

Abstract

The study focuses on the impact of foliar spraying cyanobacterium *Spirulina platensis* extract and the inoculation with the endophyte N_2-fixing *Pseudomonas stutzeri*, and their mixture in the presence of different nitrogen doses on growth and yield of onion under field conditions. Bioactive compounds of *Spirulina* and *Pseudomonas* were analyzed by GC–MC and amino acid production of *Spirulina* by the amino acid analyzer. Hydrogen cyanide (HCN), indole acetic acid (IAA), ammonia (NH_3), pectinase activity, and N_2-fixation of *Pseudomonas* were measured. Plant height (cm), leaf length (cm), number of green leaves, bulb diameter (cm), fresh and dry weight of plant (g), chlorophyll a, b of leaves, bulb weight (g), marketable bulb yield (t. ha^{-1}), cull bulb weight (t. ha^{-1}), total bulb yield (t. ha^{-1}), bulb diameter (cm), total soluble solids (TSS%), dry matter content (DM%), evaluation of storage behavior, and economic feasibility were estimated. *Spirulina* extract has several bioactive compounds. *Pseudomonas* can produce HCN, NH_3, IAA, pectinase, and nitrogen fixation. The application of mixture with recommended dose of nitrogen increases the onion plant parameters, marketable yield, total bulb yield, bulb weight, bulb diameter, TSS%, DM%, net return, benefit–cost ratio (B:C), lowest cumulative weight loss% of bulbs during storage, and reduce culls weight compared with other treatments in two seasons. Application of *S. platensis* extract and inoculation with endophyte nitrogen-fixing *P. stutzeri* enhance the growth and productivity of the onion under different doses of nitrogen fertilizer.

Keywords *Pseudomonas stutzeri* · *Spirulina platensis* extract · Growth · Productivity · *Allium cepa*

Introduction

The onion is found under the Amaryllidaceae family, the largest commercial crop in the world and used for food and medicine since ancient times (Hossain et al. 2017; Khan et al. 2017; Marrelli et al. 2019; Yang et al. 2019). China, India, USA, Turkey, Japan, Spain, Brazil, Poland, and Egypt are the largest countries of the world, producing onion (FAO 2018). Egypt's production of onion reached 2.4 million tons (FAO 2017). Application of PGPR reduces the excessive use of mineral fertilizers, stimulates growth and production of plant and reduces costs and pollution (Singh et al. 2017a, b). Plant growth-promoting rhizobacteria (PGPR) inhabited the area around the plant roots or in plant tissues, enhanced the growth and productivity of the plant, improved carbon and nitrogen of roots, and reduced N_2O release from soil (Phale 2018; Yousef 2018; Florio et al. 2019). Using PGPR saves 30% of mineral nitrogen fertilizer and enhances the economics of onion production (Yaso et al. 2007). PGPR increases the height of the plant, the number of leaves, and the dry weight of the onion bulbs (Fernandes et al. 2018). The microbial inoculation increases foliage height, the number of leaves, dry weights, bulb weights, total bulb yield, total soluble solids %, dry matter %, and NPK contents in bulb tissues (Afify et al. 2018). Biofertilizers of onion increased germination %, bulb weight, a maximum diameter of bulb, bulb dry weight, plant dry weight, harvest index, plant height, no. of leaves, chlorophyll a, chlorophyll b,

Communicated by Erko Stackebrandt.

✉ Abdelgawad Y. Elsadany
cyanogawad@gmail.com

[1] Onion Res. Dept., Field Crops Research Institute, Agric. Res. Center, Giza, Egypt

[2] Cyanobacteria Lab., Microbiology Dept., Sakha Agricultural Research Station, Soils, Water and Environment Research Institute, Agric. Res. Center, Giza, Egypt

carotenoid, leaf protein, bulb protein, and bulb sulphur (Kurrey et al. 2018). Endophytic bacteria enter the root interior and benefit the plant (Compant et al. 2005), are nonpathogenic to the plant (Santoyo et al. 2016), help in nutrient acquisition (Kandel et al. 2017), and enhance the growth and productivity of plant growth, such as increase surface area, nitrogen fixation, protection against biotic pathogens, abiotic stresses, and detoxification of harmful compounds (Adesemoye et al. 2009; Hayat et al. 2010; Lin and Xu 2013). Endophytic bacteria can secrete IAA, dissolving phosphates and help in N_2-fixation; this leads to increase germination %, root length, and the number of onion roots (Sutariati et al. 2019), ammonia production and release of degrading enzymes (Hassan 2017). Endophytic bacteria penetrate the plant through root hairs and spaces between epidermal cells and release the pectinase and cellulose to enter the plant (Rediers et al. 2003; Singh et al. 2017a, b). IAA is an essential hormone that helps regulate the growth of different processes in plants like cell division, length, and light response. IAA-producing bacteria increase growth and yield of onion (Reetha et al. 2014) and increase fresh tomato root weight (Dhungana and Itoh 2019). Endophyte *Pseudomonas stutzeri* contributes about 0.30–0.82 g N, improves the growth, and nitrogen content of the plant (Ke et al. 2019). Gallic acid enhances plant seedlings, germination, shoot and root size, decreases the leaf hydrogen peroxide content, increases residences for protection, improves plant growth stimulants, optimizes their use under field conditions (Singh et al. 2017a, b), and increases the respiration rates in soil (Davies et al. 2017). Foliar spraying of *Spirulina* extract gave a positive effect of carotene, chlorophyll a, b (Yassen et al. 2018), the plant height, plant biomass and elements of onion (Godlewska et al. 2019). The phytol is used to generate chlorophyll, tocopherol, phylloquinone and fatty acid phytyl ester (Gutbrod et al. 2019). *Spirulina* microalga is rich in organic nitrogenous components like amino acids. Amino acids have a vital role in plant such as toxin and heavy metal detoxification (Rizwan et al. 2017; Bashir et al. 2018; Hussain et al. 2018), chlorophyll synthesis (Amin et al. 2011), optimizing the nutrient uptake, translocation and metabolism, vitamin biosynthesis, production of dry matter of plant (Khalilzadeh et al. 2012), maintaining the protein structure required for cell division, helping cells divide, enlarge, differentiation, and growth-efficient polyamines (Kakkar et al. 2000), growth biostimulation and alleviation of stress conditions (Souri and Hatamian 2019). Amino acids increase the protein, N, Cu and Mn in the garlic plant tissues (Fawzy et al. 2012). Amino acids are the only source of nitrogen, which the plant can utilize more quickly than mineral nitrogen. However, external amino acids reduce the flow of ammonium and the transcription of the root tissue (Mohamed and Mohamed 2012) and enhance the dry weight of the onion plant (Shafeek and Helmy 2012). Amino acids increase the concentrations of gibberellic acid, indole acetic acid and increase NPK uptake in the plant (Talaat et al. 2005; Hua-Jing et al. 2007). Methionine enters the synthesis of growth regulator substances, e.g., cytokinins, auxins of plant, and increases NPK content and dry weight of plant shoots (Chen et al. 2005; El-Awadi et al. 2011). Tryptophan and phenylalanine enhance the surface area of the leaves of plants (Dahab and El-Aziz 2006). Cysteine has an important role in cytosol and mitochondria of plant cells and improves of hairy roots (Romero et al. 2014). Foliar spray of glycine or glutamine stimulates plant growth (Noroozlo et al. 2019). This study demonstrates the role of PGPR in improving the growth and productivity of onion to reduce nitrogen fertilizer contamination, reduce production costs and increase competitiveness in exporting onion.

Materials and methods

Microbial activities

Pseudomonas inoculum

A subculture of the *Pseudomonas stutzeri* was obtained from Microbiology Dept., Sakha Agricultural Research Station, Egypt, cultivated in the Luria broth (LB) medium for 72 h. at 30 °C under shaking and onion seedlings were inoculated by soaking the roots in the culture of *pseudomonas* (10^8 CFU ml^{-1}) and no inoculated seedlings were soaking in a medium for about 30 min. (Yaso et al. 2007).

N_2-fixation ability

The Burk salt medium was used to evaluate the ability of *pseudomonas stutzeri* to fix nitrogen qualitatively. The composition of medium per liter: (0.2 g $MgSO_4$, 0.8 g K_2HPO_4, 0.2 g KH_2PO_4, 0.13 g $CaSO_4$, 0.00145 g $FeCl_3$, 0.000253 g Na_2MoO_4, and 20 g sucrose. Sterilize by autoclaving at 15 lbs pressure (121 °C) for 15 min. Medium turbidity is evidence of nitrogen fixation (Subba Rao 1977).

Assessment of HCN potential

The capacity of *Pseudomonas stutzeri* to generate HCN as a qualitative assay test was performed (Lorck 1948).

Indole acetic acid (IAA) production

The medium Luria broth (LB) containing 1.0 (g/L) L-tryptophan was used to test the indole acetic acid (IAA) production by *pseudomonas*. 1.0 ml of 24 h old culture was used to inoculate 10 ml of Luria broth and incubated for 72 h at 30 °C. The culture was centrifuged for 10 min at 10,000

× *g*. 1.0 ml of supernatant was mixed at 30 °C for 30 min with 2 ml of Salkowski reagent. The pink color indicates the release of indole acetic acid and yellow indicate negative. The absorbance was measured at 530 nm using a UV–VIS spectrophotometer (UV-1700, Shimadzu, Kyoto, Japan) and the gradual concentrations of IAA were used to a standard curve (Patten and Glick 1996).

NH_3 production

The ammonia production of *Pseudomonas stutzeri* was conducted by peptone water (10 g peptone and 5 g NaCl). Three test tubes contain 10 ml. Peptone water inoculated with *Pseudomonas* and incubated at 30 °C for 48–72 h. After that, the culture was centrifuged at and 0.5 ml Nessler reagent was added to the supernatant. The formation of yellow to brownish color indicates ammonia production (Dey et al. 2004).

Pectinolytic activity

For the pectinase assay, the *Pseudomonas* was spotted on the nutrient agar (NA) medium supplemented with 0.5% pectin according to Ma et al. (2011). Positive enzyme activity was shown by a clearing zone. The Enzymatic Index (EI) represented the relationship between the average halo degradation halo diameter and the average colony growth diameter (Jussara et al. 2006).

Spirulina platensis cultivation

A subculture of the cyanobacterium *Spirulina platensis* was obtained from The Algae Biotechnology Unit of The National Research Center, Giza, Egypt (www.nrc.sci.eg), grown in the Zarrouk medium (Zarrouk 1966). 100 ml of the medium was autoclaved for 20 min at 121 °C. Each flask was inoculated with 10 ml culture containing 10^7–10^8 colony-forming units/ml, incubated for 7 days and then used as an inoculum for 1 L of the medium, incubated for 7 days and transported to 20-L white polyethylene container, incubated for 7 days and then inoculated in a glass basin of 120 × 60 × 60 cm containing the 100 L medium, incubated for 25 days. The cultures were grown under controlled laboratory conditions of 30 ± 2 °C and continuous illumination from 5500–6500 lx. The container received sterile air using a pump to contentiously mix/circulate/distribute the culture all over the incubation course.

Preparation of *S. platensis* extract

The cyanobacterium *S. platensis* material (1000 g) was extracted using a blender and then mortar pestle. *S. platensis* was filtered through a cotton cloth to remove debris and designated. The resulting filtrate is supplemented to a liter with distilled water, and this represents 100 % (SWC). SWC is kept in refrigerator between 0 and 4 °C until use (Pise and Sabale 2010).

GC–MS analysis

Spirulina cells were harvested by centrifugation at 10,000 rpm for 3 min and then filtered through filter paper (Whatman no. 4) and air-dried (Starr et al. 1962). For extraction, the dried cells were extracted by dissolving in methanol (1 g/10 ml) and kept overnight for complete extraction then centrifuged at 10,000 rpm for 10 min, and the supernatant was separated by filter of 0.5 µm pore size, the dry residue was re-dissolved in dimethyl sulfoxide (DMSO), and kept in fresh glass vials stored in the dark at 4 °C until use for phytochemical screening by GC–mass spectroscopy (Lefort-Tran et al. 1988).

The chemical composition of samples was identified using Trace GC-TSQ Quantum mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG-5MS (30 m × 0.25 mm × 0.25 µm film thickness). The column oven temperature was initially held at 50 °C and then increased by 5 °C/min to 200 °C, held for 2 min, increased to the final temperature of 290 °C by 30 °C /min and held for 2 min. The injector and MS transfer line temperatures were kept at 270 and 260 °C, respectively; Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 3 min and diluted samples of 1 µl were injected automatically using AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–500 in full-scan mode. The ion source temperature was set at 200 °C. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database. The chemical composition of *S. platensis* and *P. stutzeri* is illustrated in Table 1.

Amino acid analysis

The amino acids of *Spirulina* extract were measured by the high-performance amino acid analyzer (Biochrom 30) according to (AOAC 2012).

Field experiment

Two field experiments were carried out during the winter 2015/2016 and 2016/2017 at the Experimental Farm of Sakha Agricultural Research Station, Egypt. The physicochemical properties of soil were determined according to Jackson (1973). The soil of the experimental field was clay texture with pH 7.9, EC 1.75 ds/m, organic matter 1.81%, available N 27.15, available P 16.90, and available K 280 mg

Table 1 Chemical composition of *Spirulina* and *Pseudomonas stutzeri*

Compound	RT (min.)	% Area
Chemical composition of Spirulina		
Docosane	16.63	0.66
Hexadecanoic acid, methyl ester	21.49	29.26
7,10-Hexadecadienoic acid, methyl ester	21.68	8.29
Phytol	23.94	4.28
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	24.36	34.40
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	24.75	2.89
1-Nonadecene	15.25	1.59
psi.,.psi.-Carotene, 3,4-didehydro-1,2-dihydro-1-methoxy-	4.79	0.58
19-Norethindrone, O-methyloxime	7.29	1.23
Glafenin	9.92	0.92
Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-,17-[O (phenylmethyl) oxime], (3à,5à)-	10.30	0.82
Trimethylsilyl 3-methoxy-2-(2-oxo-2-((trimethylsil yl)oxy)ethoxy)benzoate	12.37	0.90
Silicone oil	15.14	0.55
Toosendanin	23.12	1.32
Propanoic acid	23.63	0.38
Linoleic acid ethyl ester	24.86	0.40
6,9-octadecadienoic acid, methyl ester	25.97	1.80
Promecarb 2,4-dinitrophenylether	30.74	1.23
Lycoxanthin	39.41	0.43
Chemical composition of *Pseudomonas stutzeri*		
Formic acid	3.23	56.78
3,5-Dimethylbenzyl3,5-dimethylbenzoate	3.92	1.24
Cumaldehyde	4.28	4.75
3,5-di-t-Butylcatechol	5.01	1.58
2-Propylphenol	6.15	0.92
Thiourea,1-[2-(benzylphenoxy)ethyl]-3-(O-tolyl)	6.47	1.19
Chromone,5-hydroxy-6,7,8-timethoxy-2,3-dimethyl	6.99	1.61
7-Hydroxy-3-methoxy-2-phenyl-4H-chromen-4-one	8.31	1.54
Rapamycin	16.22	4.12
Gentisic acid	17.88	15.96
3,4′,5,6′-tetra-tert-butylbiphenyl-2,3′-diol	18.70	3.18
Betulin	19.27	2.09
Gallic acid, propyl ester	20.93	5.03

kg^{-1}. Giza red onion seeds cultivar were hand drilled in the nursery bed on 8th and 9th October in both seasons, respectively. After 60 days, the seedling was transplanted to the field. Calcium superphosphate (15.5% P_2O_5) and potassium sulphate (48% K_2O) were applied at the rate of 107.1 kg P_2O_5/ha and 119 kg K_2O/ha, respectively before transplanting. Nitrogen fertilizer (285.6 kg N/ha) in the form of ammonium nitrate (33.5% N; 852.5 kg/ha) was added after transplanting in two equal doses, first dose 426.25 kg at 30 days after transplanting (DAT) and the second dose at 60 DAT. The plot area was 10.5 m^2 and the treatments were arranged in a randomized complete block design (RCBD), with three replications. The treatments as follows:

T_1—Recommended dose of N.
T_2—75% of the recommended dose of N.
T_3—50% of the recommended dose of N.
T_4—*Spirulina* extract + recommended dose of N.
T_5—*Spirulina* extract + 75% of the recommended dose of N.
T_6—*Spirulina* extract + 50% of the recommended dose of N.
T_7—*Pseudomonas* + recommended dose of N.
T_8—*Pseudomonas* + 75% of the recommended dose of N.
T_9—*Pseudomonas* + 50% of the recommended dose of N.
T_{10}—Mixture (*Spirulina* extract + *Pseudomonas*) + recommended dose of N.

T_{11}—Mixture (*Spirulina* extract + *Pseudomonas*) + 75% of the recommended dose of N.
T_{12}—Mixture (*Spirulina* extract + *Pseudomonas*) + 50% of the recommended dose of N.

The harvesting process was carried out on 15th and 12th May in the first and second growing seasons, respectively.

Growth parameters

At 100 and 120 days after transplanting (DAT), ten onion plants were taken randomly from each experimental plot, for investigating plant growth parameters like plant height (cm), leaf length (cm), the number of green leaves/plant, the diameter of the bulb (cm), fresh and dry weight of plant (g). Chlorophyll a, b of plant leaves were estimated according to Moran (1982). Readings were used to calculate chlorophyll a, b µg/ml based on the following equations:

Chl. a = 12.46 (A664) − 2.49 (A647) µg/ml.
Chl. b = − 5.6 (A664) + 23.26 (A647) µg/ml.

Onion bulb yield and quality

At harvesting time (~ 155 DAT), all the remaining bulbs in each plot were uprooted and bulb yield of onion expressed as average bulb weight (g), marketable bulb yield (t. ha^{-1}), culls bulb weight (t. ha^{-1}) and total bulb yield (t. ha^{-1}). At the same time, the sample of five bulbs was randomly taken to record the bulb quality properties, i.e. bulb diameter (cm), total soluble solids (TSS%) and dry matter content (DM%).

Storage losses

To evaluate tuber shelf life, ~ each of 10 kg was randomly taken from each plot. The samples were cured for 10 days after harvest to heal wounds. After curing, bulbs were stored for 180 days. The total weight loss was determined by periodical weighing of onion bulbs at 60, 120 and 180 days after storage during the 2015/2016 and 2016/2017 seasons. The differential weight losses were calculated for each interval and converted into a percentage by dividing the change with the initial weight recorded at each sampling interval. The cumulative weight loss was expressed in percent for different treatments. The difference between the initial weight and successive weights gave the rate of total weight losses (as percent) as described by Abubakar et al. (2019).

Weight loss (%) = $W_0 - W_1$ or W_2 or $W_3/W_0 \times 100$,

where W_0 initial weight, W_1 weight after 60 days, W_2 weight after 120 days, and W_3weight after 180 days.

The clean and healthy remaining marketable bulbs after 180 days were weighted after rotting and sprouting bulbs were discarded and the percentage was calculated.

Economic feasibility

Economic analysis was performed to calculate net return and the benefit cost ratio with respect to each treatment. Economic feasibility was conducted using the formulas described by Cimmyt (1988).

Cost of cultivation (L.E./ha): cost of cultivation from each treatment was calculated in Egyptian pounds (L.E.). Data on cost of inputs, which calculated as a rental cost, land preparation, seedling and planting, irrigation, fertilizers, weeding, harvesting, transportation, and other expenses.

Gross return (L.E./ha): it was estimated from the sale of harvested onion in Egyptian pounds. One ton of marketable bulbs yield = 2000 L.E. in both seasons, the average prices were taken from surveys market prices of onion.

Net return (L.E./ ha): net return was estimated as the difference between total revenue from the sale of harvested onion and total costs (fixed and variable cost of onion).

Benefit cost ratio: It was calculated by the formula, B:C ratio = Gross return/Cost of cultivation.

Statistical analysis

All data collected were subjected to statistical analysis as described by Snedecor and Cochran (1980) at 5% signiicance level. Treatments means were compared according to Duncan's multiple range test (Duncan 1955).

Results and discussion

Pseudomonas inoculum

The roots of onion seedlings were soaked in the *Pseudomonas* culture for two reasons: first, to avoid hydrogen cyanide and this is consistent with Stamenov et al. (2018) application of *Pseudomonas* sp. harm effect on onion seed germination due to its ability to produce hydrogen cyanide; secondly, *Pseudomonas* attaches to the root surface (rhizoplane) and explore the potential entry sites to access the internal plant tissues, similar findings were reported by Kandel et al. (2017).

Indole acetic acid (IAA) production

Pseudomonas can produce 9.38 µg ml^{-1} IAA. *Pseudomonas* improve the production of the plant hormones, cell division, and light response of plants (Reetha et al. 2014; Hu et al. 2017).

N_2-fixation ability

The ability of endophyte N_2-fixing *Pseudomonas* was indicated by turbidity on the Burk salt medium. However, recent research suggests that the inoculation of *Pseudomonas stutzeri* contributes about 0.30–0.82 g N/plant and improves the growth and nitrogen content of the plant (Ke et al. 2019).

Ammonia production

Pseudomonas can produce ammonia in peptone water using Nessler's reagent. The development of brown-yellow color was a positive test for ammonia production. Degradation and decarboxylation of amino acids and nitrite ammonification produce ammonia, and the production of ammonia by PGPR, particularly endophytic bacteria, influences the development of plant indirectly (Zaghloul et al. 2016; Hassan 2017).

Qualitative of *Pseudomonas* for pectinase production

The clear zone of *Pseudomonas* ranged from 4 to 7 mm. and this is an evidence of its ability to produce the pectinase. *Pseudomonas* penetrates the plant through root hairs and spaces between epidermal cells and releases the pectinase to enter the plant (Singh et al. 2017a, b).

Amino acid production by *Spirulina*

Figure 1 shows that *Spirulina* extract has eighteen amino acids, which differ in the percentages. Amino acids play a vital role in the plant where they detoxify heavy metals and toxins, chlorophyll and vitamin synthesis, and optimizing the nutrient uptake (Amin et al. 2011; Khalilzadeh et al. 2012; Rizwan et al. 2017; Bashir et al. 2018; Hussain et al. 2018). The worldwide demand for all essential amino acids must satisfy global demand and certain plants have a low level of essential amino acids such as lysine. Lysine

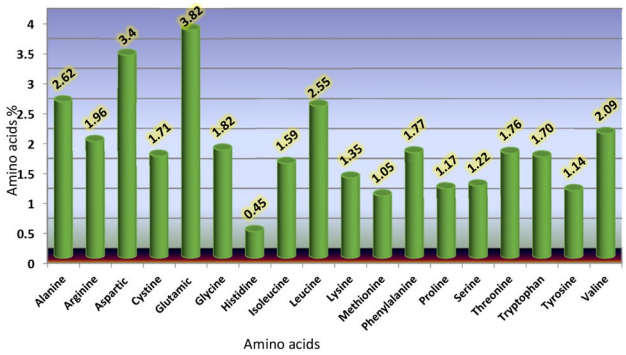

Fig. 1 Amino acid production by *Spirulina platensis*

requirement was determined to be 30 mg kg^{-1} (body-weight) d^{-1} (Kumar et al. 2019; Leinonen et al. 2019). *Spirulina* extract contains 1.35% lysine of the total amino acids, which may provide part of these requirements.

GC–mass spectroscopy analyses

The results of GC–MS in (Table 1) indicated that *Pseudomonas* and *Spirulina* contained numerous bioactive compounds belonging to various classes, e.g., phenolic, antioxidants, alkaloids, flavonoids, steroids, etc. Gallic acid is a type of phenolic acid, which enhances plant growth, decreases the leaf hydrogen peroxide content and increases residences for protection (Singh et al. 2017a, b). Phytol is used to generate chlorophyll, tocopherol, phylloquinone, and fatty acid phytyl ester (Gutbrod et al. 2019). The function of antioxidants is to protect the plant when it is subjected to drought, heavy metals, salinity, extreme temperatures, air pollutants, pesticides, and pathogen infection (Xie et al. 2019).

Growth parameters

The results in (Tables 2 and 3) show the mixture with recommended nitrogen dose gave high values of plant height, leaf length, leaf area/plant, number of leaves per plant in both seasons. This is due to the ability of *Pseudomonas* to fix N_2, secrete IAA and regulate the cell division, leaf length, and light response. Inoculation of plants with IAA-producing bacteria increase growth and yield of onion (Reetha et al. 2014; Afify et al. 2018; Fernandes et al. 2018; Sutariati et al. 2019). Also, amino acids of *Spirulina* extract such as methionine, glycine, tryptophan, and phenylalanine increase the growth substances and enhance the height, number of leaves, and leaf area/plant (Chen et al. 2005; Talaat et al. 2005; Dahab and El-Aziz 2006; Hua-Jing et al. 2007; El-Awadi et al. 2011; Yassen et al. 2018; Noroozlo et al. 2019).

It is clear from the data represented in Tables 4 and 5 that the mixture with recommended dose of N (T_{10}) enhances plant fresh, dry weight (g) and chlorophyll a, b compared with other treatments. This is a result of the foliar spray of *Spirulina* extract, which enhances chlorophyll a and b (Yassen et al. 2018), increases plant biomass (Godlewska et al. 2019), produces the phytol and amino acids responsible for synthesis of chlorophyll and increases the dry weight of plant (Amin et al. 2011; Khalilzadeh et al. 2012; Gutbrod et al. 2019) Additionally, *Pseudomonas* increases fresh and dry weights of plant (Kurrey et al. 2018; Fernandes et al. 2018; Dhungana and Itoh 2019).

Table 2 Effect of *Spirulina* extract and *Pseudomonas* on plant height and leaf length (cm) under different doses of nitrogen at 100 and 120 DAT in 2015/2016 and 2016/2017 seasons

Treatments	Plant height (cm)				Leaf length (cm)			
	2016/2017		2015/2016		2016/2017		2015/2016	
	120 DAT	100 DAT	120 DAT	100 DAT	120 DAT	100 DAT	120 DAT	100 DAT
T_1- Recommended dose of N	71.60^{cd}	68.17^{c-f}	65.13^{def}	56.67^{d}	61.83^{b}	51.50^{cd}	48.83^{def}	44.30^{cd}
T_2- 75% Recommended dose of N	70.93^{d}	66.33^{def}	62.47^{ef}	56.16^{d}	59.93^{bc}	51.50^{cd}	48.50^{def}	43.66^{cd}
T_3- 50% Recommended dose of N	62.43^{f}	47.83^{h}	53.80^{g}	50.50^{e}	54.66^{de}	47.33^{de}	42.83^{f}	35.63^{e}
T_4- *Spirulina* + T_1	78.77^{b}	72.83^{bc}	69.47^{bc}	61.83^{bc}	61.77^{b}	52.67^{cd}	56.17^{abc}	48.33^{ab}
T_5- *Spirulina* + T_2	77.27^{b}	72.00^{bcd}	67.80^{cd}	58.00^{cd}	56.67^{cd}	49.50^{cde}	54.17^{bcd}	48.00^{ab}
T_6- *Spirulina* + T_3	68.60^{de}	64.16^{fg}	61.47^{f}	55.00^{d}	51.33^{e}	42.77^{e}	47.17^{ef}	42.66^{d}
T_7- *Pseudomonas* + T_1	76.10^{b}	70.50^{b-e}	65.80^{cde}	57.00^{d}	62.67^{b}	60.33^{ab}	52.17^{b-e}	47.67^{b}
T_8- *Pseudomonas* + T_2	75.43^{bc}	69.00^{b-f}	65.47^{de}	56.83^{d}	59.83^{bc}	56.50^{bc}	50.16^{cde}	45.53^{c}
T_9- *Pseudomonas* + T_3	64.60^{ef}	58.67^{g}	56.46^{g}	53.67^{de}	56.43^{cd}	46.17^{de}	46.83^{ef}	36.00^{e}
T_{10}- Mixture + T_1	88.27^{a}	79.83^{a}	73.47^{a}	67.65^{a}	70.00^{a}	65.17^{a}	61.00^{a}	50.00^{a}
T_{11}- Mixture + T_2	79.60^{b}	74.67^{ab}	72.47^{ab}	63.00^{b}	69.07^{a}	65.00^{a}	58.17^{ab}	49.00^{ab}
T_{12}- Mixture + T_3	69.10^{d}	65.33^{ef}	61.46^{f}	55.50^{e}	57.33^{cd}	49.67^{cde}	48.17^{def}	43.33^{d}
F test	**	**	**	*	**	**	**	**

* and ** indicate significant and highly significant at $\alpha = 0.05$ level and $\alpha = 0.01$ level probability, respectively. In same column, means with the same letter (s) are not significantly different at 5% level, according to Duncan's multiple range test

Table 3 Effect of *Spirulina* extract and *Pseudomonas* on leaf area/plant (cm^2) and number of leaves/plant under different doses of nitrogen at 100 and 120 DAT in 2015/2016 and 2016/2017 seasons

Treatments	Leaf area/plant (cm^2)				Number of leaves/plant			
	2016/2017		2015/2016		2016/2017		2015/2016	
	120 DAT	100 DAT	120 DAT	100 DAT	120 DAT	100 DAT	120 DAT	100 DAT
T_1- Recommended dose of N	999.89^{cd}	602.94^{def}	673.83^{de}	529.22^{def}	9.33^{ab}	6.50^{c-f}	7.67^{bcd}	5.83^{cde}
T_2- 75% Recommended dose of N	783.78^{c-f}	537.40^{def}	619.62^{def}	461.45^{efg}	7.67^{def}	6.16^{def}	7.00^{de}	5.83^{cde}
T_3- 50% Recommended dose of N	379.74^{g}	295.15^{f}	294.92^{g}	241.33^{h}	6.33^{g}	4.68^{g}	5.67^{f}	4.83^{f}
T_4- *Spirulina* + T_1	1142.75^{bc}	968.27^{bc}	1017.66^{b}	797.83^{bc}	9.00^{bc}	7.58^{abc}	8.33^{b}	7.17^{ab}
T_5- *Spirulina* + T_2	973.07^{cd}	823.43^{cd}	907.47^{bc}	677.04^{cd}	8.67^{bcd}	7.33^{bcd}	8.17^{b}	6.50^{bc}
T_6- *Spirulina* + T_3	582.02^{efg}	490.26^{ef}	435.55^{fg}	409.47^{fg}	7.33^{efg}	6.17^{def}	7.33^{cd}	5.50^{def}
T_7- *Pseudomonas* + T_1	924.99^{cde}	676.45^{cde}	815.84^{bcd}	652.74^{cd}	8.00^{c-f}	6.83^{b-e}	7.83^{bc}	6.33^{c}
T_8- *Pseudomonas* + T_2	958.64^{cd}	648.66^{de}	762.67^{cde}	598.22^{de}	8.33^{b-e}	6.84^{b-e}	7.67^{bcd}	6.10^{cd}
T_9- *Pseudomonas* + T_3	554.93^{fg}	399.27^{ef}	396.60^{fg}	301.63^{gh}	6.83^{fg}	5.50^{fg}	6.43^{e}	5.33^{ef}
T_{10}- Mixture + T_1	1822.19^{a}	1558.70^{a}	1441.63^{a}	1084.39^{a}	10.33^{a}	8.58^{a}	9.41^{a}	7.83^{a}
T_{11}- Mixture + T_2	1427.70^{b}	1250.03^{b}	1282.10^{a}	949.07^{a}	9.50^{ab}	8.00^{ab}	9.08^{a}	7.67^{a}
T_{12}- Mixture + T_3	662.15^{d-g}	529.22^{def}	576.86^{ef}	425.86^{fg}	7.17^{efg}	6.00^{ef}	7.00^{de}	5.50^{def}
F-test	**	**	**	**	**	*	**	**

* and ** indicate significant and highly significant at $\alpha = 0.05$ level and $\alpha = 0.01$ level probability, respectively. In same column, means with the same letter (s) are not significantly different at 5% level, according to Duncan's multiple range test

Onion bulb yield

Table 6 shows clearly all the yield parameters of onion with significant variations among the treatments. Mixture treatment, accompanying recommended dose of nitrogen exhibited more results of marketable yield, total bulb yield, average bulb weight, and reduced culls compared with other treatments in the two seasons. Recent research suggests that the microbial inoculation combination with chemical fertilizers improve the growth and productivity of plants (Phale 2018; Yousef 2018; Florio et al. 2019), increases bulb weights and total bulb yield (Singh et al. 2017a, b; Afify et al. 2018).

Table 4 Effect of *Spirulina* extract and *Pseudomonas* on average plant fresh and dry weight (g) under different doses of nitrogen at 100 and 120 DAT in 2015/2016 and 2016/2017 seasons

Treatments	Plant fresh weight (g)				Plant dry weight (g)			
	2016/2017		2015/2016		2016/2017		2015/2016	
	120 DAT	100 DAT	120 DAT	100 DAT	120 DAT	100 DAT	120 DAT	100 DAT
T_1-Recommended dose of N	186.08^{e}	109.24^{def}	194.19^{def}	116.26^{def}	23.95^{d}	9.94^{bc}	22.32^{ef}	11.79^{b}
T_2-75% Recommended dose of N	171.47 ^{f}	105.38^{efg}	188.93^{efg}	114.15^{def}	21.19^{e}	9.58^{bc}	21.74 ^{f}	11.55^{b}
T_3-50% Recommended dose of N	151.11^{h}	84.11^{h}	156.92^{h}	82.38^{g}	18.21^{g}	8.28^{d}	17.58^{h}	8.65^{d}
T_4- *Spirulina* + T_1	222.19^{bc}	131.93^{b}	225.87^{b}	142.68^{b}	27.75^{b}	11.88^{a}	25.21^{bc}	12.65^{ab}
T_5- *Spirulina* + T_2	217.07^{c}	125.32^{bc}	219.33^{bc}	138.57^{bc}	25.96^{c}	10.45^{b}	24.64^{cd}	12.09^{ab}
T_6- *Spirulina* + T_3	164.52^{fg}	100.38^{fg}	177.97^{fg}	106.66^{ef}	19.78^{ef}	9.48^{bc}	18.47^{gh}	9.34^{cd}
T_7-*Pseudomonas* + T_1	200.98^{d}	120.04^{cd}	208.00^{cd}	126.30^{cd}	25.29^{cd}	10.21^{b}	23.80^{cde}	12.11^{ab}
T_8-*Pseudomonas* + T_2	193.04^{de}	113.60^{de}	200.30^{de}	120.81^{de}	24.21^{d}	10.04^{b}	23.32^{def}	12.02^{ab}
T_9-*Pseudomonas* + T_3	160.55^{g}	95.05^{gh}	172.33^{gh}	101.25^{f}	19.62^{f}	8.78^{cd}	18.08^{gh}	9.30^{cd}
T_{10}- Mixture + T_1	241.52^{a}	154.87^{a}	247.73^{a}	166.82^{a}	29.99^{a}	12.77^{a}	27.02^{a}	13.96^{a}
T_{11}- Mixture + T_2	228.81^{b}	150.98^{a}	233.05^{ab}	150.32^{b}	28.32^{b}	12.04^{a}	26.51^{ab}	12.87^{ab}
T_{12}- Mixture + T_3	168.59^{fg}	103.42^{efg}	181.92^{fg}	111.45^{def}	20.35^{ef}	9.53^{bc}	19.56^{g}	10.80^{bc}
F test	**	**	**	**	**	**	*	**

* and ** indicate significant and highly significant at $\alpha = 0.05$ level and $\alpha = 0.01$ level probability, respectively. In same column, means with the same letter (s) are not significantly different at 5% level, according to Duncan's multiple range test

Table 5 Effect of *Spirulina* extract and *Pseudomonas* on Chl. a and Chl. b (µg/ml) under different doses of nitrogen at 100 and 120 DAT in the 2015/2016 and 2016/2017 seasons

Treatments	Chl. a (µg/ml)				Chl. b (µg/ml)			
	2016/2017		2015/2016		2016/2017		2015/2016	
	120 DAT	100 DAT	120 DAT	100 DAT	120 DAT	100 DAT	120 DAT	100 DAT
T_1- Recommended dose of N	0.406^{ef}	0.326^{def}	0.342^{ef}	0.216^{def}	0.327^{g}	0.254^{cd}	0.306 ^{de}	0.217^{c}
T_2- 75% Recommended dose of N	0.475^{de}	0.300^{ef}	0.279^{fg}	0.202^{def}	0.249^{h}	0.188^{de}	0.215^{ef}	0.205^{c}
T_3- 50% Recommended dose of N	0.121^{g}	0.126^{g}	0.129^{h}	0.139^{f}	0.129^{k}	0.108^{e}	0.116^{f}	0.170^{c}
T_4- *Spirulina* + T_1	0.852^{ab}	0.608^{b}	0.727^{bc}	0.413^{abc}	0.507^{c}	0.480^{b}	0.507^{bc}	0.251^{bc}
T_5- *Spirulina* + T_2	0.788^{b}	0.525^{bc}	0.666^{c}	0.349^{a-d}	0.469^{d}	0.441^{b}	0.496^{bc}	0.243^{bc}
T_6- *Spirulina* + T_3	0.276^{g}	0.181^{g}	0.155^{h}	0.167^{ef}	0.191^{ij}	0.139^{e}	0.147^{f}	0.179^{c}
T_7- *Pseudomonas* + T_1	0.666^{c}	0.433^{cd}	0.519^{d}	0.326^{b-e}	0.406^{e}	0.458^{b}	0.458^{bc}	0.232^{bc}
T_8- *Pseudomonas* + T_2	0.551^{d}	0.384^{de}	0.421^{e}	0.263^{c-f}	0.369^{f}	0.341^{c}	0.393^{cd}	0.237^{bc}
T_9- *Pseudomonas* + T_3	0.212^{gh}	0.154^{g}	0.147^{h}	0.180^{ef}	0.174^{j}	0.124^{e}	0.133^{f}	0.160^{c}
T_{10}- Mixture + T_1	0.969^{a}	0.786^{a}	0.826^{a}	0.497^{a}	0.637^{a}	0.597^{a}	0.636^{a}	0.425^{a}
T_{11}- Mixture + T_2	0.897^{ab}	0.634^{b}	0.764^{ab}	0.480^{ab}	0.543^{b}	0.515^{ab}	0.569^{ab}	0.323^{b}
T_{12}- Mixture + T_3	0.309^{fg}	0.218^{fg}	0.204^{gh}	0.193^{def}	0.213^{i}	0.137^{e}	0.219^{ef}	0.193^{c}
F test	*	**	**	**	**	*	**	**

* and ** indicate significant and highly significant at $\alpha = 0.05$ level and $\alpha = 0.01$ level probability, respectively. In same column, means with the same letter (s) are not significantly different at 5% level, according to Duncan's multiple range test

Bulb quality

Table 7 shows the onion bulb quality, e.g., TSS%, DM%, and bulb diameter. Application of mixture with recommended dose of N was significantly higher of onion bulb quality compared with other treatments in the two seasons. Soil treatment with glycine cause higher values of N, P, Ca^{2+}, K^{1+}, Fe^{2+}, Mg^{2+} and Zn^{2+}, plant height, shoot and root fresh weights, soluble solids (TSS%), vitamin C and antioxidant activity of plant (Afify et al. 2018; Kurrey et al. 2018; Mohammadipour and Souri 2019a, b).

Table 6 Effect of *Spirulina* extract and *Pseudomonas* on onion bulb yield and its component under different doses of nitrogen in the 2015/2016 and 2016/2017 seasons

Treatments	2015/2016				2016/2017			
	Average bulb weight (g)	Total yield (t/ha)	Culls yield (t/ha)	Mark. yield (t/ha)	Average bulb weight (g)	Total yield (t/ha)	Culls yield (t/ha)	Mark.yield (t/ha)
T_1- Recommended dose of N	83.47^{cd}	39.25^{b}	4.56^{b}	34.68^{bc}	95.20^{efg}	38.53^{ef}	2.66^{de}	35.88^{ef}
T_2-75% Recommended dose of N	79.92^{de}	38.24^{b}	4.59^{b}	33.64^{c}	88.89^{g}	36.67^{f}	3.39^{a}	33.28^{fg}
T_3-50% Recommended dose of N	57.95^{f}	33.14^{d}	5.31^{a}	27.83^{e}	65.45^{i}	28.94^{h}	3.61^{a}	25.33^{i}
T_4- *Spirulina* + T_1	91.97^{bc}	40.02^{b}	4.09^{bc}	35.93^{b}	118.48^{bc}	44.51^{bc}	2.50^{ef}	42.01^{bc}
T_5- *Spirulina* + T_2	85.29^{cd}	39.80^{b}	4.12^{bc}	35.67^{b}	111.13^{cd}	43.75^{bc}	2.82^{cd}	40.94^{bc}
T_6- *Spirulina* + T_3	75.49^{de}	35.93^{c}	4.34^{b}	31.59^{d}	84.54^{gh}	33.29^{g}	2.98^{bc}	30.31^{gh}
T_7- *Pseudomonas* + T_1	84.03^{cd}	39.34^{b}	4.30^{b}	35.04^{bc}	103.52^{de}	42.66cd	3.09^{b}	39.56^{cd}
T_8- *Pseudomonas* + T_2	81.51^{cd}	39.10^{b}	4.38^{b}	34.72^{bc}	99.93^{ef}	40.70^{de}	3.49^{a}	37.21^{de}
T_9- *Pseudomonas* + T_3	69.78^{e}	35.96^{c}	4.44^{b}	31.52^{d}	74.80^{hi}	32.18^{g}	3.52^{a}	28.66^{h}
T_{10}- Mixture + T_1	106.23^{a}	42.32^{a}	3.21^{d}	39.12^{a}	133.42^{a}	47.64^{a}	2.22^{f}	45.42^{a}
T_{11}- Mixture + T_2	96.48^{ab}	39.83^{b}	3.51^{cd}	36.32^{b}	124.56^{ab}	45.93^{ab}	2.26^{f}	43.66^{ab}
T_{12}- Mixture m + T_3	76.54^{de}	35.92^{c}	4.11^{bc}	31.81^{d}	90.68^{fg}	33.52^{g}	2.26^{f}	31.26^{gh}
F test	**	**	**	**	**	**	*	**

* and ** indicate significant and highly significant at $\alpha = 0.05$ level and $\alpha = 0.01$ level probability, respectively. In same column, means with the same letter (s) are not significantly different at 5% level, according to Duncan's multiple range test

Storage losses

The percentage of total weight loss of bulbs is illustrated in Table 8. There were significant varietal differences in the percentage of total weight loss, while mixture recommended dose of N has 10.75 and 9.93% compared with 17.92 and 22.38% of N recommended dose in the two seasons, respectively. However, the lowest final loss percentage of bulbs was obtained with mixture + recommended dose of N, which achieved the maximum values of the remaining bulbs (89.25 and 90.07%) in the first and second seasons, respectively. PGPR prevents nutrient losses through a gradual release of nutrients, which is attained for the plant requirements as discussed by Mulbry et al. (2007), and enhances the bulbs dry weight of onion (Kurrey et al. 2018).

Economic feasibility

Figures 2, 3, and 4 illustrate the total income and benefit–cost with the different treatments. The highest net return of the Egyptian Pound with the maximum benefit–cost ratio was obtained with the application of mixture (*Spirulina* extract and *Pseudomonas*) with recommended dose of N. Application of microbes or their extracts reduce the nitrogen fertilizers about 30%. In this concern, Yaso et al. (2007), Singh et al. (2017a, b), Phale (2018) and Yousef (2018) displayed similar findings. Also, foliar spray of *Spirulina* extract containing the bioactive compounds increases the uptake of the macro- and micronutrients by a plant (Kandel et al.

Table 7 Effect of *Spirulina* extract and *Pseudomonas* on onion bulb quality under different doses of nitrogen in 2015/2016 and 2016/2017 seasons

Treatments	2015/2016			2016/2017		
	Bulb diameter (cm)	Dry matter %	T.S.S %	Bulb diameter (cm)	Dry matter %	T.S.S %
T_1- Recommended dose of N	5.65^{e}	16.23^{bc}	14.37^{cde}	6.73^{d}	15.50^{d}	14.63^{bcd}
T_2- 75% Recommended dose of N	5.00^{f}	16.13^{bc}	14.20^{def}	5.35^{gh}	14.45^{e}	14.40^{cd}
T_3- 50% Recommended dose of N	4.17^{g}	14.35^{e}	13.06^{g}	5.04^{h}	12.32^{g}	12.80^{g}
T_4- *Spirulina* + T_1	7.49^{bc}	16.72^{ab}	15.13^{b}	7.78^{c}	16.44^{c}	15.17^{bc}
T_5- *Spirulina* + T_2	7.01^{cd}	16.52^{bc}	14.70^{c}	6.46^{de}	16.12^{cd}	14.97^{bc}
T_6- *Spirulina* + T_3	4.62^{fg}	15.32^{d}	13.90^{f}	5.32^{gh}	13.80^{f}	13.57^{ef}
T_7- *Pseudomonas* + T_1	6.69^{d}	16.38^{bc}	14.68^{c}	6.34^{e}	15.71^{d}	14.77^{bc}
T_8- *Pseudomonas* + T_2	5.91^{e}	16.23^{bc}	14.57^{cd}	6.31^{e}	15.57^{d}	14.73^{bc}
T_9- *Pseudomonas* + T_3	4.51^{fg}	14.45^{e}	13.35^{g}	5.49^{fg}	12.72^{g}	13.10^{fg}
T_{10}- Mixture + T_1	8.64^{a}	17.35^{a}	15.80^{a}	8.72^{a}	18.22^{a}	16.20^{a}
T_{11}- Mixture + T_2	7.81^{b}	16.85^{ab}	15.77^{a}	8.13^{b}	17.50^{b}	15.20^{b}
T_{12}- Mixture + T_3	4.83^{fg}	15.75^{cd}	14.10^{ef}	5.71^{f}	14.31^{ef}	13.90^{de}
F test	**	**	*	**	*	**

* and ** indicate significant and highly significant at $\alpha = 0.05$ level and $\alpha = 0.01$ level probability, respectively. In same column, means with the same letter (s) are not significantly different at 5% level, according to 's multiple range test

Table 8 Total weight loss% and the remaining bulbs % of onion bulbs for 180 days as affected by *Spirulina* extract and *Pseudomonas* under different doses of nitrogen during the 2015/2016 and 2016/2017 seasons

Treatments	2015/2016				2016/2017			
	Remaining bulbs%	Total weight loss (%)			Remaining bulbs%	Total weight loss (%)		
		180	120	60		180	120	60
T_1- Recommended dose of N	82.08^{def}	17.92^{cde}	10.30^{c}	4.50^{d}	77.62^{g}	22.38^{f}	13.96^{f}	6.42^{e}
T_2- 75% Recommended dose of N	82.31^{def}	17.69^{cde}	10.23^{c}	4.56^{d}	75.81^{h}	24.19^{e}	15.44^{e}	6.75^{de}
T_3- 50% Recommended dose of N	73.33^{h}	26.67^{a}	16.26^{a}	8.22^{a}	66.91^{L}	33.09^{a}	21.81^{a}	9.28^{a}
T_4- *Spirulina* + T_1	86.05^{bc}	13.95^{fg}	7.82^{ef}	3.49^{e}	86.56^{c}	13.44^{j}	7.90^{j}	3.53^{h}
T_5- *Spirulina* + T_2	85.73^{c}	14.27^{f}	8.07^{ef}	3.52^{e}	85.12^{d}	14.88^{i}	9.16^{i}	3.72^{h}
T_6- *Spirulina* + T_3	80.15^{f}	19.85^{c}	12.39^{b}	5.50^{c}	71.97^{j}	28.03^{c}	18.38^{c}	7.65^{c}
T_7- *Pseudomonas* + T_1	83.95^{cd}	16.05^{ef}	8.72^{de}	3.55^{e}	82.04^{e}	17.96^{h}	11.33^{h}	4.63^{g}
T_8- *Pseudomonas* + T_2	83.22^{de}	16.78^{de}	9.60^{cd}	4.38^{d}	79.94^{f}	20.06^{g}	12.47^{g}	5.60^{f}
T_9- *Pseudomonas* + T_3	76.57^{g}	23.43^{b}	13.71^{b}	7.55^{b}	69.04^{k}	30.96^{b}	20.64^{b}	8.32^{b}
T_{10}- Mixture + T_1	89.25^{a}	10.75^{h}	5.65^{g}	2.40^{f}	90.07^{a}	9.93^{l}	5.98^{l}	1.95^{j}
T_{11}- Mixture + T_2	87.94^{ab}	12.06^{gh}	6.77^{fg}	2.79^{f}	88.30^{b}	11.70^{k}	7.06^{k}	2.64^{i}
T_{12}- Mixture + T_3	81.03^{ef}	18.97^{cd}	11.00^{c}	5.26^{c}	73.57^{i}	26.43^{d}	17.19^{d}	7.24^{cd}
F test	**	**	**	**	**	**	**	**

* and ** indicate significant and highly significant at $\alpha = 0.05$ level and $\alpha = 0.01$ level probability, respectively. In same column, means with the same letter (s) are not significantly different at 5% level, according to Duncan's multiple range test

2017; Mohammadipour and Souri 2019a, b). Additionally, *pseudomonas* releases IAA at rate 9.38 µg ml^{-1}, gallic acid, and nitrogen fixation, which improves germination, shoot and root size, and enhances the plant growth (Reetha et al. 2014; Singh et al. 2017a, b; Ke et al. 2019).

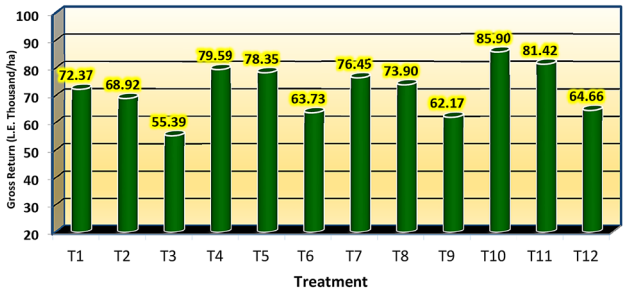

Fig. 2 Effect of *Spirulina* extract and *Pseudomonas* on total income (L.E. Thousand/ha) during 2015/2016 and 2016/2017 seasons. T_1 recommended dose of N, T_2 75% of the recommended dose of N, T_3 50% of the recommended dose of N, T_4 *Spirulina* extract + recommended dose of N, T_5 *Spirulina* extract + 75% of the recommended dose of N, T_6 *Spirulina* extract + 50% of the recommended dose of N, T_7 *Pseudomonas* + a recommended dose of N, T_8 *Pseudomonas* + 75% of the recommended dose of N, T_9 *Pseudomonas* + 50% of the recommended dose of N, T_{10} mixture (*Spirulina* extract + *Pseudomonas*) + recommended dose of N, T_{11} mixture (*Spirulina* extract + *Pseudomonas*) + 75% of the recommended dose of N, and T_{12} mixture (*Spirulina* extract + *Pseudomonas*) + 50% of recommended dose of N

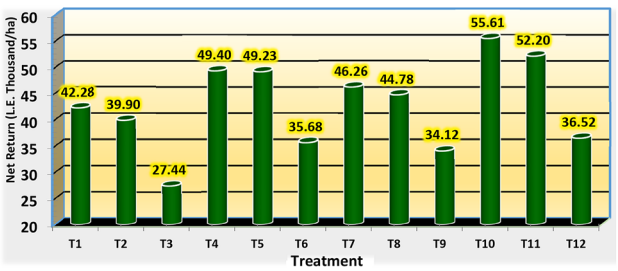

Fig. 3 Effect of *Spirulina* extract and *Pseudomonas* on total net return per ha (L.E. Thousand/ha) during 2015/2016 and 2016/2017 seasons. T_1 recommended dose of N, T_2 75% of the recommended dose of N, T_3 50% of the recommended dose of N, T_4 *Spirulina* extract + recommended dose of N, T_5 *Spirulina* extract + 75% of the recommended dose of N, T_6 *Spirulina* extract + 50% of the recommended dose of N, T_7 *Pseudomonas* + a recommended dose of N, T_8 *Pseudomonas* + 75% of the recommended dose of N, T_9 *Pseudomonas* + 50% of the recommended dose of N, T_{10} mixture (*Spirulina* extract + *Pseudomonas*) + recommended dose of N, T_{11} mixture (*Spirulina* extract + *Pseudomonas*) + 75% of the recommended dose of N, and T_{12} mixture (*Spirulina* extract + *Pseudomonas*) + 50% of recommended dose of N

Conclusions

We found that the application of *S. platensis* extract and inoculation with endophyte nitrogen-fixing *P. stutzeri* enhance the growth and productivity of the onion under different doses of nitrogen fertilizer. *S. platensis* extract and *P. stutzeri* have been bioactive compounds, thereby enhancing growth, productivity, bulb quality, and reduce the production cost of onion. This highlights the importance of applying the biofertilizers in onion agriculture for further research work in the future.

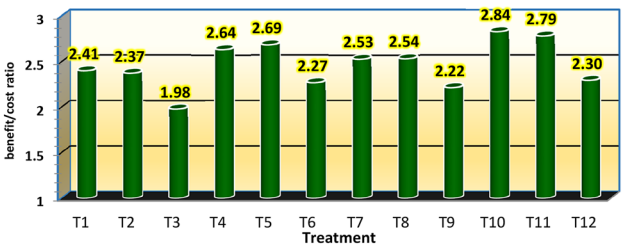

Fig. 4 Effect of *Spirulina* extract and *Pseudomonas* on benefit/cost ratio during 2015/2016 and 2016/2017 seasons. T_1 recommended dose of N, T_2 75% of the recommended dose of N, T_3 50% of the recommended dose of N, T_4 *Spirulina* extract + recommended dose of N, T_5 *Spirulina* extract + 75% of the recommended dose of N, T_6 *Spirulina* extract + 50% of the recommended dose of N, T_7 *Pseudomonas* + a recommended dose of N, T_8 *Pseudomonas* + 75% of the recommended dose of N, T_9 *Pseudomonas* + 50% of the recommended dose of N, T_{10} mixture (*Spirulina* extract + *Pseudomonas*) + recommended dose of N, T_{11} mixture (*Spirulina* extract + *Pseudomonas*) + 75% of the recommended dose of N, and T_{12} mixture (*Spirulina* extract + *Pseudomonas*) + 50% of recommended dose of N

Acknowledgements Thanks are extended to all staff members in the Cyanobacteria Research Lab., Microbiology Research Dept., Soils, Water and Environment Research Institute-Sakha Agricultural Research Station, Kafrelsheikh, Giza, Egypt, especially My Prof. Dr. Youssef Garas Yanni, for supporting me with his knowledge and experience and Onion Res. Dept., Field Crops Research Institute, Agric. Res. Center, Giza, Egypt.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Abubakar MS, Maduako JN, Ahmed M (2019) Effects of storage duration and bulb sizes on physiological losses of agrifound light red onion bulbs (*Allium cepa* L.). Agric Sci Technol 11:90–97

Adesemoye AO, Torbert HA, Kloepper JW (2009) Plant growth fertilizers. Microb Ecol 58:921–929

Afify AH, Hauka FIA, El-Sawah AM (2018) Plant growth-promoting rhizobacteria enhance onion (*Allium cepa* L.) productivity and minimize requisite chemical fertilization. Env Biodivers Soil Sec 2:119–129

Amin A, Gharib F, El-Awadi M, Rashad E (2011) Physiological response of onion plants to foliar application of putrescine and glutamine. Sci Hortic 129:353–360

AOAC (2012) Official methods of analysis of association of official analytical chemists. 19th edn, Washington, DC. USA.

Bashir A, Rizwan M, Ali S, Zia-ur Rehman MZ, Ishaque W, Riaz MA, Maqbool A (2018) Effect of foliar-applied iron complexed

with lysine on growth and cadmium (Cd) uptake in rice under Cd stress. Environ Sci Pollut Res 25:20691–20699
Chen M, Cheng B, Zhang Q, Ding Y, Yang Z (2005) Effects of applying L-methionine, L-phenylalanine and L-tryptophan on *Zea mays* growth and its nutrient uptake. J Appl Ecol 16:1033–1037
CIMMYT (1988) From agronomic data to farmer recommendation: an economic work book. CIMMYT, Mexico, DF
Compant SW, Duffy B, Nowak J, Clement C, Barka EA (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Micro 71:4951–4959
Dahab T, El-Aziz N (2006) Physiological effect of diphenylamine and tryptophan on the growth and chemical constituents of *Philodendron erubescens* plants. World J Agric Sci 2:75–81
Davies TKR, Lovelock CE, Pettit NE, Grierson PF (2017) Short-term microbial respiration in an arid zone mangrove soil is limited by availability of gallic acid, phosphorus and ammonium. Soil Biol Biochem 115:73–81
Dey R, Pal KK, Bhatt DM, Chauhan SM (2004) Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth promoting rhizobacteria. Microbiol Res 159:371–394
Dhungana SA, Itoh K (2019) Effects of co-inoculation of indole-3-acetic acid producing and -degrading bacterial endophytes on plant growth. Horticulturae 5:2–10
Duncan BD (1955) Multiple range and multiple F-tests. Biometrics 11:1–42
El-Awadi M, El-Bassiony A, Fawzy Z, El-Nemr M (2011) Response of snap bean (*Phaseolus vulgaris* L) plants to nitrogen fertilizer and foliar application with methionine and tryptophan. Nat Sci 9:87–94
FAO (2017) Onion (dried) production in 2017: crops world regions production quantity from pick lists. Food and Agriculture Organization, Statistics Division (FAOSTAT). 2018.https://www.fao.org/faostat/en/#data/QC. *Accessed 18 July 2019.*
FAO (2018) Food and Agriculture Organization of the United Nations. Rome, Italy p. 57
Fawzy Z, El-Shal Z, Yunsheng L, Zhu O, Sawan O (2012) Response of garlic (*Allium sativum* L) plants to foliar spraying of some biostimulants under sandy soil condition. J Appl Sci Res 8:770–776
Fernandes F, Abadi A, Aini LQ (2018) Utilization of plant growth promoting rhizobacteria (pgpr) in disease control important in plants onion (*Allium ascalonicum* L.). Int J Res Granthaalayah 6:126–136
Florio A, Bréfort C, Gervaix J, Bérard A, Le Roux X (2019) The responses of NO_2−- and N2O-reducing bacteria to maize inoculation by the PGPR *Azospirillum lipoferum CRT1* depend on carbon availability and determine soil gross and net N_2O production. Soil Biol Biochem 136:107524–107529
Godlewska K, Michalak I, Pacyga P, Baśladyńska S, Chojnacka K (2019) Potential applications of cyanobacteria: *Spirulina platensis* filtrates and homogenates in agriculture. World J Microbiol Biotechnol 35:1–18
Gutbrod K, Romer J, Domann P (2019) Phytol metabolism in plants. Prog Lipid Res 74:1–17
Hassan S (2017) Plant growth-promoting activities for bacterial and fungal endophytes isolated from medicinal plant of *Teucrium polium* L. J Adv Res 8:687–695
Hayat R, Ali S, Amara U, Khalid R, Ahmed I (2010) Soil beneficial bacteria and their role in plant growth promotion: a review. Ann Microbiol 60:579–598
Hossain M, Ahmed M, Ehsanul Haq M, Al Maruf M, Nabil MN (2017) Quality seed of onion: effect of micro and macronutrients. Ann Res Rev Biol 20:1–11
Hu J, Wei Z, Weidenr S, Friman V, Xu Y, Shen Q, Joussel A (2017) Probiotic pseudomonas communities enhance plant growth and nutrient assimilation via diversity-mediated ecosystem functioning. Soil Biol Biochem 113:122–129
Hua-Jing W, Liang-Huan W, Min-Yan W, Yuan-Hong Z, Qin-Nan T (2007) Effects of amino acids replacing nitrate on growth, nitrate accumulation, and macroelement concentrations in pakchoi (*Brassica chinensis* L.). Pedosphere 17:595–600
Hussain A, Ali S, Rizwan M, Zia-ur Rehman MZ, Hameed A, Hafeez F, Alamri SA, Alyemeni MN, Wijaya L (2018) Role of zinc–lysine on growth and chromium uptake in rice plants under Cr stress. J Plant Growth Regul 37:1413–1422
Jackson MI (1973) Soil chemical analysis. Prentice Hall of India Private, LTD, New Delhi
Jussara A, Carrim I, Barbosa EC, Gonçalves JD (2006) Enzymatic activity of endophytic bacterial isolates of *Jacaranda decurrens* Cham. (Carobinha-do-campo). Braz Arch Biol Technol 49:353–935
Kakkar RK, Bhaduri S, Rai VK, Kumar S (2000) Amelioration of NaCl stress by arginine in rice seedlings: changes in endogenous polyamines. Biol Plant 43:419–422
Kandel SL, Joubert PM, Doty SL (2017) Bacterial endophyte colonization and distribution within plants. Microorganisms 5:1–26
Ke Z, Feng S, Wang J, Lu W, Zang W, Chen M, Lin M (2019) Effect of inoculation with nitrogen-fixing bacterium *Pseudomonas stutzeri* A1501 on maize plant growth and the microbiome indigenous to the rhizosphere. Syst Appl Microbiol 42:248–260
Khalilzadeh R, Tajbakhsh M, Jalilian J (2012) Effect of foliar application of bio-organic fertilizers and urea on yield and yield components characteristics of mung bean. Int J Agric Res Rev 2:639–645
Khan SA, Jameel M, Kanwal S, Shahid S (2017) Medicinal importance of *Allium* species: a current review. Int J Pharmaceut Sci Res 2:29–39
Kumar V, Sharma A, Kohli SK, Yadav P, Bali S, Bakshi P, Parihar RD, Yuan H, Yan D, He Y, Wang J, Yang Y, Bhardwaj R, Thukral AK, Zheng B (2019) Amino acids distribution in economical important plants: a review. Biotechnol Res Innov 3:107–207
Kurrey DK, Lahre MK, Pagire GS (2018) Effect of *Azotobacter* on growth and yield of onion (*Allium cepa* L.). J Pharmacognos Phytochem 7:1171–1175
Lefort-Tran M, Pouphile M, Spathj S, Packer L (1988) Cytoplasmic membrane changes during adaptation of the fresh water *Cyanobacterium synechococcus* 6311 to salinity. Plant Physiol 87:767–775
Leinonen I, Iannetta PPM, Rees RM, Russell W, Watson C, Barnes AP (2019) Lysine supply is a critical factor in achieving sustainable global protein economy. Front Sustain Food Syst 3:1–11
Lin L, Xu X (2013) Indole-3-acetic acid production by endophytic *Streptomyces* sp. En-1 isolated from medicinal plants. Curr Microbiol 67:209–217
Lorck H (1948) Production of hydrocyanic acid by bacteria. Physiol Plant 1:142–146
Ma Y, Mani R, Luo Y, Freitas H (2011) Inoculation of endophytic bacteria on host and non-host plants—effects on plant growth and Ni uptake. J Hazard Mater 195:230–237
Marrelli M, Amodeo V, Statti G, Conforti F (2019) Biological properties and bioactive components of *Allium cepa* L.: focus on potential benefits in the treatment of obesity and related comorbidities. Molecules 24:1–18
Mohamed E, Mohamed S (2012) Improvement the growth and quality of green onion (*Allium Cepa* L.) plants by some bioregulators in the new reclaimed area at Nobaria region, Egypt. N Y Sci J 5:114–120
Mohammadipour N, Souri MK (2019a) Effects of different levels of glycine in the nutrient solution on the growth, nutrient composition and antioxidant activity of coriander (*Coriandrum sativum* L.). Acta Agrobot 72:6–9

Mohammadipour N, Souri MK (2019b) Beneficial effects of glycine on growth and leaf nutrient concentrations of coriander (*Coriandrum sativum*) plants. J Plant Nutr 42:1637–1644

Moran R (1982) Formula for determination of chlorophyllous pigments extracted with N, N-dimethylformamide. Plant physiol 69:1376–1381

Mulbry W, Kondrad S, Pizarro C (2007) Biofertilizers from algal treatment of dairy and swine manure effluents: characterization of algal biomass as a slow release fertilizer. J Veg Sci 12:107–125

Noroozlo YA, Souri MK, Delshad M (2019) Stimulation effects of foliar applied glycine and glutamine amino acids on lettuce growth. Open Agric 4:164–172

Patten CL, Glick BR (1996) Bacterial biosynthesis of indole-3-acetic acid. Can J Microbiol 42:207–220

Phale S (2018) Isolation and screening of multifunctional plant growth promoting rhizobacteria (PGPR) from onion rhizosphere (*Allium cepa*). J Bioprocess Biotech 8:1–3

Pise NM, Sabale AB (2010) Effect of seaweed concentrates on the growth and biochemical constituents of *Trigonella foenum-graecum* L. J Phytol 2:50–56

RediersBonnecarre`r HV, Rainey PB, Hamonts K, Vanderleyden J, De Mot R (2003) Development and application of a dapB-based in vivo expression technology system to study colonization of rice by the endophytic nitrogen-fixing bacterium Pseudomonas stutzeri A15. Appl Environ Microbiol 69:6864–6874

Reetha S, Bhuvaneswari G, Thamizhiniyan P, Ravi Mycin T (2014) Isolation of indole acetic acid (IAA) producing rhizobacteria of *Pseudomonas fluorescens* and *Bacillus subtilis* and enhance growth of onion (*Allium cepa*.L). Int J Curr Microbiol App Sci 3:568–574

Rizwan M, Ali S, Hussain A, Ali Q, Shakoor MB, Zia-ur-Rehman M, Farid M, Asma M (2017) Effect of zinc-lysine on growth, yield and cadmium uptake in wheat (*Triticum aestivum* L.) and health risk assessment. Chemosphere 187:35–42

Romero I, Téllez J, Yamanaka L, Steindel M, Romanha A, Grisard EC (2014) Transsulfuration is an active pathway for cysteine biosynthesis in *Trypanosoma rangeli*. Parasites Vectors 7:2–11

Santoyo G, Moreno-Hagelsieb G, Mosqueda MCO, Glick BR (2016) Plant growth-promoting bacterial endophytes. Microbiol Res 183:92–99

Shafeek M, Helmy MS (2012) Response of onion plants to foliar application of sources and levels of some amino acid under sandy soil conditions. J Appl Sci Res 8:5521–5527

Singh A, Gupta R, Pandey R (2017a) Exogenous application of rutin and gallic acid regulate antioxidants and alleviate reactive oxygen generation in *Oryza sativa* L. Physiol Mol Biol Plants 23:301–309

Singh M, Kumar A, Singh R, Pandey KD (2017b) Endophytic bacteria: a new source of bioactive compounds. Biotech 7:1–14

Snedecor GV, Cochran WG (1980) Statistical methods, 12th ed. Iowa State Univ. Press, Amer. Iowa, USA.

Souri MK, Hatamian M (2019) Aminochelates in plant nutrition: a review. J Plant Nutr 42:67–78

Stamenov D, Djurić S, Jafari TH (2018) Effect of PGPR on the germination and early growth of onion (*Allium cepa*). Proceedings of ISER 137th International Conference, Paris, France, 12th–13th July 2018.

Starr TJ, Die EF, Church KK, Allen MB (1962) Antibacterial and antiviral activities of algal extracts studies by acridine orange staining. Tex Rep Biol Med 20:271–278

Subba Rao NS (1977) In: soil microorganisms and plant growth. Oxford & IBH Publishing Co., New Delhi, pp 254–255

Sutariati GAK, Khaeruni A, Muhidin, Madiki A, Rakian TC, Mudi, L, Fadillah N (2019). Seed biopriming with indigenous endophytic bacteria isolated from Wakatobi rocky soil to promote the growth of onion (*Allium ascalonicum* L.). IOP Conf. Series: Earth and Environmental Science 260: 1–8.

Talaat I, Bekheta M, Mahgoub M (2005) Physiological response of periwinkle plants (*Catharanthus roseus* L.) to tryptophan and putrescine. Int J Agric Biol 7:210–213

Xie X, He Z, Chen N, Tang Z, Wang Q, Cai Y (2019) The roles of environmental factors in regulation of oxidative stress in plant. Biomed Res Int 2019:1–11

Yang W, Kim J, Lee JY, Kim C, Hwang C (2019) Antihyperlipidemic and antioxidative potentials of onion (*Allium cepa* L.) extract fermented with a novel *Lactobacillus casei* HD-010. Evid Based Complement Altern Med 2019:1–10

Yaso IA, Abdel-Razzak HS, Wahb-Allah MA (2007) Influence of biofertilizer and mineral nitrogen on onion growth, yield and quality under calcareous soil conditions. Agric Env Sci Alex Univ 6:245–246

YassenAbou ELNour AAEAA, Abou Seeda MA, Abdallah MMS, El-Sayed SAA (2018) Effect of potassium fertilization levels and algae extract on growth, bulb yield and quality of onion (*Allium cepa* L.). Middle East J Agric Res 7:625–638

Yousef NMH (2018) Capability of plant growth-promoting rhizobacteria (PGPR) for producing indole acetic acid (IAA) under extreme conditions. Eur J Biol Res 8:174–182

Zaghloul RA, Abou-Aly HE, Tewfike TA, Ashry NM (2016) Isolation and characterization of endophytic bacteria isolated from legumes and non-legumes plants in Egypt. J Pure Appl Microbiol 10:277–290

Zarrouk C (1966) Contribution à l'étuded'unecyanophycée. Influence de Divers Facteurs Physiques et Chimiques Sur la Croissance et la Photosynthèse de *Spirulina maxima*. Ph.D. Thesis, Université De Paris, Paris.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.