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Utilization of actinobacteria to enhance the production and quality of date palm (*Phoenix dactylifera* L.) fruits in a semi-arid environment

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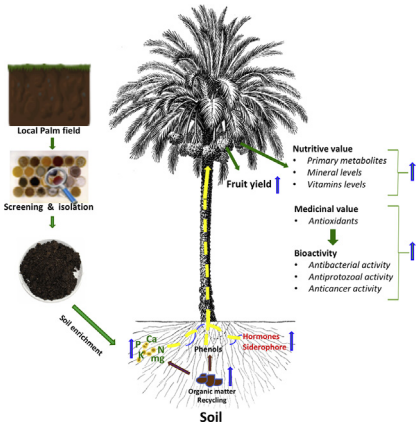
HIGHLIGHTS

- Utilization of actinobacteria resulted in improved soil fertility and yield of date palms.
- Treated palm fruits showed enhanced nutritive and health-promoting values.
- Major phenolics, flavonoids, vitamins, EAA and PUFA have significantly improved.
- Actinobacteria are a promising approach for supporting functional food value of plants.

GRAPHICAL ABSTRACT



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ABSTRACT

Actinobacteria have received much attention due to their capacity for plant growth promotion, a promising approach in sustainable development of agriculture. Date palm (*Phoenix dactylifera* L.) is an important crop, particularly in semi-arid regions of the world, due to the high nutritional and health-promoting values of its fruits. The present study was conducted to investigate the utilization of actinobacteria as an approach to support soil fertility and enhance production and functional food value of date palm fruits in a semi-arid environment. To achieve this purpose, actinobacterial strains were isolated from palm rhizosphere, characterized and screened for bioactivity. Then the potent isolates, based on plant growth promoting assays, were inoculated into the soil rhizosphere of five-target palms (Ajwa, Sokary, Khodry, Rashodia and Saffawy) before flowering and during fruiting stages in

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two successive seasons. Interestingly, the actinobacterial inoculants increased soil fertility and improved fruit yield of the tested palms. The treated date fruits accumulated higher levels of valuable phytochemicals such as sugars, organic acids, essential amino acids, unsaturated fatty acids, phenolic acids, flavonoids, vitamins and minerals, as compared with the untreated ones. Moreover, actinobacterial treatment induced the biological activities (antioxidant, antibacterial, antifungal and anticancer) of the produce dates. Conclusively, results presented herein suggest the promising application of actinobacteria for supporting the production and functional food value of date palms in semi-arid regions.

1. Introduction

Worldwide, there were interesting attempts in addition to the urgent need to promote sustainable agriculture, combining high productivity, resource use efficiency and better nutritive value (Garnett et al., 2013; Hassan et al., 2018). Organic farming is a substantial aspect that minimizes the environmental hazards of the artificial fertilizers and pesticides in the horticultural systems. From the socio-economic point of view, searching for innovative approaches to improve agriculture without application of synthetic agro-chemicals is a worthwhile demand (Cordeau et al., 2016; Peigné et al., 2016). Organic agriculture provides several merits as an agriculture practice if compared with the conventional one (Luttikholt, 2007), for instance, organic fruits and vegetables showed better yield and nutritional value (Gomiero, 2018).

Date palm (*Phoenix dactylifera* L.) is one of the most widely used and commercially important crops in the hot arid and semi-arid regions of the world, e.g., the Arabian Peninsula, Middle East and North Africa (Al-Farsi and Lee, 2008; Baron et al., 2006; Hamad et al., 2015). The products of date palm fruits are commonly used in human diet for its high nutritional and health promoting values, whereas it provides a good source for sugars, vitamins, minerals, phenolics, flavonoids, antioxidants and fibers (Al-Farsi and Lee, 2008; Baron et al., 2006; Hamad et al., 2015). Thus, the approach to induce the yield, nutritional value and the health benefits of the date fruits represents a great interest.

A promising approach in organic farming is the utilization of soil microflora as biofertilizers to support the nutrient availability and uptake, particularly under unfavorable conditions (Numan et al., 2018). In this regard, application of plant growth-promoting rhizobacteria (PGPR) was the most likely used tool (Armada et al., 2014). Application of PGPR as soil inoculants was reported to participate in nutrient cycling and to improve crop productivity (Singh et al., 2011).

Actinobacteria are Gram-positive bacteria with high G + C content that exhibiting various morphologies from unicellular to filamentous forms and constituting a major phylum within the domain Bacteria (Goodfellow, 2012). They are remarkably known for their capacity to produce diverse groups of bioactive secondary metabolites including antimicrobials, immunosuppressive agents, antioxidants, enzymes and plant growth hormones (Barka et al., 2016; Fiedler et al., 2008; Igarashi et al., 2007). Many actinobacteria are reported as endophytes in various plants or as inhabitants of the rhizospheric soil. In this context, several studies have reported the growth-promoting traits of actinobacteria due to their ability to solubilize phosphates, fix nitrogen and produce phytohormones such as indole acetic acid (IAA) and gibberellic acid (GA) (Anwar et al., 2016). Moreover, some members of the genus *Streptomyces*, the most common and prolific actinobacterial genus, have been proved to play a substantial role in inducing plant resistance against phytopathogens (Saeed et al., 2017) and in plant growth promotion (El-Tarabily et al., 2009).

Field studies addressing the plant growth-promoting behavior of actinobacteria are increasing all over the world (Franco-Correa et al., 2010; Jog et al., 2012). However, none of these studies has deeply undertaken the effects of the soil inoculated with actinobacteria on the functional food vales of plants, including date palm. Therefore, the current study was undertaken to evaluate the influence of plant growth promoting actinobacteria on the accumulation of nutritionally and medicinally important phytochemicals. To fulfill this aim, the levels of individual sugars, organic acids, essential amino acids, unsaturated fatty acids, phenolic acids, flavonoids, vitamins and minerals were assessed in the fruits of date palm grown in soil inoculated with plant growth promoting actinobacteria and compared with those grown in actinobacteria free soil. Moreover, the associated changes in antioxidant, antibacterial, antifungal and anticancer activities of the produced fruits were evaluated.

2. Materials and methods

2.1. Study area

This region is located in the northern part of KSA. It is located between latitudes 29° and 32° N, longitudes 37° and 42° E and altitude of about 684 m a.s.l. It occupies about 107,794 km^2, representing about 5% of the total area of Saudi Arabia. Al-Jouf province is one of the most important agricultural centers in KSA. The cultivated area of this territory is about 4600 km^2. The region is characterized by the cultivation of economically- and nutritionally-based crops usually cultivated in semi-arid regions such as olive and date palm. In addition, other field crops could be also found such as wheat, barley, alfalfa, sorghum, and watermelon. This area is marked by a semi-arid climate, with hot summer and cool winter. The average monthly air temperature ranges from 9.8 °C in January to 33.8 °C in August. The mean monthly relative humidity varies from 16% during June to 53% during January. The rainfall in the region is sporadic and irregular and the mean annual rainfall is 55 mm, with the rainy season extending from October to May. Soils suitable for cultivation in this region are mainly loamy sand and sandy loam texture classes. In general, the soils possess poor fertility represented in the lower contents of organic matter and available soil nutrients, in addition to the higher contents of $CaCO_3$ and soluble salts (Hussain et al., 2010). Therefore, special fertility management practices are being adopted to increase land productivity. For this reason, we tested the potential application of the growth-promoting actinobacteria to enhance soil fertility as much as possible safely.

2.2. Experimental setup

A field study was carried out during two successive growing seasons of 2016 and 2017 in a private orchard located at Al-Jouf province. Healthy date palm cultivars (Ajwa, Sokary, Khodry, Saffawy and Rashodia) were cultivated in sandy soil at the space of 8 × 8 m apart. Studied trees were mostly similar in age and growth. The number of leaves bunch was adjusted for both control and treated cultivars. The trees tested have not received any fertilizers before the initiation of this study.

A group of actinobacterial strains, isolated from soil samples collected from the study area, were grown on a Glycerol-Yeast Extract Agar medium (Glycerol 5 mL, Yeast extract 2 g, K_2HPO_4 1 g, Agar 15 g, Distilled water 1000 mL) supplied with nystatin (50 μg/mL) (Waksman et al., 1961). The selected colonies of actinobacteria were purified and sub-cultured on the same agar medium and incubated at

27 °C for a week. The pure cultures were kept in starch casein agar medium at 4 °C and as 20% glycerol suspensions at −20 °C. Morphological characterization was done by examining the cover-slip cultures of the isolates with light and electron microscopes as described by Shirling et al. (1966), while the biochemical characterization (e.g., carbon and nitrogen utilization and enzymatic activities) were carried out according to the methods of Williams et al. (1989). Plant growth promoting capacity of the different isolates was elucidate by determination of IAA and siderophore production (Gordon and Weber, 1951; Schwyn and Neilands, 1987). A mixture of the four actinobacterial isolates producing the highest levels of IAA and siderophore (isolates 1, 3, 7 and 9) were applied twice as soil drenches in 5 L/replicate (10^8 colony forming units (CFU) mL^{-1}) before and during date palm fruiting. The experiment was conducted in a triplicate design for each treatment, and 3 trees for each replicate. The routine agro-technical operations were performed and soil water content was adjusted for around 60%. At the full fruiting stage, fresh dates were harvested from treated and untreated cultivars at the tamar stage. Date fruits of uniform size, free of physical damage and injury from insects and fungal infection, were selected and used for measurements. Clean dates were stored at −80 °C for metabolic, mineral and biological activity analyses.

2.3. Soil analysis

To analyze the change in soil physical and chemical properties, soil samples were collected from target date palm rhizosphere zone. The pH value (pH meter (AD 3000)) and EC (conductivity meter (Jenway 3305)) were measured. Total phenols and organic matter determined by spectrophotometer (A_{700}) and Walkely and Black rapid titration method (Black et al., 1965). Micro and macro-minerals in soil and plant were extracted in aqueous HNO_3 solution (80%) and were detected by using ICP-MS (Finnigan Element XR, Scientific, Germany) (Agusa et al., 2005).

2.4. Metabolite profiling

2.4.1. Primary metabolites

The changes in primary metabolites including sugars, amino acids, organic acid and fatty acids were measured by using High-performance liquid chromatography (HPLC) methods. Mono and disaccharides levels were extracted by homogenizing the fresh fruits (0.5 g) in aqueous acetonitrile (50%) at 60 °C. After filtration, the supernatant was injected to the HPLC (SUPELCOSIL LC-NH2 column (250 × 4.6 mm)) and was eluted with aqueous acetonitrile (75%). Sugar forms were determined based on peak area of each corresponding standard. To measure the total soluble and insoluble sugars were extracted in aqueous ethanol (80%) and measured by Nelson's method (Al Jaouni et al., 2018).

Individual amino acids were extracted by homogenizing the fresh fruits (0.5 g) in ethanol:water (80%) using MagNALyser equipment. After centrifugation at 15,000 rpm for 25 min, supernatants were dried and re-dissolved in chloroform and the aqueous phase were separated and then filtered through 0.22 μM filter paper. The level of amino acids determined by using HPLC (Waters Acquity UPLC-tqd system) equipped with a BEH amide2.1 × 50 column (Sinha et al., 2013). Different amino acids forms were determined based on peak area of each corresponding standard.

To measure individual organic acids, 0.1 g fresh fruits were homogenized in 10 mL of phosphoric acid mixed with butylated hydroxyanisole. After centrifugation at 12,000 rpm for 30 min, the supernatant were filtered and eluted by 0.1% phosphoric acid at 0.45 mL/min flow rate. Different organic acids were then measured at 210 nm (HPLC, LaChrom L-7455 diode array) (Hamad et al., 2015). The mobile phase, 0.1% phosphoric acid:water (v/v), was applied at a flow rate of 0.45 mL/min. Different amino acids concentrations were determined based on standards calibration curve.

Fatty acids were extracted by mixing 0.25 g of fresh fruits with chloroform/methanol (2:1, v/v) at 25 °C to obtain lipophilic fractions. Samples were centrifuged at 16,000 rpm for 30 min and the filtered supernatants were quantified using GC–MS analysis (Hewlett Packard 6890, MSD 5975 mass spectrometer, equipped with an HP-5 MS column) (Torras-Claveria et al., 2014). Individual fatty acids were identified using the database of NIST 05 and GolmMetabolome (http://gmd.mpimp-golm.mpg.de).

2.4.2. Secondary metabolites

Individual secondary metabolites including phenols, flavonoids, antioxidants and vitamins were quantified using HPLC methods. To extract phenols and flavonoids, about 0.1 g of fresh date samples were mixed with 2 mL of aqueous methanol (80%) at 60 °C for 30 min. After centrifugation at 12,000 rpm for 30 min, methanol in supernatants was evaporated and the aqueous solution was re-extracted with diethyl ether. Samples were then dried and redisolved in methanol:water (80%) and filtered through a 0.45 μm filter for purity before injection to HPLC system. Individual phenolic and flavonoid compounds were measured according to Hassan (2018) (Shimadzu HPLC system, Japan). To measure total phenolic and flavonoids contents, aluminum chloride and Folin–Ciocalteu spectrophotometric methods were applied (Al Jaouni et al., 2018).

Regarding vitamins, different forms of tocopherol and carotene as well as thiamine, β-cryptoxanthin and phylloqinone were measured by normal or reversed phase HPLC methods (Shimadzu, Hertogenbosch, Netherlands). Tocopherols were mixed with hexane in MagNALyser and the homogenized samples were centrifuged for 20 min at 12,000 rpm. The supernatants were dried and re-dissolved in 0.1 mL of hexane. Tocopherols were separated in Particil Pac column material according to AbdElgawad et al. (2015). Carotene and β-cryptoxanthin content was extracted by homogenizing the fresh fruits (0.1 g) in 2 mL of acetone using MagNALyser. Homogenized samples were evaporated and the residue were re-dissolved in 0.2 mL of ethanol and filtered for high purity through 0.45 μm filter. Samples were analyzed by a reversed phase HPLC with diode array detector (Al Jaouni et al., 2018). 20 μL of supernatants was injected to a silica-based C18 column and eluted by aqueous acetonitrile:methanol:water as well as methanol:ethyl acetate mixture at 1.2 mL/min flow rate. Phylloquinone was detected by using reversed phase HPLC (Jakob and Elmadfa, 1996). Phylloquinone was extracted in methanol and after centrifugation at 14,000 rpm for 25 min, it was filtered and 20 μL of supernatants was injected to RP18 column and eluted with a mobile phase consisted of aqueous methanol and dichloromethane (90 and 10%, respectively) as well as methanolic solution containing $ZnCl_2$, Na acetate and acetic acid. Phylloquinone was detected by fluorescence detection (excitation, 243 nm; emission, 430 nm) and its concentrations were determined based on a standard calibration curve.

2.4.3. Biological activities

In vitro antioxidant capacity was measured by DPPH and FRAPS methods (Hamad et al., 2015). About 0.2 g of fresh fruits was homogenized in ethanol (80%) at 4 °C, then samples were centrifuged for 20 min at 14,000 rpm. To measure the DPPH % values, 0.1 mL of samples supernatants was mixed with 0.25 mL of DPPH solution. Sample and reagent mixture was shacked at room temperature and the absorbance of the mixture was measured at 517 nm. For FRAP assay, 150 μL of freshly prepared FRAP reagent (10 mM TPTZ and 20 mM $FeCl_3$ in 0.25 M acetate buffer, pH 3.6) was mixed with 50 μL of diluted samples. After 30 min of incubation at room temperature, absorbance was measured at 600 nm.

Antibacterial potential of fresh fruit ethanolic extracts was performed by the disc diffusion method (bacterial suspension containing 10^8 CFU/mL of bacteria spread on Muller Hinton agar) (Al Jaouni et al., 2018). Sterilized extracts were loaded on sterilized filter paper discs (5 μg/disc). These discs were putted on the top of agar media and

incubated for 24 h at 37 °C. Ethanol solvents were used as a negative. The inhibition zones were measured by Vernier caliper.

Antiprotozoal potential of fruit ethanol extract against *Trypanosoma cruzi* was measured (Al Jaouni et al., 2018). Rat skeletal myoblasts (L-6 cells) were used to assay the viability of *T. cruzi* trypomastigotes. The chlorophenol red-β-D-galactopyranoside (CPRG)-Nonidet substrate were mixed in micro-plates at 35 °C under 5% CO_2 for 72 h. The color of reaction was measured at A_{540} and reduction % was calculated.

Regarding anticancer activities of ethanolic extracts of fresh fruit, different human cancer cell lines were incubated in Dulbecco's Modified Eagle Medium (DMEM) contained 10% fetal calf serum (10%), sodium pyruvate, streptomycin (10 mg/1 mL) and penicillin (10 U/1 mL). The anticancer activities against carcinoma of colon (Colo205), kidney (293), hepatocellular (HepG2), and bladder (T24P) were detected by harvesting the incubated cancer cells (37 °C and 5% CO_2) using trypsinization (0.25% trypsin). CellTiter-Blue reagent used to determine vital cells (Solowey et al., 2014).

2.5. Statistical analyses

Normality and homogeneity of variances were carried out using the Kolmogorov–Smirnoff and Levene's tests, respectively. The field data presenting normality and homoscedasticity were analyzed by the parametric statistic, throughout the independent-Samples *t*-test. When field data were not normally distributed, the non-parametric Mann-Whitney *U* test was applied. Tukey's test was employed for multiple comparisons of means. The analyses were performed using the SPSS statistics software package, version 20.0 (IBM Corporation, USA) at $P \leq 0.05$ and $P \leq 0.01$ as probability levels.

3. Results

3.1. Characterization of the isolated actinobacteria

The target actinobacteria were isolated from palm tree rhizosphere and characterized at the morphological, biochemical and molecular levels (Table 1). All actinobacterial isolates possess aerial and substrate mycelia and can produce diffusible pigments. The isolates showed different substrate mycelial colors and their spores were long, either straight or flexuous, with mostly smooth and rarely spiny or hairy surfaces. Moreover, they could utilize different carbon and nitrogen sources (Table 1). The activity of antioxidant enzymes (catalase and peroxidase) and those involved in lipid hydrolysis, nitrogen utilization, nucleic acids and organic acids degradation were detected for the majority of the isolated actinobacteria.

Regarding the plant growth promoting assays, the different isolates vary in their capacity to produce IAA and siderophore, whereas the highest values were recorded for isolates 1, 3, 7 and 9 (Fig. 1). Those four isolates were selected for the subsequent experiments and were fully identified to the genus level based on their morphological characteristics and phylogenetic analysis of the 16S rRNA gene sequences. The results revealed that isolates 1, 7 and 9 belong to genus *Streptomyces* with the characteristic spore chain morphology of the genus (S1a, c and d), while the isolate number 3 was found to belong to the genus *Nocardiopsis* with fragmented mycelia and long spore chains with variable spore size (S1b). This taxonomical affiliation was supported by the results of phyllogenetic analysis (Data not shown).

3.2. Soil analysis

The inoculated actinobacteria improved soil chemical properties (Table 2). The actinobacteria did not affect pH and EC, but they increased organic matter and total phenolic contents in treated soils. Application of actinobacterial isolates also improved the nutrients availability, for instance, elevated levels of macronutrients e.g., N, P, Mg, Ca and K were recorded.

Table 1
Morphological and biochemical characterization of the actinobacterial strains isolated from local palm fields. The signs + and − indicate presence or absence, respectively. Three independent replicates were conducted for each assay.

	Isolates	1	2	3	4	5	6	7	8	9
Spore chain	Aerial mycelium	+	+	+	+	+	+	+	+	+
	Pigmentation	+	+	+	+	+	+	+	+	+
	Spiral	+	−	+	−	−	+	+	+	+
	Rectiflexibiles	+	+	−	+	+	−	−	−	−
	Verticillat	−	−	−	−	−	−	−	−	−
Substrate Mycelia	Brown	+	−	−	−	−	−	−	+	−
	Yellow	−	−	−	+	+	−	−	−	−
	Orange	−	+	−	−	−	+	−	−	+
	Gray	−	−	−	−	−	−	+	−	−
	Red	−	−	+	−	−	−	−	−	−
N source utilization	L-Cysteine	−	−	+	+	+	−	+	+	+
	L-Phenylalanine	+	+	+	+	+	+	+	−	−
	L-Histidine	+	−	+	−	+	−	+	+	+
	L-Lysine	+	−	+	+	−	−	−	+	−
	L-Asparagine	+	+	+	−	−	−	+	−	+
	L-Arginine	+	+	+	+	+	+	+	−	+
	L-Proline	+	−	+	+	−	+	−	−	+
	L-Valine	+	−	−	+	+	+	−	+	−
	Tyrosine	−	−	+	−	+	−	+	+	+
C source utilization	D-Fructose	+	−	+	+	+	+	+	+	+
	D-Glucose	+	+	+	−	+	−	+	+	−
	Sucrose	+	+	−	+	−	+	+	+	+
	Maltose	−	−	+	−	−	+	+	+	+
	Raffinose	−	+	+	+	+	+	+	−	+
	Lactose	+	−	+	+	−	−	+	+	−
	Galactose	+	−	−	+	+	−	−	+	+
	Meso-Inositol	+	−	−	+	+	−	+	+	+
	Celullose	−	+	+	−	−	+	−	−	+
	Xylose	−	−	−	+	+	+	+	+	+
	Dextran	+	−	−	+	−	+	−	−	+
Enzymes activity	Catalase	−	+	+	−	+	−	+	−	−
	Peroxidase	+	−	+	+	−	−	−	+	+
	Starch hydrolysis	+	−	+	−	+	+	−	−	−
	Gelatin liquefication	−	+	+	+	−	−	−	+	+
	Casein hydrolysis	−	+	−	+	−	−	+	+	−
	Lipolysis	+	+	+	+	+	−	+	−	+
	Citrate utilization	+	+	−	−	+	+	−	+	+
	Nitrate reduction	−	−	+	+	−	−	−	+	−
	Urease	−	+	−	−	−	+	+	−	−
	H_2S Production	+	−	−	+	+	+	−	+	+
	DNase	+	−	+	+	+	+	+	+	+

3.3. Date's yield and chemical composition

The date's yield of the treated cultivars was significantly induced (Table 2). The highest and lowest increases were recorded for Sokary and Ajwa cultivars (increases of 40 and 10 kg/tree, respectively).

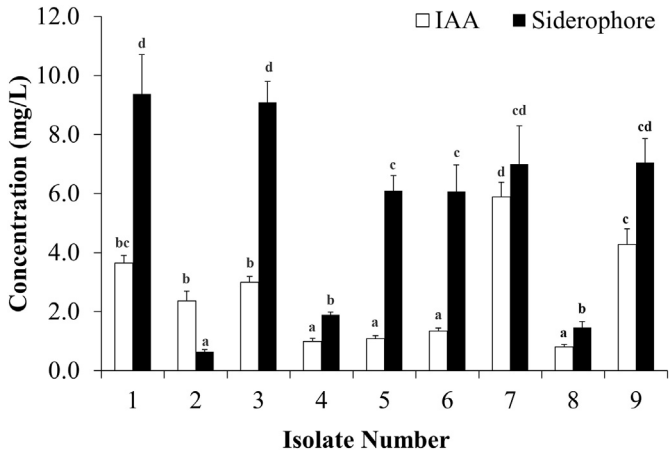

Fig. 1. Production of IAA and siderophore by the different actionbacterial isolates. Values are mean ± standard error of three independent replicates. Different lower-case letters within the same parameter indicate significant difference at the 0.05 probability level.

Table 2
Effect of actinobacterial inoculants on the properties of rhizospheric soil and the yield of five palm cultivars. Values are mean ± standard error of three independent replicates.

	Ajwa		Sokary		Khodry		Saffawy		Rashodia	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Soil properties										
EC ds/mL	3.7 ± 0.5	3.6 ± 0.4	3.1 ± 0.6	3.4 ± 0.7	3.8 ± 0.1	3.4 ± 0.5	4.1 ± 0.7	3.9 ± 0.5	3.7 ± 0.3	4.0 ± 0.8
pH	7.3 ± 0.6	7.7 ± 0.4	7.4 ± 0.5	7.3 ± 0.4	7.7 ± 0.6	7.5 ± 0.9	7.6 ± 0.8	7.4 ± 0.5	7.5 ± 0.1	7.7 ± 0.3
Ca^{++} (meq/L)	19.7 ± 4.0	21.5* ± 0.5	17.6 ± 0.7	23.1 ± 3.1	17.5 ± 1.1	22.1* ± 1.9	21.1 ± 0.7	31.7* ± 2.3	26.6 ± 2.1	28.3* ± 3.1
Mg^{++} (meq/L)	8.9 ± 0.4	10.2* ± 0.9	11.1 ± 0.2	16.5** ± 1.4	10.9 ± 0.2	17.1** ± 0.5	9.4 ± 0.3	12.5* ± 0.7	13.5 ± 0.7	17.4* ± 1.1
K^{+} (meq/L)	2.3 ± 0.2	3.4* ± 0.9	2.1 ± 0.7	3.6* ± 0.9	4.2 ± 0.2	6.2* ± 0.5	3.7 ± 0.6	5.2* ± 1.2	5.1 ± 0.4	7.4* ± 1.2
Na^{+} (meq/L)	8.1 ± 0.3	9.6* ± 0.9	17.3 ± 0.9	15.6* ± 0.5	13.8 ± 1.3	17.1* ± 1.2	11.8 ± 0.2	18.1* ± 1.0	12.0 ± 0.6	17.2* ± 1.5
Fe (ppm)	16.5 ± 1.0	19.5* ± 2.0	13.7 ± 0.9	15.3* ± 1.3	10.6 ± 0.8	18.5* ± 2.5	13.1 ± 1.1	21.1** ± 1.0	22.7 ± 0.2	27.6* ± 1.2
Mn (ppm)	15.6 ± 2.1	17.5* ± 1.0	16.3 ± 1.1	19.5* ± 1.1	21.7 ± 0.9	26.4* ± 0.5	16.3 ± 1.2	19.7* ± 1.1	17.4 ± 1.7	23.5* ± 0.8
Zn (ppm)	11.6 ± 1.1	14.1* ± 1.1	12.6 ± 0.8	16.7* ± 0.3	17.7 ± 1.2	23.5** ± 01.3	20.5 ± 2.1	33.5** ± 2.8	17.5 ± 2.5	23.1** ± 1.3
Cu (ppm)	9.5 ± 0.7	12.6* ± 1.7	10.3 ± 0.7	11.5* ± 1.9	9.45 ± 1.6	10.2* ± 0.9	11.3 ± 0.7	9.3* ± 0.8	10.6 ± 0.7	12.2* ± 2.2
P (ppm)	35.0 ± 2.3	40.2** ± 1.4	56.6 ± 3.3	65.3** ± 2.7	31.2 ± 1.8	40.5** ± 2.7	55.1 ± 2.1	59.5* ± 1.3	53.1 ± 2.9	71.3** ± 6.5
N (ppm)	100.0 ± 5.1	127.0* ± 8.2	117 ± 5.9	139.0* ± 7.1	102 ± 8.2	177.0** ± 8.1	92 ± 5.9	120.0** ± 6.3	125.0 ± 9.0	161.0** ± 11.9
Total phenols (mg/g soil)	90.0 ± 4.6	117.0* ± 6.4	98 ± 7.1	113.0* ± 6.6	97 ± 2.4	166.0* ± 6.2	97 ± 8.2	158.0* ± 3.2	125.0 ± 7.7	168.0** ± 8.3
OM (%)	1.3 ± 0.1	2.1* ± 0.3	2.15 ± 0.6	2.92* ± 0.7	1.7 ± 0.2	2.88* ± 0.3	1.09 ± 0.5	1.56* ± 0.8	2.38 ± 0.2	3.14* ± 0.5
Yield										
kg/tree	76.5 ± 3.2	86.6** ± 7.1	81.7 ± 6.9	119.8* ± 6.2	91.2 ± 7.9	112.4* ± 3.7	92.5 ± 6.9	117.7* ± 6.1	123.9 ± 18.9	152.0** ± 14.7

* Sign indicates significant difference from control at $P \le 0.05$.
** Sign indicates significant difference from control at $P \le 0.01$.

3.4. Primary metabolites levels in fruits of date palm

The inoculated actinobacteria significantly increased the levels of fructose, glucose and total soluble sugars for all treated cultivars (Fig. 2, S2). In contrast, the sucrose content in Khodry cultivar was significantly reduced. On fold change basis, the highest accumulation for soluble sugars was recorded in Sokary cultivar, while the lowest accumulation was detected in Rashodia and Saffawy (Fig. 2). Most of the detected organic acids were unaffected or reduced by the actinobacterial treatment (Fig. 2, S2). However, fumaric acid showed about 0.53-fold accumulation in the treated Khodry cultivar and citric acid was accumulated in Saffawy (Fig. 2).

The effect of the selected actinobacteria on the levels of amino acids in date palms was cultivar dependent (Fig. 2, S3). Amongst the essential amino acids, increased level of isoleucine was observed in treated Khodry dates (1-fold) and lysine in Saffawy and Rashodia (0.64 and 0.74-fold, respectively) compared to untreated palms. In Ajwa and Sokary, inoculation with actinobacteria significantly increased the contents of leucine and methionine. Similarly, the accumulation of phenylalanine was significantly induced in the treated Rashodia, Ajwa and Saffawy (0.86, 1.36 and 10.65-fold, respectively). Moreover, threonine content was higher in both treated Sokary and Rashodia dates, while tyrosine was only stimulated in Ajwa dates compared to control. In case of the non-essential amino acids, soil inculcation with actinobacteria significantly increased the alanine and asparagine contents in Khodry, cysteine and asparagine contents in Sokary, arginine content in Ajwa and Saffawy and glutamine in Rashodia dates compared to untreated palms (Fig. 2, S3).

Soil inoculation with actinobacteria affected the saturated (SFA) and unsaturated fatty acids (USFA) levels, where the effect was cultivar dependent (Fig. 2, S4). Regarding SFA, the majority of treated palm cultivars significantly contained lower contents of dodecanoic (C12:0), pentadecanoic (C15:0), octadecanoic (C18:0), docosanoic (C22:0) and tetradecanoic (C24:0) acids as compared with the respective control. In contrast, higher amounts of all USFA relative to the control were recorded in date fruits of all treated cultivars, except for eicosadienoic acid (C20:2) in Ajwa.

3.5. Secondary metabolites levels in fruits of date palm

Soil inoculation with actinobacteria differentially affected phenolic and flavonoids levels in the tested date cultivars (Fig. 2, S5). The treated palm dates attained elevated levels of ferulic, protocatechuic and syringic acids when compared to untreated palm dates. Majority of the treated dates had higher contents of ellagic acid, β-glucogallin and the total phenolic content ($P \le 0.01$). For the treated Ajwa, Sokary and Khodry, the increase in flavonoids content was non-significant. However, Saffawy and Rashodia cultivars grown in soils inoculated with actinobacteria were richer in flavonoids, particularly apigenin, isoquercitrin, luteolin, quercetin, rutin and velutin.

Regarding the concentrations of vitamins and/or pro-vitamins, actinobacterial treatment showed cultivar specific responses. Actinobacteria significantly induced α-tocopherols in Khodry and Saffawy dates and β-, ɣ-and δ-tocopherols in Ajwa, Sokary and Khodry (Fig. 2, S6). Besides, the ɣ-type was stimulated in the treated Saffawy dates, while the δ- type was elevated in those of Rashodia. Interestingly, all of the inoculated cultivars were abundant in the total tocopherols when compared with the control. Except for Saffawy, the dates of the treated cultivars contained significantly higher contents of β-carotene and thiamine. Significant induction in the β-cryptoxanthin contents was also manifested in all treated cultivars but for Sokary. Similarly, all cultivars except Khodry contained significantly higher amounts of vitamin K (phylloqinone), relative to the respective control.

3.6. Minerals content in fruits of date palm

In all tested cultivars, the dates of palms grown in actinobacteria-inculcated soil showed plentiful K and Mg contents, whereas Na content was not significantly altered (Fig. 2, S6). Except for Saffawy, dates of actinobacteria-inoculated palms contained a higher P content. Also, other minerals were induced in most of palm dates. On fold change basis, Cu was the most accumulated mineral in response to actinobacterial treatment, with the most accumulation detected in Rashodia cultivar (14.49-fold).

3.7. Biological activities of ethanolic extracts of date palm fruits

Fruits of date palm are rich in antioxidants and thus they are used traditionally to treat human diseases in many African and Asian countries. Here we measured the changes in antioxidant, antibacterial, anti-fungal antiprotozoal and anticancer activities as compared to control date fruits (Fig. 2, S7). Overall, the treated dates showed significantly higher antioxidant (FRAP and DPPH scavenging activity), antibacterial, antiprotozoal and/or anticancer activities. In particular,

Sugars	Ajwa	Sokary	Khodry	Saffawy	Rashodia
Glucose	0.85	0.78	0.76	0.74	0.70
Fructose	0.74	0.82	0.71	0.68	0.61
Sucrose	0.06	0.22	-0.12	-0.03	-0.06
Total soluble sugars	0.75	0.91	0.86	0.61	0.60
Total carbohydrates	0.76	0.81	0.74	0.64	0.63
Organic acids					
Citric acid	0.07	0.05	0.04	0.00	0.42
Fumaric acid	-0.10	0.05	0.53	-0.12	-0.23
Isobutyric acid	0.07	-0.05	-0.22	-0.02	-0.03
Malic acid	-0.11	-0.12	-0.16	-0.10	0.10
Oxalic acid	-0.15	-0.11	-0.24	-0.09	0.00
Succinic acid	0.07	-0.16	0.05	0.28	0.21
Essential amino acids (EAAs)					
Histidine	0.45	0.29	0.06	0.33	0.32
Leucine	0.51	0.60	0.46	0.26	0.25
Isoleucine	0.29	0.29	1.01	0.39	0.37
Lysine	0.45	0.14	0.42	0.74	0.64
Methionine	0.51	0.60	0.46	0.43	0.33
Phenylalanine	1.36	0.37	0.47	0.86	10.65
Threonine	0.14	0.81	0.31	0.20	0.58
Valine	0.71	0.86	0.54	0.40	0.64
Non-essential amino acids (NEAAs)					
Glutamlic acid	0.31	0.38	0.18	0.41	0.54
Glutamine	0.38	0.36	0.32	0.42	0.73
Alanine	0.22	0.43	1.07	0.20	0.04
Arginine	0.68	0.55	0.50	0.47	0.38
Ornithine	0.47	0.62	0.42	0.34	0.20
Proline	0.43	0.34	0.20	0.59	0.58
Asparagine	0.49	0.81	0.70	0.20	0.19
Serine	0.29	0.27	1.01	0.39	0.50
Glycine	0.27	0.40	0.19	0.56	0.32
Aspartate	0.35	0.36	0.43	0.31	0.21
Cystine	0.33	18.13	-0.03	0.41	0.38
Tyrosine	1.13	0.28	0.12	0.41	0.40
Saturated Fatty acids (SFA)					
C12:0	-0.30	-0.25	-0.35	-0.25	-0.26
C14:0	-0.28	-0.20	-0.30	-0.33	-0.15
C15:0	-0.24	-0.36	-0.20	-0.05	-0.19
C16:0	-0.49	-0.32	0.06	-0.37	-0.83
C17:0	-0.17	-0.32	-0.55	-0.24	-0.19
C18:0	-0.41	-0.28	0.08	-0.38	-0.44
C20:0	-0.12	-0.23	-0.20	-0.20	-0.21
C22:0	-0.22	-0.11	-0.18	-0.33	-0.40
C23:0	-0.26	-0.38	-0.43	-0.08	-0.08
C24:0	-0.26	-0.90	-0.09	-0.30	-0.35
C26:0	-0.44	13.67	-0.43	-0.25	-0.26
C25:0	1.08	1.55	0.87	0.60	0.60
Unsaturated fatty acids (USFA)					
C16:1	2.38	0.83	1.13	1.37	18.49
C18:1	0.94	1.00	0.91	0.66	0.71
C24:1	1.40	1.64	0.68	0.88	1.13
C16:2	0.91	0.77	0.95	0.85	1.27
C18:2	0.73	0.90	0.57	1.16	0.90
C20:2	0.36	1.09	0.67	0.59	0.94
C16:3	0.65	0.60	1.66	0.95	0.93
C18:3	1.23	0.86	1.02	1.06	0.66
Phenolic acids					
Caffeic acid	0.10	0.11	0.10	0.09	0.10
Chlorogenic acid	0.09	0.09	0.08	0.10	0.10

	Ajwa	Sokary	Khodry	Saffawy	Rashodia
Ellagic acid	1.06	0.66	0.89	0.82	0.66
Ferulic acid	0.08	0.12	0.15	0.10	0.13
Galic acid	0.10	0.10	0.09	0.08	0.10
p-Coumaric acid	0.10	0.08	0.11	0.10	0.09
Protocatechuic acid	0.09	0.12	0.15	0.10	0.13
Resorcinol	1.52	0.10	0.07	0.10	0.11
Syringic acid	0.11	0.12	0.10	0.09	0.12
β-glucogallin	0.98	0.44	0.30	0.54	0.59
Flavonoids					
Apigenin	0.70	0.56	0.75	0.68	0.61
Catechin	0.10	0.12	0.11	0.14	0.10
Quercetin	0.70	0.70	0.85	0.80	0.65
Quercetrin	0.09	0.86	0.10	0.15	0.14
Isoquercetrin	0.53	0.65	0.85	0.61	0.66
Luteolin	0.70	0.62	0.75	0.68	0.73
Rutin	0.80	0.65	0.87	0.72	0.66
Velutin	0.72	0.67	0.76	0.68	0.66
Total flavonods	0.37	0.66	0.50	0.83	1.20
Total Phenolics	1.01	-0.16	0.68	1.12	2.12
Minerals					
N	0.50	-0.04	1.48	-0.09	1.73
K	0.24	0.25	0.22	0.27	0.28
Mg	0.27	0.22	0.32	0.28	0.30
P	0.18	-0.66	-0.39	0.04	1.49
Na	0.27	0.27	0.23	0.25	0.24
Cu	2.90	8.46	8.97	0.76	14.49
Fe	0.31	0.28	0.28	0.27	0.25
Mn	0.27	0.30	0.26	0.25	0.22
Zn	0.27	0.33	0.31	0.22	0.22
Vitamin E					
α-tocopherol	0.24	0.24	0.37	0.25	0.25
β-tocopherol	0.28	0.29	0.27	0.23	0.23
γ-tocopherol	0.28	0.29	0.27	0.26	0.24
δ-tocopherol	0.21	0.33	0.24	0.22	0.29
Total tocopherol	0.31	0.34	0.28	0.26	0.30
Vitamin A					
α-Carotene	0.24	0.23	0.37	0.25	0.27
β-Carotene	0.43	0.25	0.27	0.34	2.11
β-Cryptoxanthin	0.23	0.26	0.22	0.29	0.24
Vitamin B					
Thiamine	0.25	0.25	0.26	0.24	0.22
Vitamin K					
Phylloquinone	0.24	1.80	0.18	0.26	0.25
Antioxidant Activity					
FRAP	0.46	0.74	0.81	0.79	0.78
DPPH (%)	0.37	0.92	0.65	3.18	8.74
Antiprotozoal					
Trypanosoma cruzi	0.54	0.54	0.64	1.34	3.02
Antibacterial					
Streptococcus sp.	0.75	0.68	0.62	1.00	1.63
Escherichia coli	0.27	0.33	0.67	1.75	6.19
Antifungal					
Candida albicans	0.38	0.45	0.64	1.34	3.02
Anticancer					
HepG2	0.55	-0.09	0.28	0.16	0.07
Colo205	0.21	0.18	0.15	0.34	0.49
293	0.06	0.20	0.09	0.21	0.33
T24P	0.11	0.32	0.29	0.34	0.39

Fig. 2. Heatmap of fold change ratios in metabolite, vitamins, minerals and biological activities in the fruits of five date palm cultivars in response to soil inoculation with plant growth promoting actionbacteria. Fold change in each metabolite was calculated relative to its corresponding mean (n = 3) in the control plant. As shown in the color scale, red indicates inhibition, white no change and blue improvement in metabolite levels as affected by actinomycetes.

Khodry, Saffawy and Rashodia grown in soil enriched with actinobacteria, had the strongest antiprotozoal capacity (0.64, 1.34 and 3.02-fold, respectively). Moreover, the antibacterial activity against *Streptococcus* sp., *Escherichia coli* and *Candida albicans* was also higher in the treated cultivars where the highest increases were recorded for Rashodia and Ajwa, respectively.

The anticancer activities were improved in fruits of treated date palms in a cultivar dependent manner. The activity against hepatocellular carcinoma (HepG2) was only increased in the treated Ajwa, Ruthan and Saffawy fruits. Significant inhibitory effects against colon carcinoma (Colo205) were recorded for the extract of the treated Ajwa and Sokary. Moreover, Sokary, Saffawy and Rashodia dates produced in

actinobacteria-treated soil showed improved activities against embryonic kidney adenocarcinoma (293), when compared with control palms. On the other hand, all treated dates manifested higher activity against the urinary bladder carcinoma (T24P cell line).

4. Discussion

In this study, we have investigated the biofertilization effect of actinobacteria on five different cultivars of date palm in a semi-arid region, Kingdom of Saudi Arabia (KSA), with respect to fruit yield, nutritional and health-promoting values. In addition, the concomitant changes in biological activities of the treated fruits were elucidated. To our knowledge, this is the first study indicating the possible application of actinobacteria for these purposes.

4.1. Actinobacteria treatment improves date palm yield by enhancing soil fertility

Growing date palm cultivars in soil inoculated with actinobacteria promoted fruit yield for all the tested cultivars (Table 2). Such enhanced fruit yield points to the biofertilization capacity of the applied actinobacteria. Supporting this hypothesis, actinobacteria-inoculated soils showed higher organic matter and nutrients availability (Table 2). In this regard, actinobacteria are known to improve the bioavailability of soil nutrients through its potential role in organic recycling, nitrogen fixation and phosphate solubilization (Battini et al., 2017). The production of organic acids, phenols and ammonia has been also reported for several actinobacteria (Jog et al., 2012; Rodríguez and Fraga, 1999).

Moreover, actinobacteria can produce growth-promoting metabolites such as phytohormones and siderophores (Anwar et al., 2016). Phytohormones such as IAA, are positively correlated with a better yield of crops, as they indirectly enhance the uptake of water and soil nutrients by stimulating root growth and formation (Idris et al., 2007). Besides, the produced siderophores by actinobacteria can bind Fe^{3+} from the environment and make it available for the target plants (Anwar et al., 2016). From another point of view, the higher productivity of the treated palms may be attributed to the increase in phenolic ingredients of the soil, which could reduce the susceptibility of plants to soil-borne pathogens, and therefore maintain the yield. In this context, several actinobacterial strains have been reported as powerful biocontrol agents against a wide range of root pathogenic fungi (Franco-Correa et al., 2010).

4.2. Soil enrichment with actinobacteria supports the nutritional value of date palm fruits

In general, food nutritional quality is determined by various factors, including sugars, organic acids, proteins, lipids, minerals and vitamins, as well as secondary metabolites such as phenolic acids and flavonoids. Therefore, it is important to have an adequate knowledge of the changes in chemical composition of crops for the sake of the human health.

In the present study, palm dates grown in actinobacteria-inoculated soil showed higher levels of primary metabolites such as sugars, amino acids and unsaturated fatty acids (Fig. 2). Physiologically, the strong correlation between the photosynthetic efficiency of plants and the availability of soil nutrients is well understood (Evans, 1989). In this context, the induced fertility of actinobacteria-inoculated soil was concomitant with elevated levels of the different sugar fractions. Such higher availability of sugars could improve the synthesis of other metabolites, whereas, by their degradation during the process of dark respiration, sugars provide the C-skeleton and energy required for the synthesis of other classes of phytochemicals, including amino acids, fatty acids, vitamins and various secondary plant metabolites (Brown et al., 2010; Nunes-Nesi et al., 2010).

From a nutritional prospective, the accumulation of soluble sugars could improve the taste and flavor of the treated fruits (Malundo et al., 2001). Moreover, the higher levels of essential amino acids substantially enhanced the nutritive value of the treated dates, being one of the most important dietary sources of the plant-based proteins (Al-Alawi et al., 2017). Interestingly, we recorded the accumulation of USFA at the expense of SFA. USFA were considered as cardioprotective ingredients, whereas replacing foods rich in SFA by foods rich in USFA was of significant effect in reducing the cardiovascular disease (CVD) risk by reducing blood cholesterol (Wang and Hu, 2017). Moreover, the reduction in the levels of oxalic acid in actinobacteria-treated fruits reported herein for some date palm cultivars supports its functional food value, whereas higher levels of dietary oxalate is linked to formation of oxalate kidney stones (Bsc and Bsc, 1999).

4.3. Actinobacteria treated-date fruits accumulated higher levels of bioactive compounds and possessed induced biological activities

Plants are characterized by rational utilization of their resources, whereas the surplus of C and N skeletons is exploited in the synthesis of a variety of biologically active compounds such as vitamins and phenolic compounds (Herms and Mattson, 1992). In accordance, the accumulation of C and N compounds reported herein, in response to actinobacteria treatment, was concomitant with improvement in the content of bioactive compounds, especially flavonoids, and vitamins (Fig. 2). This could support the health promoting value of the produced fruits due to the significant contribution of these phytochemicals in the therapeutic potential of date palm (Al-Alawi et al., 2017). Supporting this interpretation, the present study revealed that the treated palm trees could produce dates with enhanced biological activities with respect to their antioxidant, antibacterial, antifungal and anticancer activities.

The enhanced antioxidant capacity of the treated date fruits, ascertained by FRAP and DPPH scavenging activities, could be attributed to induction of some phenolic acids, flavonoids, vitamins B and E and minerals like Cu, Zn, and Mn. Higher levels of antioxidants prevent the accumulation of free radicals which could damage cell compartments and, consequently, may induce cancer and aging related diseases (Zhang et al., 2013). In addition to tocopherols (vitamin E), the health benefits of thiamine (vitamin B_1) and phylloquinone (vitamin K) were heavily reported (Hamad et al., 2015). Moreover, the polyphenolic ingredients of food products have been linked to a number of potential health benefits (Muthukumaran et al., 2017). They were also reported to have antioxidant, antiallergic, anti-inflammatory, immunomodulator and other pharmaceutical applications (Bidlack et al., 2000). Besides, the health benefits of dietary flavonoids have been reported (Yao et al., 2004). Our findings suggest a positive correlation between the antibacterial, antiprotozoal and anticancer activities of the treated dates and their total contents of phenolics and flavonoids.

5. Conclusion

Based on the present results, the potential application of actinobacteria to ameliorate the overall soil fertility is recommended. Date fruits of the actinobacteria-treated palms attained higher contents of many ingredients of nutritional interest such as sugars, inorganic minerals, amino acids, USFA, vitamins and antioxidants and reduced levels of anti-nutritional factors such as SFA and oxalic acid. Furthermore, treated palm fruits had better biological activities with respect to their antioxidant, antimicrobial, antiprotozoal and anticancer properties. Such improved activities could be attributed to the induced accumulation biologically active metabolites such as tocopherols, carotenes, phenolic acids and flavonoids. Overall, incorporation of actinobacteria in soil is a promising eco-friendly approach for the sustainable development of date palms with respect to their yield and nutritional and health-promoting values.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.02.140.

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