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EL-SALAM CANAL WATER AUTOCHTHONOUS MICROBIOME SELF-BIOREMEDIATES THE ENTERIC PATHOGENIC BACTERIA AND SUPPORTS THE *IN-SITU* LETTUCE DEVELOPMENT

Helal I.M. Nagwa[1], Ibrahim A. K.Heba[1], I.R. Refaea[2] and M. Fayez[2]

[1]Department of Soil Fertility and Microbiology, Desert Research Center, Cairo, Egypt.
[2]Department of Agricultural Microbiology, Faculty of Agriculture, Cairo University, Giza, Egypt.

Abstract

El-Salam canal water is planned to cultivate an acreage of 400,000 acre^{-1} located in north Sinai. In the present study, chemical (pH, EC, DO, BOD, COD, NH_4, NO_2, NO_3, Ca, Mg, Na, K, SAR, Cd, Cu, Fe, Zn) and bacteriological (total bacteria, total and faecal coliforms) analyses were related to the permissible limits of FAO, WHO and Mediterranean countries for irrigation water. Representatives of pathogen and PGPR isolates were secured from the canal water and rhizospheres of some plants cultivated in El-Qantara Shark fields irrigated with such water. Based on biochemical properties and 16S rRNA gene sequencing, the pathogens were identified as *Escherichia fergusonii, Klebsiella pneumoniae* and *Shigella sonnei* while PGPR were belonging to *Bacillus altitudinis, Brevibacillus brevis, Paenibacillus xylanexedens* and *Stenotrophomonas maltophilia*. These strains did possess the ability to produce the plant growth accelerating compounds, abscisic acid (15.0 – 45.0 µg/100ml), cytokinins (1.2 – 90.2 µg/100ml), gibberellins (2.7 – 4.0 mg/100ml), indole acetic acid (73.0 – 203.0 µg/100ml), and polysaccharides (1.1 – 5.6 mg/ml). They successfully antagonized the pathogen *E. fergusonii* in nutrient agar culture media. The lettuce growth traits were found to be PGPR inoculation-dependent rather than irrigation water type-dependent.

Key words: EL-Salam canal water, pathogenic bacteria, PGPR, 16S rRNA gene sequencing, antibiosis, lettuce development.

Introduction

Over the years, the Sinai peninsula has been subjected to numerous investigations in respect to its flora (Gibbali, 1988; Boulos, 1999; Serag and Khedr, 2001) and microflora (Othman *et al*., 2003 a, b and Hanna *et al*., 2011). Actually, the major limitation in the area is the very low rainfall that hardly exceeding 100 mm a year. To overcome this shortage in water supply, Egypt is currently adopting new strategies to secure additional water resources, of those is the reuse of agriculture drainage water. Based on the governmental plans, El-Salam canal is expected to deliver a total amount of 4.45 milliard m^3 water represented by 2.11 milliard of Nile water mixed with 2.34 milliard from drainage water (El-Serw and Hadous drains) (Mostafa, 2002).

This, indeed, necessitates that great efforts have to be done to expound how far this particular water resource could be safely used for cultivation with no harmful impacts on the agricultural products.

It should be realized that diverse members and species of pathogenic microbiota are highly expected to present in this type of water supplies, that when used for irrigation may severely affect the crop quality and consequently the human health. But, fortunately, bioremediation in most cases is capable to reduce the microbial loads (Dattamudi *et al*., 2018).

The microbial community accommodating the plant root theatre includes a variety of micro-residents with different metabolisms and mechanisms of antagonistic actions with potentials for suppressing some pathogens probably present in the rhizospheric soil (Coelho *et al*., 2007). Of the total community structure, the plant growth-promoting rhizobacteria (PGPR) are of special concern as they capable to produce compounds that can inhibit and suppress the development of pathogenic organisms *via* producing a wide spectrum of antimicrobial substances (Santos *et al*., 2006; Kavino *et al*., 2010; Marroni and Germani, 2011).

The present study monitors the presence of pathogenic bacteria in El-Salam canal water and

introduces information on to what extent the PGPR could be successfully used in bioremediation programs to minimize the expected hazard impacts of irrigation with such water on development of lettuce plant.

Materials and Methods

Sampling site

The sampling region stretches along South El-Qantara Sharq, and characterized by an open plain of gravely desert and scanty quantities of rainfall with very few inland salines.

Water samples

Surface water (*ca*. 1 m ashore) samples were aseptically collected in sterile brown bottles. For chemical analyses, glass stopped oxygen sampling bottles (300 ml capacity), for biochemical oxygen demand and dissolved oxygen examinations, were filled carefully with water samples and fixed immediately on the spots by adding 2 ml $MnSO_4$ followed by 2 ml alkaline KI (APHA, 1995). This is beside one-liter plastic bottles used for the other chemical determinations. In respect to trace elements, water samples were preserved with 5 ml concentrated nitric acid on the spot and stored in refrigerator (APHA, 1995). For bacteriological analyses, samples were stored at 4 ^{O}C until examinations within 48 hr of sampling.

Soil samples

Soil samples from the rhizospheres of barley (*Hordeum vulgare*), bean (*Phaseolus vulgaris*), cucumber (*Cucmis sativus*), lettuce (*Lettuce sativa*), maize (*Zea mays*), onion (*Allium cepa*), pepper (*Capsium annuum*), tomato (*Lycopersicum esculentum*) and wheat (*Triticum aestivum*) were collected in autoclaved paper bags. Those plants are cultivated in private farms at different locations of El- Qantara Sharq and regularly irrigated with El-Salam canal water. Samples were kept in a refrigerator until analyses within 2-3 days of sampling.

Bacteriological analyses

In water

a. Total bacteria were enumerated on nutrient agar medium at 30 ^{o}C incubation temperature adopting the pour plate technique of Parkinson *et al*., (1971).

b. Total and faecal coliforms were counted using the procedure mentioned by Eaton *et al*., (1995).

In soil

Roots with adhering soils were divided into two sub-samples prepared for counting ectorhizospheric- and endorhizospheric- microbiomes. For the first, 10 gram soils (in close contact to root system) were used to prepare the first dilution in 250 ml capacity conical flasks containing 90 ml distilled water, agitated at 180 rpm for 10 min., then further serial dilutions were prepared for culturing representative groups of epiphytic PGPR on nutrient agar medium. Roots and shoots of the second sub-samples were carefully washed with tap water, treated with 95% ethanol for 30 sec. followed by 3% sodium hypochlorite for 30 min., then thoroughly washed five times with sterile distilled water. Thereafter, the plant materials were triturated for 5 min. in Warring blender using sufficient amount of sterile distilled water. Further serial dilutions were prepared for enumeration of endorhizosphere and endorhizospheric (endophytic) populations.

Isolation of pathogens and PGPR antagonists

Single - colony isolation was performed for a number of colonies developed on MacConkey, eosin methylene blue (EMB) and S.S (S*almonella-Shigella*, Ewing, 1976) agar plates to secure a number of pathogenic isolates, in addition to PGPR developed on nutrient agar plates.

Antibiosis of PGPR isolates towards pathogenic bacteria

A series of laboratory experiments was carried out to report on the ability of a number of pure PGPR isolates to suppress three pathogenic organisms isolated from El-Salam canal water as well as rhizospheres of pepper and tomato cultivated in neighbored soils irrigated with such water.

Preliminary screening of PGPR against *Escherichia fergusonii*

Loopfuls of one-day old *Escherichia fergusonii* broth culture were surfacelly swabbed on nutrient agar plates. Plates were spot inoculated by freshly prepared PGPR liquid cultures. Incubation took place at 30^{o}C for 24 hr, then the pathogen growth inhibition was recorded as positive or negative.

Antagonism of superior PGPR candidates against pathogenic bacteria

The most active 45 PGPR isolates that possessed the ability to restrict *E. fergusonii* growth were tested to antagonize the three isolated pathogens in addition to *Proteus vulgaris* and *Salmonella typhimurium* obtained from the Culture Collection of Faculty of Agriculture, Ain Shams University (MERCIN) adopting the agar diffusion method of Liu *et al.,* (2006). One hundred microliter suspensions of pathogenic cultures were added to Petri dishes containing nutrient agar medium, gently mixed and left for solidification. Filter paper discs of 5 mm diameter were moistened with PGPR suspensions and seeded on the agar plates. Incubation took place at 30^{o}C for 24 hr, thereafter, the inhibition zone diameters

were measured.

Identification of pathogens and superior PGPR antagonists

Representatives of pathogenic (3) and PGPR (4) isolates were subjected to morphological and biochemical characterization. The morphological properties included cell shape, Gram stain, and motility. The following biochemical tests were carried out: a) production of catalase b) citrate utilization, c) hydrolysis of casein, gelatin and starch, d) methyl red e) Voges-Proskauer tests, and f) nitrate reduction.

For further identification, the isolates were analyzed by 16S rRNA sequencing adopting the procedures of Higgins *et al.* (2007).

The pioneeric and identified 4 PGPR strains were assessed for their biochemical activities, including abscisic acid, cytokinine, gibberellins and indole acetic acid (Shindy and Smith, 1975) as well as polysaccharide (Emtiazi *et al*., 2004) production.

Growth of lettuce as affected by PGPR inoculation and irrigation with El-Salam canal water

Plastic pots (25 cm diameter, 25 cm depth) were filled with sandy soil, previously cultivated with wheat and irrigated with the canal water, were taken from El-Qantara Shark fields at the rate of 5 kg pot^{-1}. Soil is characterized by: coarse sand, 85.5%; fine sand, 11.3%; clay, 1.1%; organic carbon, 0.26%; total nitrogen, 0.08%; pH, 7.9 and EC, 4.9 dSm^{-1}. The NPK fertilization regimes recommended for lettuce cultivation were added, those equivalent to 37 kg $acre^{-1}$ superphosphate (P_2O_5, 15.5%) and 50 kg $acre^{-1}$ potassium sulfate (K_2O, 48%). The potting soil was supplemented with nitrogen fertilizer in the form of calcium ammonium nitrate (N, 33.3%) at the rate of 90 kg $acre^{-1}$ in two successive equal doses at planting and after 40 days. Organic fertilizer as a plant waste compost was incorporated into soil at planting as 10 ton $acre^{-1}$.

Lettuce (*Lactuca sativa*, *cv.* Longifolia) seeds, obtained from the Horticulture Research Institute, Agricultural Research Center, Giza, were carefully washed in tap water and tested for germination rate. Seeds, either inoculated or not, were sown at the rate of 10 pot^{-1}, thinned to two well developed and healthy seedlings after 60 days.

For bacterial inoculation, seeds were soaked in freshly prepared heavy inoculum (*ca*.10^9 cells ml^{-1}) of either PGPR member for 30 min just prior to sowing. In case of consortium inoculation, equal portions of the individual bacterial liquid cultures were gently mixed before seed soaking. Experimental treatments included mono-culture-inoculated seeds beside those soaked in the composite inoculum of all. Potting soils were regularly watered with either El-Salam canal or tap water when needed. Pots were arranged in the greenhouse in a complete randomized design with four replications. Boost representative PGPR inocula were added twice, after 45 and 65 days of planting, this is to guarantee dense populations of microbiota in the plant root theater.

At 30, 60 and 90 day growth periods, one plant from each pot was gently uprooted without tearing the root system as possible, then the adhering soil was taken for the enumeration of total bacteria as well as total and faecal coliforms. Roots and shoots were separated and carefully washed in tap water, surface sterilized and prepared for counting the endophytic populations as previously mentioned. After 100 days of planting, the other plant was taken from each pot, and the various growth parameters were determined. Those encompassed plant lengths, fresh and dry weights, number and area of leaves as well as head characteristics.

Statistical analysis

Data were statistically analyzed using analysis of variance (ANOVA) according to Hardle and Simar (2007) using SPSS Inc., 2009 computer program.

Results

Chemical and bacteriological composition of El-Salam canal water

Table 1 summarizes the chemical and bacteriological profiles of El-Salam canal water. The pH was in the alkaline side of 8.9. The canal water seems to be non-saline with EC of 2.72 dSm^{-1}. The estimates for the biotic criterion BOD was 5.98 mg l^{-1} against the chemical corresponding COD of 16.02 mg l^{-1}. Very little amounts of ammoniacal-and nitrite-N of < 1.0 were scored being 0.96 and 0.81 mg l^{-1} respectively. On the contrary, nitrate-N was present in high level exceeding 4.0 mg l^{-1}. Among water cations, samples showed conspicuously high level of sodium (43.88 mg l^{-1}), the situation was different with calcium, magnesium and potassium where considerably respective lower quantities of 3.90, 8.10 and 6.81 mg l^{-1} were recorded. Chemical analysis of the water sample indicated SAR of 9.82 meq l^{-1}. The heavy metals; cadmium, copper, iron and zinc were detected in variable amounts of 0.36, 0.24, 11.71 and 0.18 mg l^{-1} respectively.

Dense bacterial population of > 2 X 10^6 CFU ml^{-1} was encountered after incubation at 37°C. Pollution indicators were detected in relatively lower numbers, total coliforms as 1.4 X 10^4 and faecal coliforms as 1.7 X 10^3

MPN/100 ml.

Table 1: Chemical and bacteriological analyses of El- Salam canal water in relation to international permissible limits*.

Parameters	Canal water	Permissible limits	
		Irrigation water	Drinking water
Chemical			
PH	8.9	6.5 – 8.5	6.5 – 8.5
EC (dSm^{-1})	2.72	<0.7 - <3	0.4^{EC}
DO (mg l^{-1})	4.66	-	-
BOD (mg l^{-1})	5.98	10^{WEF}, 40	NA
COD (mg l^{-1})	16.02	75^{WEF}, 80	NA
NH_4^+ (mg l^{-1})	0.96	0 – 5	1.5
NO_2^- (mg l^{-1})	0.81	NA	1.0
NO_3^- (mg l^{-1})	4.24	<5 - <30	50
Ca^{++} (mg l^{-1})	3.90	0 – 400	100^{EC}
Mg^{++} (mg l^{-1})	8.10	0 – 60	30^{EC}
Na^+ (mg l^{-1})	43.88	0 – 920	20^{EC} - 200
K^+ (mg l^{-1})	6.81	0 – 2	NA
SAR (meq l^{-1})	9.82	0 – 15	NA
Cd (mg l^{-1})	0.36	0.01	0.003
Cu (mg l^{-1})	0.24	0.2	2.0
Fe (mg l^{-1})	11.71	5.0	0.3
Zn (mg l^{-1})	0.18	2.0	5.0
Bacteriological			
Total bacteria at 37°C (CFU/ml^{-1})	2.06 x 10^6	NA	10^{EC}
Total coliforms (MPN/100ml^{-1})	1.40 x 10^4	NA	0
Faecal Coliforms (MPN/100ml^{-1})	1.70 x 10^3	(≤10^3)^{WHO}	0

* permissible limits provided by FAO for irrigation water and WHO for drinking water. EC, European Economic Community 1998; WEF, Water Environmental Federation USA 1998. NA, not available.

Table 2: Potential metabolites produced by the superior PGPR strains in nutrient broth medium.

Metabolites	*Bacillus altitudinis*	*Brevibacillus brevis*	*Paenibacillus xylanexedens*	*Stenotrophomonas maltophilia*
Abscisic acid (µg/100ml)	38	45	15	31
Benayl (µg/100ml)	59.96	90.15	45.04	48.83
Gibberellins (mg /100ml)	4.01	3.36	2.69	2.93
Indole Acetic Acid (µg/100ml)	203	73	126	149
Kinten (µg/100ml)	55.88	40.72	15.98	1.23
Polysaccharides (mg/ml)	5.58	3.72	1.12	3.14
Zeatin (µg/100ml)	64.53	20.16	17.62	29.04

Growth of lettuce plants inoculated with PGPR bio-preparates and irrigated with El-Salam canal water.

Both chemical and bacteriological characteristics of El-Salam canal water were compared and discussed with those registered in the international standards.

Screening of antagonistic PGPR against pathogenic bacteria using agar disc method

On hundred eighty five colonies developed on nutrient agar plates were selected for single colony isolation and experimented for antagonistic activity towards *E. fergusonii*. Further screening was performed for the most active 45 isolates against the three pathogens isolated from El-Salam canal water and rhizosphere soils of pepper and tomato, in addition to two type culture pathogenic candidates. They exhibited varying zones of inhibition ranging from 6 to 33 mm. (data not shown). Up to 33.3% of the culturable bacterial isolates did obviously suppress the growth of all the examined pathogens. Twelve isolates, representing 26.6% of total, slightly injured the pathogens.

Identification of the bacterial isolates

The three pathogenic isolates and the four PGPR antagonists were subjected to cell morphology, biochemical properties and 16S rRNA gene sequencing for identification. The pathogen isolates were found closely related to the species *Eschericia fergusonii*, *Klebsiella pneumonia* and *Shigella sonnei* of the GenBank with similarity levels of 99.90, 99.90 and 99.51% respectively. With similarity percentages of 99.90, 99.90, 99.60 and 99.46, the PGPR isolates were identified as *Bacillus altitudinis*, *Brevibacillus brevis, Paenibacillus xylanexedens* and *Stenotrophomonas maltophilia*, respectively.

***In vitro* production of plant growth-promoting substances by PGPR antagonists**

Apart from the test strain, the plant hormone Polysaccharides were produced in extraordinary quantities compared to other promoting compounds, its level ranged from 5.58 to 1.12 mg/ ml, this was followed by gibberellins in amounts of 4.01 – 2.69 mg/100 ml table 2. As low as 55.88 - 1.23 µg/100 ml of Kinten were detected in the bacterial culture medium. Irrespective of the produced materials, *Bacillus altitudinis* deemed the superior while *Paenibacillus xylanexedens* was the inferior.

The experimental design included cultivation of lettuce in sand soil of El-Qantara Shark irrigated with El-Salam canal and tap waters, and irrigated with the potent PGPR inocula.

Bacteriological analyses of lettuce-soil systems

Among the introduced PGPR inocula, the

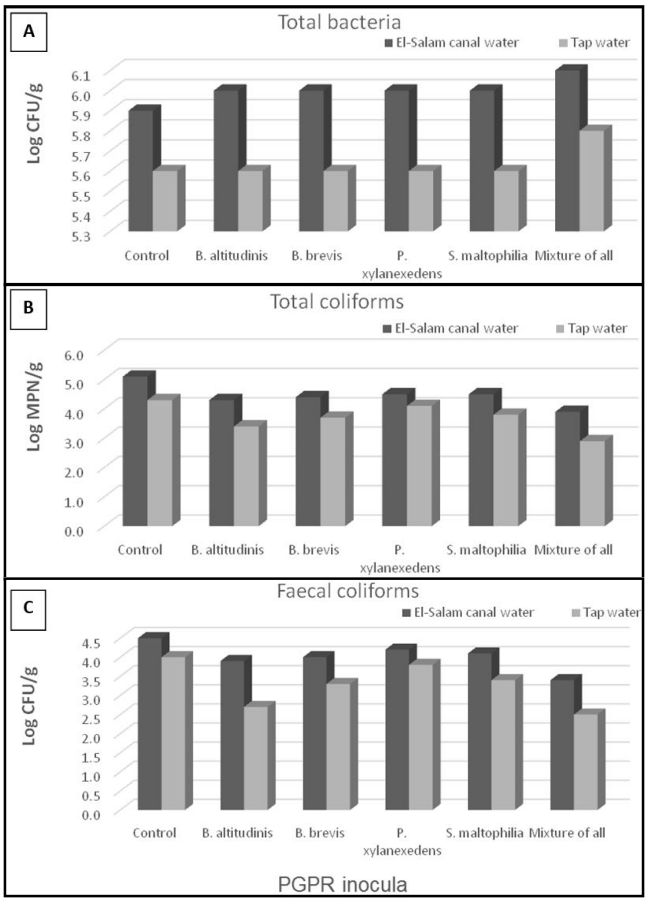


Fig. 1: Comparative populations of total bacteria (A); total coliforms (B) and faecal coliforms (C) attributed to PGPR inoculation and irrigation with El-Salam canal and tap waters.

composite of all resulted in the highest total bacterial population particularly in soil irrigated with El-Salam canal water Fig. 1, a phenomenon not observed in case of either total or faecal coliforms. No obvious differences were attributed to other mono-bacterial candidates.

Total and faecal coliform loads in soils received El-Salam canal water exceeded those due to irrigation with tap water Fig. 2. However, no conspicuous variations in total bacterial populations were attributed to irrigation water type.

Bacteriological examinations of surface sterilized

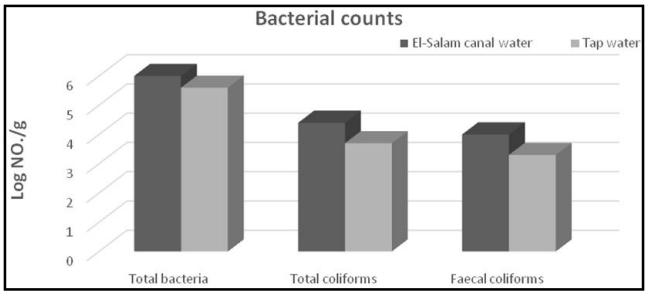
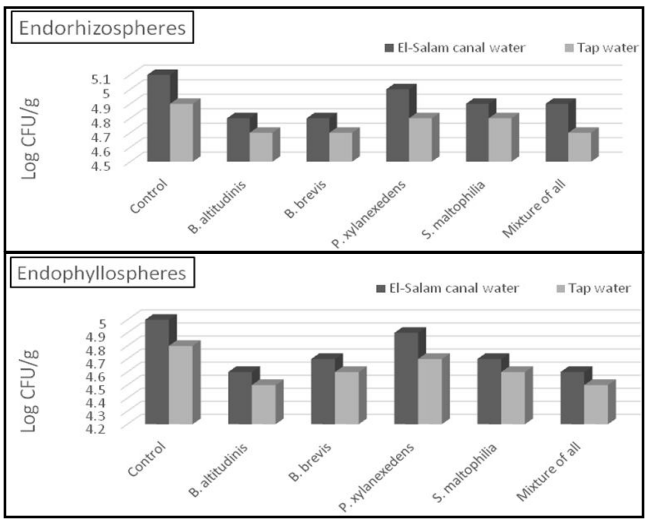
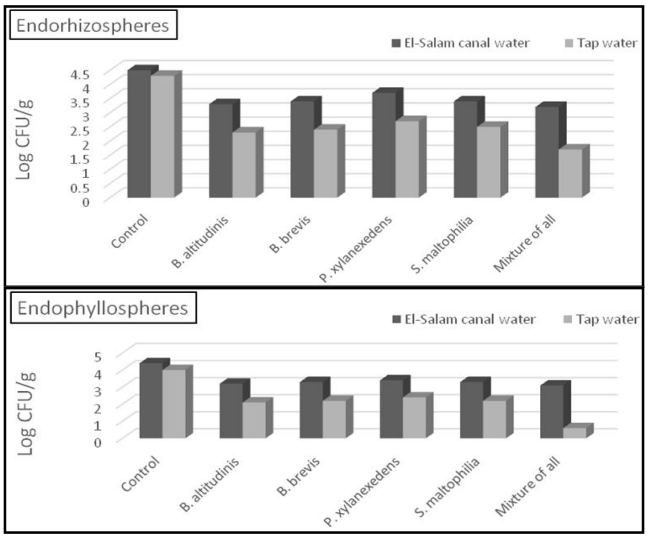


Fig. 2: Effect of irrigation water type on lettuce rhizosphere populations.

A. Total bactdria



B. Total coliforms



C. Faecal coliforms

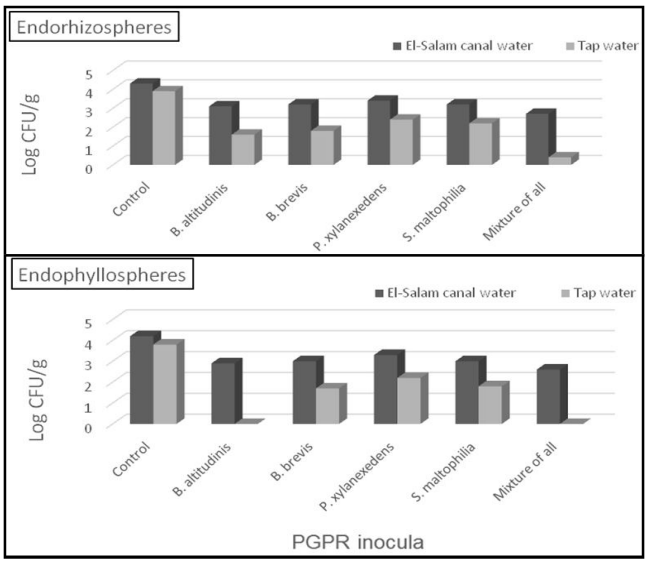


Fig. 3: Endophytic communities of lettuce inoculated with PGPR preparations and irrigated with El- Salam canal water and Tap water.

roots (endorhizospheres) and shoots (endophyllospheres) indicated the presence of endophytic communities Fig. 3. The highest colonization of > 10^4 CFU/g total bacteria was reported for the endorhizosphere of plants received El-Salam canal water. Total and faecal coliforms successfully invaded the root system and established endophytic association with lettuce, lower numbers of <10^3/g were scored. Vegetative parts of lettuce accommodated as well dense populations indicating the mobility of endorhizosphere microbiota to the plant endophyllosphere, but in relatively lower numbers.

Lettuce growth traits and yield of the various PGPR antagonist-irrigation water interweaves

The 100-day old lettuce plants were the tallest when inoculated with the composite inoculum of all PGPR strains, being 57.2 and 59.9 cm for El-Salam canal water- and tap water –irrigated plants respectively table 3. The consortium of the antagonists significantly reflected on the leaf area, but no significant differences were attributed to irrigation water type.

Table 3: Growth parameters of 100-day old lettuce plants inoculated with PGPR and irrigated with El- Salam canal and tap waters.

PGPR inocula	Length (cm plant^{-1})	Leaf area (cm^2 plant^{-1})	Number of leaves plant^{-1}	Fresh weight (g plant^{-1})	Dry weight (g plant^{-1})
El- Salam canal water					
Control	28.8	99.00	12	24.35	4.88
Bacillus altitudinis	42.3	271.74	31	51.37	9.32
Brevibacillus brevis	40.4	234.27	29	38.02	5.98
Paenibacillus xylanexedens	23.4	102.18	12	23.38	4.80
Stenotrophomonas maltophilia	39.1	217.61	20	30.50	5.22
Mixture of all	57.3	421.40	34	76.62	14.64
Tap water					
Control	26.7	90.77	12	23.27	4.47
Bacillus altitudinis	43.6	273.48	31	50.58	9.29
Brevibacillus brevis	40.9	227.77	29	37.29	5.72
Paenibacillus xylanexedens	24.9	99.18	13	23.55	4.15
Stenotrophomonas maltophilia	37.9	209.32	20	30.83	5.21
Mixture of all	59.9	418.71	34	70.33	10.29
L.S.D (P<0.05)	2.8	19.15	2	1.76	0.43

Number of leaves per plant was inoculum-dependent rather than irrigation water-dependent. Numerous leaves (34 plant^{-1}) were produced for mixed culture-treated plants irrigated with either water type. The effect of PGPR inoculation overcame the effect of irrigation water in respect to plant biomass yields. Among the single inocula, *Bacillus altitudinis* supported the highest fresh weights of lettuce irrigated with either water type (average of 51.37 g plant^{-1}) followed by *Brevibacillus brevis* (38.02 g plant^{-1}). Variations among the experimental treatments in respect to plant dry weights followed a trend akin to that for the fresh weights.

Table 4: Head characteristics of harvested lettuce plants under different inoculation and irrigation treatments.

PGPR inocula	Dia-meter (cm)	Fresh weight (g plant^{-1})	Dry wight (g plant^{-1}	Com-pact-ness*
El- Salam canal water				
Control	1.2	7.3	1.1	+
Bacillus altitudinis	2.7	17.4	4.1	+++
Brevibacillus brevis	2.2	12.81	2.8	+++
Paenibacillus xylanexedens	1.3	9.1	1.6	-
Stenotrophomonas maltophilia	1.8	13.2	3.1	++
Mixture of all	3.2	18.6	5.6	+++
Tap water				
Control	1.2	7.2	1.1	+
Bacillus altitudinis	2.5	16.2	3.5	+++
Brevibacillus brevis	2.2	12.1	2.5	+++
Paenibacillus xylanexedens	1.2	8.9	1.6	_
Stenotrophomonas maltophilia	1.8	11.4	2.34	++
Mixture of all	3.1	18.1	5.5	+++
L.S.D (P<0.05)	0.3	0.9	0.5	

* contiguous of leaves.

The mixed culture of PGPR kept its superiority, over single ones, regarding lettuce head characteristics table 4. Statistically significant higher head diameter (3.2 cm) was attributed to the composite inoculum against 1.2-2.7 cm for single culture-treated plants. Changes in head fresh and dry weights among the bacterial inocula behaved in trends almost similar to those reported with head diameter. In the majority of cases, irrigation water type had no significant influence on head traits.

Discussion

Due to limited water supplies, the government implemented El-Salam Canal Project, therefore, the major target of the present study is to introduce some information on the physico-chemical and bacteriological assessments of canal water, in addition to document the presence of enteric

pathogenic microorganisms in the water-soil system of El-Qantara Shark area and to what extent the plant growth-promoting rhizobacteria (PGPR) could be used for decontaminating these pathogens and consequently its reflection on lettuce development. Collected water sample had EC of 2.72 dSm^{-1} falling within the international permissible limits table 1. The low DO estimate (4.66 mg l^{-1}) indicates the presence of substances liable to oxidation such as biodegradable organic materials and ions in lower oxidation states, e.g., Fe^{2+} and Mn^{2+} (Von Sperling, 1996). On the other hand, the high DO concentration indicates good aeration conditions and absence of chemicals capable of consuming the dissolved oxygen. As low as BOD (5.98 mg^{-1}) was recorded, this could be attributed to decreases in water temperature which diminish the microbial growth and metabolism as well as oxidation rates. This level of BOD is in conformity with the findings of El-Degwi *et al*., (2003) that BOD records along El-Salam canal do comply with Egyptian environmental regulations. In this context, the international permissible limits for irrigation waters are in the average of 10 mg l^{-1} (WEF, 1998) to 40 mg l^{-1} (Ayers and Wescot, 1994). As expected, the COD value of the water examined (16.02 mg^{-1}) was higher than that scored for BOD as the latter expresses only the oxidation of biodegradable organic matter. The very low levels of NH_4^+ and NO_2^- (0.96 and 0.81 mgl^{-1}, respectively) indicate the absence of biodegradable organic pollutants in the region. These low concentrations might be attributed to the fast conversion to NO_3^- ions by nitrifying bacteria. The nitrate-N concentration of the water sample was high (4.24 mgl^{-1}), such relatively high level might be a result of NO_2^- and NO_3^- from fertilizers and biocides during irrigation of agricultural lands surrounding the canal before Suez Canal crossing point. At such area, leakage from overloaded sewage networks is among the nutrient and heavy metal pollution. As to mineral contents in canal water, Ca^{++} (3.90 mg l^{-1}), Mg^{++} (8.10 mg l^{-1}) and Na^+ (43.88 mg l^{-1}) were detected in quantities within the international permissible limits. As excessive solutes in irrigation water are a common problem in semi-arid areas, FAO recommended the use of sodium adsorption ratio (SAR) to be in the range of 0-15 meq l^{-1} (Jurdi *et al*., 2002). The reported SAR in the examined canal water of 9.82 meq l^{-1} comply with such permissible limits and proved the suitability for irrigation. The concentrations of Cu and Zn are within the permitted levels for irrigation and drinking waters. But both Cd and Fe existed in amounts higher than those recorded in the international standard providing an additional evidence for the industrial pollution of the drainage water used.

Dense populations of 37 °C growing bacteria (2.06×10^6 CFU ml^{-1}) as well as total coliforms (1.40×10^4 MPN/ 100 ml^{-1}) and faecal coliforms (1.70×10^3 MPN/ 100 ml^{-1}) were encountered in the examined El-Salam canal water sample. On the assessment of the canal water suitability for agriculture in principal, and for drinking if possible, Othman *et al*., (2008) followed the spatial distribution along the first 55 km extended in north Sinai during the seasons of the two successive years 2003-2004 and 2004-2005. They reported that the chemical and microbiological analyses were related to the permissible levels of FAO (Ayers and Wescot, 1985), WHO (2006) and Mediterranean countries (Bahir and Brissaud, 2004).

Three pathogenic isolates were secured from the canal water and soil, this is beside 185 PGPR isolates taken from the rhizospheres of representative plants cultivated in the area and regularly irrigated with such water. Adopting the agar diffusion method, results demonstrated that a great portion of isolates possessed antibacterial activities against all of the tested pathogens. Here, the mechanism of antimicrobial activity of bacterial antagonists was studied by Liu *et al*., (2017) who detected some secondary metabolites such as bacillibactin, bacilysin, microcin, haloduracin and bacillomycin in the culture media of *Bacillus altitudinis* and *Bacillus* sp. They added that *Bacillus altitudinis* has frequently been used as a representative commercialized strain for biological control against a broad spectrum of plant pathogens, including *Pythium* spp., *Rhizoctonia* spp. and *Xanthomonas* spp. In the present study, the bacterial isolates having the greatest antibiosis efficiencies were identified based on biochemical characteristics and 16S rRNA gene sequencing. They belonged to *Bacillus altitudinis*, *Brevibacillus brevis*, *Paenibacillus xylanexedens* and *Stenotrophomonas maltophilia*. As well, the pathogenic members were identified as *Eschericia fergusonii*, *Klebsiella pneumonia* and *Shigella sonnei*.

The bacterial antagonists were evaluated for their capabilities to produce a number of plant growth-promoting compounds. All successfully produced indole acetic acid, gibberellins, abscisic acid, cytokinins and polysaccharides in variable quantities. In an agreement with these findings, a vast array of literature confirmed the ability of PGPR to produce a variety of plant growth accelerating compounds (Sivasakthi *et al*., 2014, Huang *et al*., 2016, Xiang *et al*., 2017).

For lettuce inoculation experiment, all the tested bioformulations displayed a decontaminating potency towards the enteric pathogens, a phenomenon that

indicated by the decreased numbers of total and faecal coliforms in soils of PGPR-inoculated treatments, the consortium of all strains was the superior. The antagonistic influence was also extended to the endophytic pathogen community. Several studies indicated the application of microorganisms as bioremediation agents in various contaminated environments. Olguin (2012) stated that soil microbiota, algae in particular, are important agents in bioremediation programs since they successfully suppressed the growth of total and faecal coliforms.

The PGPR promoted the growth of lettuce where inoculated plants nicely developed with better leaves and biomass yields. In accordance with these findings, Singh and Jha (2017) reported that *Stenotrophomonas maltophilia* provided bio-control for *Fusarium graminearum* and stimulated shoot and root lengths of wheat. Several crops responded as well to PGPR treatments, e.g. cucumber (Gamalero *et al*., 2008) and chickpea (Shahzad *et al.*, 2010). It is noteworthy to mention that, root morphology parameters play a role in plant development because nutrient uptake is more dependent on total root length and root surface area than on total root biomass. Hence, increasing nutrient uptake sometimes results from PGPR treatment, as found by Gamalero *et al*., (2004). Additionally, the studies of Liu *et al*., (2018) indicated that increasing the root biomass may be caused by the PGPR-associated enhancement of photosynthesis which provide the basic energy to the roots, which in turn absorb more water and nutrients from soil to support the growth of whole plant.

Finally, results of the present study proved the ability of the PGPR inocula to overcome, to an extent, the relative in-suitability of El-Salam canal water for proper plant development and prospective yield. In this respect, great efforts are necessary to be done, regularly, to monitor the physic-chemical and bacteriological qualities of the canal water to guarantee a safe irrigation water supply for the agricultural expansion in Sinai peninsula.

References

APHA, (American Public Health Association) (1995). Standard Methods for Examination of Water and Waste Water. 19th ed. APHA – AWWA-WEF, Inc., Washington, D.C.

Ayers, R.S. and D.W. Wescot (1985). Water Quality for Agriculture, FAO Irrigation and Drainage Paper NO. 29., Rev. I, FAO, Rome.

Ayers, R.S. and D.W. Wescot (1994). Water Quality for Agriculture, FAO Irrigation and Drainage Paper 29. Rome, Italy.

Bahir, A. and F. Brissaud (2004). Setting up microbiological water reuse guidelines for the Mediterranean. *Water Sci. Technol.,* **50:** 39-46.

Boulos, L. (1999). Flora of Egypt. Al Hadara Publishing, Cairo, Egypt. pp.419.

Coelho, L.F., S.S. Freitas, A.M.T.M. Melo and G.M.B. Ambrosano (2007). Interação de bactérias ûuorescentes do gênero Pseudomonas e de *Bacillus spp*. com a rizosfera de diferentes plantas. *Rev. Bras. Cienc. Solo.,* **31:** 1413-33.

Dattamudi, S., G. Banta and B.S. Sidhu (2018). Hargopal Singh and Saoli Chanda. Effect of Treated Sewage Water on Survival of Microbial Community and Rice Production in Northwest India. *Acta. Sci. Agri.,* **2(12):** 103-111.

Eaton, A.D., L.S. Clesceri and A. Greenberg (1995). Standard Methods for the Examination of Water and Waste Water. 19th ed American Water Works Association, Washingtons, D.C., publisher.

El-Degwi, A.M.M., F.M. Ewida and S.M. Gawad (2003). Estimating BOD pollution rates along El-Salam canal using monitored water quality data (1998–2001). In: Proceedings of 9th International Drainage Workshop, Paper No 50. The Netherlands, Utrecht, September 10–13.

Emtiazi, G., Z. Ethemadifara and M.H. Habib (2004). Production of extra-cellular polymer in *Azotobacter* and biosorption of metal by exopolymer. *Afr. J. Biotechnol.,* **3(6):** 330-333.

European Economic Community (EC): Dir. 98/83 (1998). Council Directive of 3 November 1998 on the quality of water intended for human consumption. *Off. J. Eur. Commun. L.,* **330:** 32–56.

Ewing, E.H. (1976). Isolation and identification of *Salmonella* and *Shigella*. HEW Publication no. (CDC) 76-8098 Atlanta, PP.3-5.

Gamalero, E., G. Berta, N. Massa, B.R. Glick and G. Lingua (2008). Synergistic interactions between the ACC deaminase-producing bacterium *Pseudomonas putida* UW4 and the AM fungus *Gigaspora rosea* positively affect cucumber plant growth. *FEMS Microbiol. Ecol.,* **64:** 459-467.

Gamalero, E., A. Trotta, N. Massa, A. Copetta, M. Martinotti and G. Berta (2004). Impact of two fluorescent pseudomonads and an arbuscular mycorrhizal fungus on tomato plant growth, root architecture and P acquisition. *Mycorrhiza,* **14:** 185-192.

Gibbali, M.A. (1988). Studies on the flora of north Sinai. M.Sc. Thesis. Fac. Science, Cairo Univ., Egypt.pp. 393.

Hanna, L. Amira, Yossef H. Hanan, Amer M. Wafaa, M. Monib, M. Fayez and N.A. Hegazi (2011). Diversity of bacteria nesting the plant cover of north Sinai deserts, Egypt. *J. Adv. Res.,* **4:** 13-26.

Hardle, W. and L. Simar (2007). Applied Multivariate Statistical Analysis. 2nd ed, Springer,420 pp.

Higgins, K.L., A.E. Arnold, J. Miadlikowska, S.D. Sarvate and F. Lutzoni (2007). Phylogenetic relationships, host affinity, and geographic structure of boreal and arcticendophytes from three major plant lineages. *Mol. Phylogen. Evol.,* **42:**

543-555.

Huang, P., L. de-Bashan, T. Crocker, J.W. Kloepper and Y. Bashan (2016). Evidence that fresh weight measurement is imprecise for reporting the effect of plant growth-promoting (rhizo) bacteria on growth promotion of crop plants. *Biol. Fertil. Soils,* **53:** 199-208.

Jurdi, M., S.I.Y. Korfali Karahagopian and B. Davies (2002). Evaluation of water quality of the Qaraaoun reservoir, Lebanon suitability for multipurpose usage. *Environ. Monit. Assess,* **77:** 11–30.

Kavino, M., S. Harish, N. Kumar, D. Saravanakumar and R. Samiyappan (2010). Eûect of chitinolytic PGPR on growth, yield and physiological attributes of banana (*Musa* spp.) under field conditions. *Appl. Soil Ecol.,* **45:** 71-78.

Liu, H., S. Wang, Y. Cai, X. Guo, Z. Cao and Y. Zhang (2017). Dietary administration of *Bacillus subtilis* enhances growth, digestive enzyme activities, innate immune responses and disease resistance of tilapia, *Oreochromis niloticus*. *Fish and Shellfish Immunology,* **60:** 326-33.

Liu, Ke, J.A. McInroy, Chi. Hu and J.W. Kloepper (2018). Mixtures of Plant-Growth-Promoting Rhizobacteria Enhance Biological Control of Multiple Plant Diseases and Plant-Growth Promotion in the Presence of Pathogens. *Journal of Plant Disease,* **102:** 67-72.

Liu, X., H. Zhao and S. Chen (2006). Colonization of maize and rice plants by strain *Bacillus megaterium* C4, *Curr. Microbio.,* **52:** 186-190.

Marroni, I.V. and J.C. Germani (2011). Efciência de rizobactérias *Bacillus* spp. no controle *in vitro* de *Macrophomina phaseolina* agente etiológico da podridão de tronco da mamona (*Ricinus communis* L.). *Rev. Bras. Agroecol.,* **6:** 159-226.

Mostafa, A.M. (2002). Development of water quality indicators and atlas of drainage water quality using GIS tools. CIDA-DRTPC-MWRI, Technical reported submitted to the NAWQAM project, Egypt, 85 p.

Olguin, E.J. (2012). Dual purpose microalgae-bacteria based systems that treat wastewater and produce biodiesel and chemical products within a biorefinery. *Biotechnol. Adv.,* **30(5):** 1031-1046.

Othman, A.A., M.W. Amer, M. Fayez, M. Monib and N.A. Hegazi (2003a). Biodiversity of microorganisms in semi-arid soils of north Sinai deserts. *Arch. Agron. Soil Sci.,* **49:** 241–260.

Othman, A.A., M.W. Amer, M. Fayez, M. Monib and N.A. Hegazi (2003b). Biodiversity of diazotrophs associated to the plant cover of north Sinai deserts. *Arch. Agron. Soil Sci.,* **49:** 683–705.

Othman, A.A., S.A. Rabeh, M. Fayez, M. Monib and N.A. Hegazi (2008). The water quality of El-Salam canal, north Sinai, Egypt. *J. Egypt. Acad. Soc. Environ. Develop.,* **9(2):** 47-64.

Parkinson, D., T.R.G. Gray and S.T. Williams (1971). Methods for Study the Ecology of Soil Micro- organisms. IBP Handbook No.19.

Santos, E.R., E.R. Gouveia, R.L.R. Mariano and A.M. Souto-Maior (2006). Controle biológico da mancha-aquosa do melão por compostos bioativos produzidos por *Bacillus* spp. *Summa Phytopathol.,* **32:** 376-384.

Serag, M.S. and A.A. Khedr (2001). Vegetation-environment relationships along El-Salam Canal, Egypt. *Environmetrics,* **12:** 219–251.

Shahzad, S.M., A. Khalid, M. Arshad, J. Tahir and T. Mahmood (2010). Improving nodulation, growth and yield of *Cicer arietinum* L. through bacterial ACC-deaminase induced changes in root architecture. *Eur. J. Soil Biol.,* **46:** 342-347.

Shindy, W.W. and O.E. Smith (1975). Identification of plant hormones from cotton ovules. *Plant Physiol.,* **55:** 550-554.

Singh, R.P. and P.N. Jha (2017). The PGPR *Stenotrophomonas maltophilia* SBP-9 augments resistance against biotic and abiotic stress in wheat plants, *Front. Microbiol.,* **8:** 1945.

Sivasakthi, S., G. Usharani and P. Saranraj (2014). Biocontrol potentiality of plant growth promoting bacteria (PGPR)-*Pseudomonas fluorescens* and *Bacillus subtilis*: *A review. Afr. J. Agric. Res.,* **9:** 1265-1277.

Von Sperling, M. (1996). IntroduÇão á Qualidade das Águas e ao Tratamento de Esgotos, Segrac, Belo Horizonte, Brasil. Cited by Jordão *et al.,* (2002).

WEF (1998). Water Environment Federation. Using reclaimed water to augment potable water resources. Alexandria, VA, USA.

Weisburg, W.G., S.M. Barns, D.A. Pelletier and D.J. Lane (1991). 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.,* **173:** 697-703.

WHO (2006). Guidelines for Drinking-Water Quality, incorporating first addendum. Recommendations. 3rd ed. Vol. 1. Geneva: World Health Organization; P. 515

Xiang, N., K. Lawrence, J. Kloepper, P.A. Donald and J. McInroy (2017). Biological control of *Heterodera glycines* by spore-forming plant growth promoting rhizobacteria (PGPR) on soybean. *PLoS One,* **12:** e 018120.