Egyptian Journal of Biological Pest Control

RESEARCH Open Access

Influence of hydrogen cyanide-producing rhizobacteria in controlling the crown gall and root-knot nematode, *Meloidogyne incognita*

A. F. Abd El-Rahman*, Hanan A. Shaheen, Rabab M. Abd El-Aziz and Dina S. S. Ibrahim

Abstract

Out of 39 isolates of rhizobacteria, recovered from economic plants grown in 8 locations in Egypt, 6 isolates were able to produce Hydrogen Cyanide (HCN). 16S rRNA sequence analysis identified these isolates as: *Pseudomonas japonica* strain NBRC 103040, *Bacillus megaterium* strain CtST3.5, *Pseudomonas* sp. strain Gamma-81, *P. tolaasii* strain ATCC 33618, *P. chlororaphis* strain Lzh-T5, and *P. mosselii* strain CV25. These HCN producers were able to inhibit growth of *Agrobacterium tumefaciens* and affect viability of *Meloidogyne incognita* juveniles in vitro. The isolates of *P. japonica* and *Pseudomonas* sp. Gamma-81 prevented the gall formation on tomato plants by *A. tumefaciens*, regardless of the presence of *M. incognita*. The isolates of *B. megaterium*, *P. chlororaphis*, *P. tolaasii*, and *P. mosselii* decreased the weight and number of galls produced by *A. tumefaciens* in the presence or absence of *M. incognita*. The 6 HCN producers decreased the population of *M. incognita* and the number of nematode galls than the positive control, when used against *M. incognita*. A similar effect was achieved against mixed infections with *M. incognita* and *A. tumefaciens*. The HCN-producing rhizobacteria, in the presence of *A. tumefaciens* and/or *M. incognita*, caused obvious increment in all growth parameters of tomato than the negative control and healthy plants. The only exception was found in case of *Pseudomonas* sp. Gamma-81 against *M. incognita* and against mixed infection, where growth parameters of tomato were decreased. Although the isolates were naturally isolated from the rhizosphere of economic plants, it must be cautiously considered since the isolate identified as *P. japonica* has been reported as a human pathogen. Also, *P. tolaasii* was reported causing a bacterial blotch on cultivated mushrooms under certain environmental conditions. Further investigations are needed.

Keywords: Crown gall, Root-knot nematode, Antagonistic bacteria, Hydrogen cyanide (HCN), Biological control

Background

Crown gall disease caused by *Agrobacterium tumefaciens* is a major bacterial disease in nurseries and orchards and is being considered one of the most important disorders that causes high losses in nurseries (Pulawska 2010). Biological control of crown gall is more effective in controlling the disease rather than chemical control (Tolba and Soliman 2013). Root-knot nematodes, *Meloidogyne* spp., are among the most important plant fauna that limit the productivity of many economic crops. The search for biological means for controlling root-knot nematodes (RKNs) is important because of inability of plants to resist most types of RKNs, and the chemical control is subject to debate due to the environmental hazards and human health damage (Mostafa et al. 2014). The interrelation between nematode densities and increasing of crown gall on the roots was reported for many crops references. Occurrence of wounds by nematodes allows incorporation of T DNA (the transferred DNA of the tumor-inducing (Ti) plasmid) of bacteria into the genome of plant cell and development of crown gall disease (Rubio-Cabetas et al. 2001).

Cyanide forms stable complexes with the essential elements (Cu^{2+}, Fe^{2+}, and Mn^{2+}) for the protein function and therefore is considered a toxic substance to most living

* Correspondence: aabdelrahman2012@gmail.com
Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt

organisms. Hydrogen cyanide (HCN) is a volatile secondary metabolite that is synthesized by many rhizobacteria and has a powerful effect on many organisms. HCN inhibits the electron transport and disrupts the energy supply to the cell, which leads to death of living organisms. Many bacterial genera have the ability to produce HCN including species of *Alcaligenes*, *Aeromonas*, *Bacillus*, *Pseudomonas*, and *Rhizobium* (Alemu 2016).

The ability of some bacterial strains to control nematodes was attributed to their ability to produce HCN. Moreover, a direct influence of contact between HCN-producing bacteria and the nematode is essential in the effective control of target nematode (Siddiqui et al. 2006). HCN may affect plant establishment or inhibit development of plant disease, with great potential for controlling plant bacterial diseases (Lanteigne et al. 2012). Some studies have questioned of HCN based on the lack of correlation of the level of HCN produced by rhizobacteria with the biocontrol effects in vitro. These studies have suggested that HCN does not act directly in the process of biocontrol, but involved in geochemical processes in the substrate (e.g., chelation of metals) leading to an indirect increase in the nutrients availability for the rhizobacteria and their plant hosts (Rijavec and Lapanje 2016).

The purposes of this work were (1) to isolate and identify HCN-producing rhizobacteria from different locations in Egypt, screen the ability of these rhizobacteria in inhibiting *A. tumefaciens,* and evaluate their influence on *Meloidogyne incognita* in vitro; (2) to study the correlation of HCN levels produced by rhizobacteria with the biocontrol effects in vitro; and (3) to study the influence of these HCN-producing rhizobacteria on the growth of tomato plant, the crown gall disease, the root-knot nematode infection, and the mixed infection by these two pathogen in vivo.

Materials and methods

Sources of the pathogens

The crown gall bacterium, *A. tumefaciens* (virulent isolate), was previously isolated from peach and identified by Abd El-Rahman (2012). The pathogenicity test was re-performed to check virulence. In order to confirm the identification, the 16S rDNA analysis was used. The sequencing process was conducted at the Potato Brown Rot Project laboratories (Giza, Egypt), using forward primer (U968-f) 5′-AACGCGAAGAACCTTAC-3′ and reverse primer (L1401-r) 5′-CGGTGTGTACAAGACCC-3′ as described by Farag et al. (2017). The RKN, *M. incognita* inoculum, was obtained from Nematode Diseases Research Department, Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

Isolation of rhizobacteria

Rhizobacteria were isolated from rhizosphere of different crops from 8 locations in Egypt. Serial dilution plate count technique was followed, using soil adhering to the roots of screened plants as described by Kifle and Laing (2016). Nutrient glucose agar and King's B agar media were used for the isolation of bacteria. The plates were incubated at 28 °C for 3 days. The separate colonies were selected and stored as pure cultures.

Screening hydrogen cyanide (HCN) produced by rhizobacterial isolates

Assessment of HCN produced by rhizobacterial isolates was carried out. Appropriate amount of each isolate was inoculated in 250-ml flask (3 flasks/isolate), containing King's B broth medium (100 ml) amended with 4.4 g/l glycine. Non-inoculated flasks were used as a control. Sterile filter paper strip was dipped in picric acid solution (0.5% picric acid in 2% sodium carbonate) and was attached to the neck of the flask. The flask was plugged and sealed off with Parafilm. Incubation was made at (28 ± 2 °C) for 4 days, with shaking at 140 rpm. A change in color of the filter paper strips from yellow to light brown, brown, or brick red was recorded as weak (+), moderate (++), or strong (+++) reaction, respectively. No change in color was recorded as a negative (−) reaction (Abd El-Rahman and Shaheen 2016).

Identification of selected isolates of rhizobacteria

Identification of selected rhizobacteria was made, using 16S rRNA analysis at Sigma Scientific Services Co., Giza, Egypt, as described by Abd El-Ghany et al. (2017). DNA extraction was made, using of GenJet™ genomic DNA purification Kit (ThermoK0721), according to the manufacturer's protocol by gram-negative bacteria genomic DNA purification protocol. PCR was made, using Maxima Hot Start PCR Master Mix (Thermo K 1051), according to the manufacturer's protocol by using forward primer 5′-AGAGTTTGATCCTGGCTCAG-3′ and reverse primer 5′-GGTTACCTTGTTACGACTT-3′ (Eden et al. 1991). The PCR products were purified using GeneJet™ PCR purification Kit (Thermo K0701). Finally, the PCR products were sequenced, using ABI 3730 × 1 DNA sequencer, using forward and reverse primers. Obtained sequences were compared to those available in the database GeneBank network services at http://blast.ncbi.nlm.nih.gov/Blast.cgi for significant alignments.

Quantitative assay of hydrogen cyanide (HCN) production

Suspension (3 ml of 10^6 cfu/ml) of each bacterial isolate was inoculated in (250 ml) flask containing King's B broth medium (100 ml) amended with glycine (4.4 g/l). Three replicates were used for each tested isolate. Non-inoculated flasks were used as control. Strips of filter

paper (10 × 0.5 cm) were soaked in alkaline picrate solution and kept hanging to the neck of the flask (one strip for each flask). The flasks was plugged and sealed off with Para film. Three replicates were used for each isolate. After incubation at 28 ± 2 °C for 4 days (with shaking at 140 rpm), the color of sodium picrate strips was changed to a reddish compound in proportion to the amount of HCN evolved. The color was eluted by placing the changed filter paper in a test tube containing 10 ml of distilled water, and its absorbance was read by the UV/Visible spectrophotometer (MODEL:2000 UV – UNICO INSTRUMENTS CO., LTD,USA) at 625 nm. Distilled water was used to adjust the zero absorbance reading at 625 nm before reading (Reetha et al. 2014).

Preparation of *A. tumefaciens* and rhizobacteria inoculum

Each bacterial isolate was grown in King's B agar medium plates for 48 h at 28 °C, and then, bacterial growth was harvested into a saline solution (0.85% NaCl) to prepare bacterial suspension that was adjusted to 10^8 cfu/ ml.

Preparation of *M. incognita* inoculum

Fresh second-stage juveniles (J_2) of *M. incognita* were obtained from pure culture maintained on coleus (*Coleus blumei*) roots. Roots were incubated for 5–7 days in a modified Baermann method for hatching at room temperature (Ibrahim et al. 2013).

In vitro antagonistic determination of rhizobacteria against gall-forming bacteria and second-stage juveniles

Certain rhizobacterial isolates were screened for their ability to inhibit the growth of *A. tumefaciens* on KBA medium plates. A loopful of the tested isolate (24 h old culture) was placed at the center of inoculated plate with *A. tumefaciens*, using 3 ml of 10^8 cfu/ml suspension for flask containing 250 ml medium. Three replicates were used for each tested isolate. The plates were incubated at 28 °C for 48 h, and the inhibition zone diameter was measured to the nearest millimeter (Abd El-Rahman and Shaheen 2016).

Detrimental effect of bacterial isolates against (J_2) of *M. incognita* was evaluated. One milliliter (10^8cfu/ml) of bacterial isolates was added to 4 ml of *M. incognita* suspension containing 100 juveniles (J_2) in a Petri dish (6 cm diameter). One milliliter of saline solution (0.85% NaCl) instead of bacterial suspension was used as control. Three replicates from each treatment were made. All dishes were sealed off with Parafilm and kept at the room temperature (30 °C). The dishes were examined under a microscope after 24, 48, and 72 h and then left for 7 days and checked for the last time. The number of surviving and dead larvae was counted. Percentage mortality due to the effect of bacterial isolate was calculated as follows:

Mortality % = [(number of live nematode juveniles in control – number of live nematode juveniles in treatment)/number of live nematode juveniles in control] × 100 (Abdel-Salam et al. 2018).

In vivo experiment

The effect of selected isolates of rhizobacteria on growth of tomato plant, crown gall disease, the RKN, and the mixed infection by these 2 diseases was tested in a greenhouse (22 ± 3 °C). Each pot (20 cm in diameter) containing 800 g clean soil (clay to sand to compost at 2:1:1 ratio) was planted by one of 3 weeks old tomato (cv. Super Strain B) seedlings. The first group of pots was allocated to study the effect of rhizobacteria on crown gall disease, the second one for the effect of rhizobacteria on RKN, and the third was to test the effect of rhizobacteria on the mixed infection with crown gall bacteria and root-knot nematode. Pots were arranged in a completely randomized design and treated as needed experimentally. Five replicates per treatment were used. All treatments were run 10 days after planting. Five pots were left free of nematode, *A. tumefaciens*, and rhizobacteria as un-inoculated (healthy plant) treatment for the 3 experiment groups. Five pots pricked at the crown region of tomato plants were used as negative control for the first and third experiment groups.

For the first group, the seedlings were pricked at the crown region of tomato plants, and the soil was drenched with suspension (10^8 cfu/ml) of bioagent (100 ml/pot) along with suspension (10^8 cfu/ ml) of *A. tumefaciens* isolate (100 ml/pot) together at the same time. Five inoculated pots with *A. tumefaciens* were served as a positive control. For the second group, the soil was drenched by the suspension (10^8 cfu/ml) of bioagent at the rate of 100 ml/pot (five pots treated with oxamyl 0.3 ml/pot was used for comparison), and after 48 h, inoculation was made with 2000 s-stage juveniles/plant of *M. incognita*. Five inoculated pots with *M. incognita* were served as a positive control. The same treatments in the first group were prepared as a third trial, and after 48 h, the seedlings were inoculated by 2000 of the second-stage juveniles/plant of RKN. Five inoculated pots with *A. tumefaciens* and *M. incognita* served as a positive control.

Pathological determination

Determination for *A. tumefaciens* and *M. incognita* syndromes were recorded after 45 days of inoculation. Weight (g) and number of galls of *A. tumefaciens* were recorded, in the first and the third groups. Percentage of decreasing in crown gall disease (PDD) was calculated from the weight and number of galls as follows: PDD = [(C – T (/

C] × 100 where C = weight or number of galls in control and T = weight or number of galls in treatment.

Nematode population in soil (250 g), number of developmental stages and females in roots, number of galls, and number of egg masses of *M. incognita* were recorded in the second and the third groups of treatments. The final population (Pf) of nematode was calculated as follows: nematode population in soil + no. of developmental stages + no. of females. Reproduction factor (RF) was calculated as follows: final population (Pf) in treatment/no. of treatment initial inoculum. The percentage of reductions in nematode population (red %) was calculated as follows: Red% = $[(Pf_c - Pf_t)/Pf_c] \times 100$ where Pf_c = the final population in control and Pf_t = the final population (Pf) in treatment. Root gall index (RGI) or egg masses index (EI) was determined according to the scale given by Taylor and Sasser (1978) as follows: 0 = no galls or egg masses, 1 = 1–2 galls or egg masses, 2 = 3–10 galls or egg masses, 3 = 11–30 galls or egg masses, 4 = 31–100 galls or egg masses, and 5 = more than 100 galls or egg masses.

Plant growth parameters

Growth parameters (shoot length, shoot fresh weight, and root fresh weight) of tomato plants were recorded after 45 days of inoculation at all treatments. The dry weight of the whole plant is estimated after drying the vegetative growth and roots of plant in oven at 100 °F (37.8 °C) overnight.

Statistical analysis

Completely randomized design was used in all in vitro and in vivo pot experiments. Collected data were subjected to analysis of variance. Comparisons among treatment means were made, using Duncan multiple range test at 0.05 level of probability, using MINITAB software (version 16, MINITAB, Inc., State College, PA).

Results and discussion

Confirmation the identity of the crown gall bacterium *Agrobacterium tumefaciens* (virulent isolate)

Bacterial isolate, previously identified as *A. tumefaciens* on the basis of the traditional bacteriological methods, was further confirmed by 16S rDNA analysis. The results showed that *A. tumefaciens* (virulent isolate) showed (98.41%) similarity with *A. tumefaciens* strain IAM 12048 and *A. tumefaciens* strain NCPPB2437. Distance tree, using rRNA type strains/Bacteria and Archaea_16S_ribosomal RNA sequences database, is illustrated in (Fig. 1).

Ability of rhizobacteria to produce hydrogen cyanide (HCN)

Many bacterial genera had the ability to produce HCN embracing many species of *Alcaligenes*, *Aeromonas*, *Bacillus*, *Pseudomonas*, and *Rhizobium* (Alemu 2016). Thirty-nine isolates of rhizobacteria, recovered from different locations and different crops in Egypt, were tested for their ability to produce hydrogen cyanide (HCN). Only 6 isolates were able to produce HCN. The 6 HCN producing isolates were isolated from rhizosphere soil of

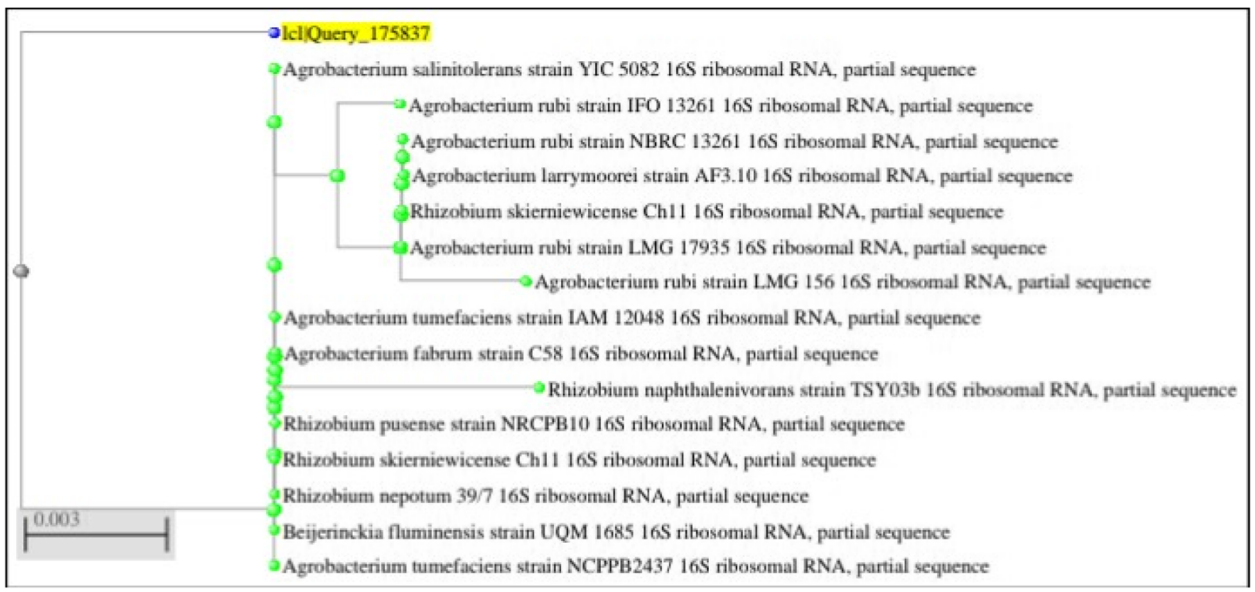

Fig. 1 Distance tree using rRNA type strains/bacteria and Archaea_16S_ribosomal RNA sequences database. lcl|Query _ 175837 refers to the *A. tumefaciens* (virulent isolate)

guava plants grown in Behiera governorate (BR11 and BR13), peach plants grown in Giza governorate (GA21), potato plants grown in Ismailia governorate (SW1 and SW4), and corn plants grown in Beni-Seuf governorate (BW3). A strong (+++) HCN production was recorded by the isolate BR13 from guava; while a moderate (++) reaction was recorded by BR11, GA 21, and SW1 from guava, peach, and potato, respectively. A weak (+) reaction was recorded by BW3 and SW4 from corn and potato, respectively (Table 1).

16S rRNA analysis

Results of DNA sequence, obtained from 16S rRNA analysis of the 6 HCN isolates collected from different plant rhizosphere and locations in Egypt compared to DNA sequence available at the NCBI database, are shown in (Table 2). Comparison of the DNA sequences of SW1, BW3, SW4, BR 11, BR 13, and GA 21 with DNA sequence in the NCBI database identified these isolates as: *Pseudomonas japonica* strain NBRC 103040, *Bacillus megaterium* strain CtST3.5, *Pseudomonas* sp. strain Gamma-81, *P. tolaasii* strain ATCC 33618, *P. chlororaphis* strain Lzh-T5, and *P. mosselii* strain CV25 by percent of 97, 98, 100, 99, 99, and 99), respectively.

Quantitative assay of HCN production by bacterial isolates

Quantitative assay of HCN production by bacterial isolates showed that *P. chlororaphis* greatly produced HCN and recorded the maximum absorbance value of (0.047), followed by *P. mosselii*, *P. tolaasii*, and *P. japonica*, which recorded absorbance values of 0.027, 0.024, and 0.020), respectively. The lowest absorbance values (0.002 and 0.005) were recorded by *B. megaterium* and *Pseudomonas* sp. Gamma-81, respectively (Table 3).

Efficacy of HCN-producing rhizobacteria on growth of *A. tumefaciens* and *M. incognita* in vitro

The results reported herein (Table 4) showed that the 6 isolates of HCN-producing rhizobacteria could inhibit the growth of *A. tumefaciens* in vitro. The inhibition zone diameter ranged from 13.7 to 49.7 mm. The maximum inhibition (49.7 mm) was recognized by the *P. japonica* isolate, followed by *P. mosselii*, *Pseudomonas* sp. strain Gamma-81., *P. chlororaphis*, *P. tolaasii*, and *B. megaterium*, which recorded 33.7, 30.7, 25.7, 14.3, and13.7 mm), respectively.

Efficacy of 6 isolates of HCN-producing rhizobacteria on *M. incognita* under laboratory conditions was shown in (Table 5) as a percentage of mortality of 100 juveniles (J_2) of *M. incognita* after different exposure periods to bacterial suspension. The results showed that *P. japonica*, *P. chlororaphis*, and *P. mosselii* had influenced J_2 survival after 24 h incubation. The mortality rate of juveniles reached 24.3, 38.0, and 23.0%, respectively. By increasing the exposure time, all bacterial isolates affected the life of nematode juveniles. The rate of mortality of the juveniles ranged from 23.7 to 49.2% and from 32.3 to 68.6% after 48 and 72 h, respectively. After 7 day of incubation, the maximum mortality percent was achieved by *P. chlororaphis* isolate, recording 96.4 with significant differences with *Pseudomonas* sp. Gamma-81, *P. mosselii*, *P. japonica*, *B. megaterium*, and *P. tolaasii*, which recorded mortality percent of 63.9, 54.6, 51.8, 43.9, and 42.8, respectively.

In this regard, some studies have questioned the biocontrol effect of HCN based on the findings on the lack of correlation between the level of HCN produced by rhizobacteria and the antagonistic effects in vitro (Rijavec and Lapanje 2016). From these results, the correlation of the level of HCN, produced by rhizobacteria with the biocontrol effects in vitro, was more clearly in the case of *M. incognita* nematode than the case of *A.*

Table 1 Source of rhizobacterial isolates and their tentative ability to produce HCN

Governorate	Location	Type of crop	No. of tested isolates	No. of HCN producing isolates	HCN producing isolate code	HCN producing reaction*
Beni Suef	Al-Wasta	Corn	4	1	BW3	+
Monufia	Cairo–Alex. desert Rd(Dina farms)	Corn	5	0	–	–
Minya	Mallawi	Onion	4	0	–	–
Beheira	Rashid	Guava	7	2	BR11	++
					BR13	+++
Beheira	International Coastal Rd	Apple	4	0	–	–
Beheira	Abu Al Matamir	Banana	4	0	–	–
Giza	Abu Rawash	Peach	5	1	GA 21	++
Ismailia	Wadi El-Mollak	Potato	6	2	SW1	++
					SW4	+

*Negative (–), weak (+), moderate (++) or strong (+++) reaction

Table 2 Molecular identification of six plant rhizosphere isolates using 16S rRNA gene sequence

Isolates code.	Identification by 16S rRNA sequence analysis	Max score	Total score	Query cover	E. value	Max ident.
SW1	*Pseudomonas japonica strain* NBRC 103040	1524	1524	99%	0.0	97%
BW3	*Bacillus megaterium* strain CtST3.5	1334	1334	99%	0.0	98%
SW4	*Pseudomonas* sp. strain Gamma-81	1314	1314	100%	0.0	100%
BR11	*Pseudomonas tolaasii* strain ATCC 33618	1149	1149	100%	0.0	99%
BR13	*Pseudomonas chlororaphis* strain Lzh-T5	1528	7615	100%	0.0	99%
GA 21	*Pseudomonas mosselii* strain CV25	1650	1650	99%	0.0	99%

tumefaciens bacteria, although this correlation was not a complete correlation relationship in the both cases.

Effect of HCN-producing rhizobacteria on incidence of crown gall on tomato plants

Effect of 6 isolates of HCN-producing rhizobacteria on incidence of crown gall caused by *A. tumefaciens* on tomato plants in the absence or presence of *M. incognita* after 45 days of inoculation was shown in (Table 6). Results proved that the isolates of *P. japonica* and *Pseudomonas* sp. Gamma-81 prevented completely gall formation on tomato plants in both cases. In the absence of *M. incognita*, the isolates of *B. megaterium*, *P. chlororaphis*, *P. tolaasii*, and *P. mosselii* decreased the weight of galls caused by *A. tumefaciens* on tomato plants by 92.1, 67.0, 60.8, and 49.9%, respectively. Also, a pronounced decrement in the number of galls by 93.3, 52.0, 56.0, and 48.0% was recognized, respectively. In the presence of *M. incognita*, the isolates of *B. megaterium*, *P. chlororaphis*, *P. tolaasii*, and *P. mosselii* decreased the gall weight produced by *A. tumefaciens* on tomato plants by 80.2, 81.7, 64.8, and 53.0% and decreased the number of galls by 81.7, 73.9, 62.5, and 57.0%, respectively. Although the isolate of *B. megaterium* showed a high ability to control crown gall disease in the absence of *M. incognita*, however, this capability was decreased significantly in the presence of *M. incognita* by 11.9% for gall weight and by 11.6% for number of galls. Meanwhile, though the isolates of *P. tolaasii*, *P. chlororaphis*, and *P. mosselii* showed a moderate ability to control crown gall in the absence of *M. incognita*, the capability increased in the presence of *M. incognita* by 4.0, 14.0, and 3.1% for gall weight and by 6.5, 21.9, and 9.0% for the number of galls, respectively. This is despite the fact that *P. chlororaphis* and *P. mosselii* produced HCN and inhibited the growth of *A. tumefaciens* better than *Pseudomonas* sp. Gamma-81 in vitro. These results denoted that HCN can play a role in controlling the crown gall disease, in addition to possible other factors produced by antagonistic bacteria that are more potent in controlling the disease. There are many mechanisms that are used by *Pseudomonas* spp. to control plant diseases such as production of some secondary metabolites (hydrogen cyanide, Fe-chelating siderophores, and antibiotics), induction of plant defense responses and competition for nutrients (Lukkani and Reddy 2014).

Table 3 Quantitative assay of HCN produced by bacterial isolates at 625 nm wavelength

Rhizobacteria	Absorbance at wavelength 625 nm
P. japonica	0.020^{C}
B. megaterium	0.002^{D}
Pseudomonas sp. strain Gamma-81	0.005^{D}
P. tolaasii	0.024^{BC}
P. chlororaphis	0.047^{A}
P. mosselii	0.027^{B}
Control	0.001^{D}

Means in the table shared a letter are not significantly different ($p \leq 0.05$)

Effect of HCN-producing rhizobacteria on incidence of root-knot nematode on tomato plants

The potential of some bacterial strains to control nematodes is related to their ability to produce HCN. Moreover, a direct contact between HCN-producing bacteria and the nematode is essential in the effective control of nematode (Siddiqui et al. 2006). Data in Table 7 showed that HCN-producing rhizobacteria decreased the final population of *M. incognita* and the number of nematode galls than the positive control, when used against *M. incognita* and mixed infection with *M. incognita* and *A. tumefaciens*. The lowest final population count of nematodes (411.0 and 342.4) was recorded by *P. mosselii,* when used against *M. incognita* and mixed infection, respectively. The *M. incognita* and mixed infection (positive controls) recorded final population counts of 5574.0

Table 4 Effect of six isolates of HCN- producing rhizobacteria on growth of *Agrobacterium tumefaciens* in vitro

Rhizobacteria	Inhibition zone diameter (mm)
P. japonica	49.7^{A}
B. megaterium	13.7^{D}
Pseudomonas sp. Gamma-81	30.7^{BC}
P. tolaasii	14.3^{D}
P. chlororaphis	25.7^{C}
P. mosselii	33.7^{B}

Means in the table shared a letter are not significantly different ($p \leq 0.05$)

Table 5 Effect of different exposure time with certain HCN-producing rhizobacteria on mortality rate of *Meloidogyne incognita*

Rhizobacteria	Exposure period							
	24 h		48 h		72 h		7 days	
	No. of live juveniles*	Mortality %**	No. of live juveniles*	Mortality %**	No. of live juveniles*	Mortality %**	No. of live juveniles*	Mortality %**
P. japonica	75.7^{B}	24.3^{K}	54.3^{EF}	44.8^{EFG}	52.0^{FGH}	45.9^{D-G}	45.0^{GHI}	51.8^{CDE}
B. megaterium	100.0^{A}	00.0^{L}	75.0^{B}	23.7^{K}	65.0^{CD}	32.3^{IJ}	52.3^{FGH}	43.9^{EFG}
Pseudomonas sp. Gamma-81	100.0^{A}	00.0^{L}	51.0^{F-I}	48.1^{C-F}	44.0^{HI}	54.2^{C}	33.7^{JK}	63.9^{B}
P. tolaasii	100.0^{A}	00.0^{L}	73.0^{BC}	25.8^{JK}	62.0^{DE}	35.4^{HI}	53.3^{EFG}	42.8^{FGH}
P. chlororaphis	62.0^{DE}	38.0^{GHI}	50.0^{F-I}	49.2^{C-F}	30.0^{K}	68.6^{B}	03.3^{L}	96.4^{A}
P. mosselii	77.0^{B}	23.0^{K}	64.0^{CD}	34.9^{HI}	45.0^{GHI}	53.2^{CD}	42.3^{IJ}	54.6^{C}
Control	100.0^{A}	00.0^{L}	98.3^{A}	00.0^{L}	96.0^{A}	00.0^{L}	93.3^{A}	00.0^{L}

Means in the columns which marked with* or ** shared a letter are not significantly different ($p \leq 0.05$)

and 4560.4, respectively. The nematicidal oxamyl recorded a final population count of 288.0, when used as a comparison treatment against *M. incognita.*

The lowest population counts of nematode in soil (396.0 and 324.0) were recorded by *P. mosselii*, when used against *M. incognita* and mixed infection, respectively. The *M. incognita* and mixed infection (positive controls) recorded population counts in soil of 5412.0 and 4456.0, respectively. Oxamyl recorded a population count in soil of 244.0 in soil, when used as a comparison treatment against *M. incognita.*

The number of nematode galls decreased by using HCN-producing rhizobacteria against *M. incognita* and mixed infection than the positive control. The lowest numbers of galls (11.2 and 11.0) were recorded by *P. japonica* and *P. mosselii*, when used against *M. incognita* and mixed infection, respectively. The *M. incognita* and mixed infection (positive controls) recorded a number of galls of 63.8 and 53.0, respectively. Oxamyl recorded a number of galls (21.6), when used as a comparison treatment against *M. incognita*.

These results are remarkably consistent with the results obtained in the laboratory, where a quantitative assay of producing HCN by tested bacterial isolates showed that *P. japonica*, *P. tolaasii*, *P. chlororaphis*, and *P. mosselii* strongly produced HCN compared to *B. megaterium* and *Pseudomonas* sp. Gamma-81. Only *P. japonica*, *P. chlororaphisi,* and *P. mosselii* isolates affected the life of juveniles (J_2) of *M. incognita* after 24 h of incubation. This conclusion is clear in spite of the observation that isolate of *P. japonica* did not give good results that match with laboratory experiments.

Effect of HCN-producing rhizobacteria on growth parameters of tomato plants

The 6 isolates of HCN-producing rhizobacteria in the presence of *A. tumefaciens* and/or *M. incognita* increased

Table 6 Effect of six isolates of HCN-producing rhizobacteria on incidence of crown gall disease on tomato plants in the absence or presence of *Meloidogyne incognita* after 45 days of inoculation

Rhizobacteria	Incidence of crown gall disease							
	A. tumefaciens				*A. tumefaciens* + *M. incognita*			
	Galls weight (g)/plant*	Decreasing in galls weight %**	No. of galls/ plant***	Decreasing in no. of galls %****	Galls weight (g)/plant*	Decreasing in gall weight %**	No. of galls/ plant***	Decreasing in no. of galls %****
P. japonica	0.00^{G}	100.0^{A}	0.0^{F}	100.0^{A}	0.00^{G}	100.0^{A}	0.0^{F}	100.0^{A}
B. megaterium	0.05^{FG}	92.1^{AB}	0.4^{EF}	93.3^{AB}	0.14^{DEF}	80.2^{B}	1.4^{DE}	81.7^{BC}
Pseudomonas sp. Gamma-81	0.00^{G}	100.0^{A}	0.0^{F}	100.0^{A}	0.00^{G}	100.0^{A}	0.0^{F}	100.0^{A}
P. tolaasii	0.24^{BCD}	60.8^{CDE}	2.4^{CD}	56.0^{EF}	0.25^{BC}	64.8^{CD}	2.6^{CD}	62.5^{DE}
P. chlororaphis	0.20^{CDE}	67.0^{C}	2.6^{CD}	52.0^{EF}	0.13^{EF}	81.7^{B}	1.8^{CD}	73.9^{CD}
P. mosselii	0.31^{BC}	49.9^{E}	2.8^{C}	48.0^{F}	0.33^{B}	53.0^{DE}	3.0^{C}	57.0^{EF}
Control(+)	0.61^{A}	0.00^{F}	5.4^{B}	0.0^{G}	0.71^{A}	0.00^{F}	7.0^{A}	0.0^{G}
Control(–)	0.00^{G}	–	0.0^{F}	–	0.00^{G}	–	0.0^{F}	–
Healthy plant	0.00^{G}	–	0.0^{F}	–	0.00^{G}	–	0.0^{F}	–

Means in the columns which marked with*, **, *** or **** shared a letter are not significantly different ($p \leq 0.05$)

Table 7 Effect of six isolates of HCN-producing rhizobacteria on incidence of root-knot nematode on tomato plants in the absence or the presence of *A. tumefaciens* after 45 days of inoculation

Treatments	Pathogen	Incidence of root-knot nematode disease									
		Nematode population in			Final population (Pf)	Reproduction factor (RF)	Reductions in nematode population %(Red %)	No. of galls	Root gall index (RGI)	No. of egg masses	Egg masses index (EI)
		Soil (250 g)	Root								
			Developmental stages	Females							
P. japonica	*M. incognita*	784.0^{EF}	0.0^{E}	15.0^{G}	799.0^{E}	0.40	85.7	11.2^{F}	3.0	12.8^{ABC}	3.0
B. megaterium		1808.0^{C}	5.0^{DE}	44.2^{B}	1857.2^{C}	0.93	66.7	37.0^{C}	4.0	10.0^{C}	2.0
Pseudomonas sp. Gamma-81		500.0^{FG}	0.0^{E}	26.0^{DE}	526.0^{EF}	0.26	90.6	13.2^{F}	3.0	0.0^{D}	0.0
P. tolaasii		564.0^{FG}	0.0^{E}	29.2^{CD}	593.2^{EF}	0.30	89.4	16.0^{EF}	3.0	3.0^{D}	2.0
P. chlororaphis		428.0^{FGH}	0.0^{E}	16.0^{FG}	444.0^{EFG}	0.22	92.0	13.0^{F}	3.0	0.0^{D}	0.0
P. mosselii.		396.0^{FGH}	0.0^{E}	15.0^{G}	411.0^{EFG}	0.21	92.6	12.8^{F}	3.0	0.0^{D}	0.0
Control(+)		5412.0^{A}	87.4^{A}	74.6^{A}	5574.4^{A}	2.79	0.0	63.8^{A}	4.0	15.8^{A}	3.0
Oxamyl		244.0^{GH}	16.2^{CD}	27.8^{CD}	288.0^{FG}	0.14	94.8	21.6^{DE}	3.0	10.0^{C}	2.0
P. japonica	*M. incognita* + *A. tumefaciens*	1216.0^{DE}	10.0^{CDE}	31.6^{CD}	1257.6^{D}	0.63	72.4	31.4^{C}	4.0	12.0^{BC}	3.0
B. megaterium		608.0^{FG}	0.0^{E}	27.0^{D}	635.0^{EF}	0.32	86.1	23.2^{D}	3.0	0.0^{D}	0.0
Pseudomonas sp. Gamma-81		1348.0^{D}	19.4^{C}	35.2^{C}	1402.6^{D}	0.70	69.2	36.0^{C}	4.0	3.0^{D}	2.0
P. tolaasii		436.0^{FG}	0.0^{E}	25.8^{DE}	461.8^{EF}	0.23	89.9	22.2^{DE}	3.0	0.0^{D}	0.0
P. chlororaphis		486.0^{FG}	0.0^{E}	23.8^{DEF}	509.8^{EF}	0.25	88.8	21.4^{DE}	3.0	0.0^{D}	0.0
P. mosselii.		324.0^{GH}	0.0^{E}	18.4^{EFG}	342.4^{FG}	0.17	92.5	11.0^{F}	3.0	0.0^{D}	0.0
Control(+)		4456.0^{B}	59.6^{B}	44.8^{B}	4560.4^{B}	2.28	0.0	53.0^{B}	4.0	13.4^{AB}	3.0
Control(−)		0.0^{H}	0.0^{E}	0.0^{H}	0.0^{G}	0.0	0.0	0.0^{G}	0.0	0.0^{D}	0.0
Healthy plants		0.0^{H}	0.0^{E}	0.0^{H}	0.0^{G}	0.0	0.0	0.0^{G}	0.0	0.0^{D}	0.0

Means in the same column shared a letter are not significantly different ($p \leq 0.05$); RF = final population in treatment/2000; Red% (reductions in nematode population%) = $[(Pf_c - Pf_t)/Pf_c] \times 100$ where Pf_c = final population in control and Pf_t = final population (Pf) in treatment; RGI or EI was determined according to the scale given by Taylor and Sasser (1978)

growth parameters of tomato (shoot and root length, shoot and root fresh weight, total fresh weight of plant, and total dry weight of plant) than the negative control and healthy plants (Table 8). The only exception was found in case of *Pseudomonas* sp. Gamma-81, when used against *M. incognita* and the mixed infection (*A. tumefaciens* and *M. incognita*), where growth parameters of tomato were decreased. On the other hand, *A. tumefaciens* (positive control treatment) increased all growth parameters than the negative control and healthy plants, while *M. incognita* (positive control treatment) decreased all growth parameters than the healthy plants.

When the tested HCN-producing rhizobacteria were used against *A. tumefaciens*, all isolates increased shoot weight, root length, total fresh weight, and total dry weight of tomato plants than the positive control treatment (*A. tumefaciens*). The only exception was recognized in case of root length and total fresh weight in *P. japonica* isolate treatment. The isolate of *P. japonica* recorded root length and total fresh weight of 28.2 cm and 49.3 g compared to *A. tumefaciens* (positive control treatment) that recorded 29.7 cm and 52.9 g, respectively. Also, *P. japonica*, *B. megaterium*, and *P. chlororaphis* increased shoot length compared to the positive control treatment (*A. tumefaciens*) and recorded 58.4, 56.3, and 61.8 cm, respectively, while *Pseudomonas* sp. Gamma-81, *P. tolaasii*, and *P. mosselii* decreased shoot length than the positive control treatment (*A. tumefaciens*) and recorded 52.5, 51.7, and 54.3 cm, respectively. The positive control treatment (*A. tumefaciens*) recorded shoot length of 55.0 cm. Meanwhile, root weight of tomato plants was increased by using *P. mosselii* against *A. tumefaciens* than the positive control treatment (*A. tumefaciens*) and recorded 18.0 g. At the same time, root weight of tomato plants was decreased by using *P. japonica*, *B. megaterium*, *Pseudomonas* sp. Gamma-81, *P. tolaasii*, and *P. chlororaphis*, which recorded 8.3, 12.6, 12.9, 14.0, and 11.0 g, respectively. The positive control treatment (*A. tumefaciens*) recorded root weight of 14.8 g.

Meanwhile, the tested HCN-producing rhizobacteria against *M. incognita* increased all growth parameters of tomato plants than the positive control treatment (*M. incognita*). The only exception was found in case of *Pseudomonas* sp. Gamma-81 that decreased all growth parameters of tomato plants.

Table 8 Effect of HCN-producing rhizobacteria on growth parameters of tomato plants infected with of *Agrobacterium tumefaciens* and/or *Meloidogyne incognita* after 45 days of inoculation

Treatment	Pathogen	growth parameters					
		Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Total fresh weight (g)	Total dry weight (g)
P. japonica	*A. tumefaciens*	58.4^{B-F}	41.0^{ABC}	28.2^{E-I}	8.3^{E-I}	49.3^{A-F}	24.8^{AB}
B. megaterium.		56.3^{C-G}	46.5^{A}	37.7^{A-D}	12.6^{B-F}	59.1^{A}	22.5^{A-D}
Pseudomonas sp. Gamma-81		52.5^{E-I}	40.8^{ABC}	30.5^{C-H}	12.9^{B-E}	53.7^{A-E}	19.8^{A-E}
P. tolaasii		51.7^{F-J}	42.4^{ABC}	39.3^{AB}	14.0^{ABC}	56.4^{ABC}	23.5^{ABC}
P. chlororaphis		61.8^{BCD}	46.9^{A}	43.8^{A}	11.0^{B-H}	57.9^{AB}	25.6^{AB}
P. mosselii		54.3^{D-H}	43.1^{AB}	39.0^{AB}	18.0^{A}	61.1^{A}	27.2^{A}
Control(+)		55.0^{C-G}	38.1^{BCD}	29.7^{D-H}	14.8^{AB}	52.9^{A-E}	18.4^{B-F}
Control(–)		37.8^{KL}	11.2^{I}	21.3^{IJ}	4.0^{IJ}	$15.2^{J\ K}$	6.8^{G}
P. japonica	*M. incognita*	58.8^{B-F}	31.2^{DEF}	34.3^{B-E}	11.6^{B-G}	42.8^{EFG}	25.5^{AB}
B. megaterium.		57.3^{C-F}	34.7^{CDE}	32.8^{B-G}	9.5^{C-H}	44.2^{D-G}	21.0^{A-D}
Pseudomonas sp. Gamma-81		43.7^{JKL}	16.9^{HI}	23.7^{HIJ}	6.5^{HIJ}	23.4^{IJK}	11.5^{FG}
P. tolaasii		60.5^{B-E}	37.2^{B-E}	32.2^{B-G}	7.4^{GHI}	44.6^{C-G}	22.0^{A-D}
P. chlororaphis		63.2^{ABC}	30.1^{EF}	34.5^{B-E}	7.2^{G-J}	37.3^{G-H}	26.4^{A}
P. mosselii		54.7^{D-G}	26.5^{FG}	$29.0^{E\ -I}$	7.9^{F-I}	34.4^{GHI}	27.0^{A}
Control(+)		45.5^{IJK}	$17.6^{H\ I}$	25.0^{G-J}	6.7^{HIJ}	24.3^{IJK}	15.0^{DEF}
Oxamyl		$48.0^{G\ -J}$	$20.1^{G\ H}$	28.9^{E-I}	7.3^{G-J}	27.4^{HI}	16.0^{C-F}
P. japonica	*A. tumefaciens* and *M. incognita*	71.0^{A}	42.8^{AB}	30.0^{C-H}	12.9^{B-E}	55.7^{A-D}	26.8^{A}
B. megaterium.		58.0^{B-F}	30.8^{DEF}	39.2^{AB}	10.0^{B-H}	40.8^{FG}	18.5^{BF}
Pseudomonas sp. Gamma-81		36.0^{L}	11.0^{I}	17.5^{J}	2.5^{J}	13.5^{K}	5.5^{G}
P. tolaasii		61.5^{BCD}	39.4^{ABC}	29.3^{E-I}	13.2^{A-D}	52.6^{A-F}	20.0^{A-E}
P. chlororaphis		65.8^{AB}	36.2^{B-E}	33.3^{B-F}	8.6^{D-I}	44.8^{C-G}	$18.1^{B\ -F}$
P. mosselii		57.0^{C-F}	38.3^{BCD}	29.0^{E-I}	8.0^{F-I}	$46.3^{B\ -G}$	$24.0^{A\ B}$
Control(+)		54.3^{D-H}	36.5^{B-E}	37.8^{ABC}	9.7^{C-H}	46.2^{B-G}	18.0^{B-F}
Control(–)		37.8^{KL}	11.2^{I}	21.3^{IJ}	4.0^{IJ}	15.2^{JK}	6.8^{G}
Healthy plants		46.0^{H-K}	$18.5^{H\ I}$	25.3^{F-J}	7.0^{G-J}	25.5^{HI}	12.4^{EFG}

Means in the same column shared a letter are not significantly different ($p \leq 0.05$)

HCN-producing rhizobacteria gave various results when used against mixed infection with *A. tumefaciens* and *M. incognita*. The isolates of *P. japonica* and *P. tolaasii* increased all plant growth parameters of tomato than the positive control treatment (*A. tumefaciens* and *M. incognita*), the only exception was found in case of root length. The isolate of *B. megaterium* increased all tomato plant growth parameters than the positive control treatment (*A. tumefaciens* and *M. incognita*), the only exception was found in case of shoot weight and total fresh weight. Also, *P. mosselii* increased all tomato plant growth parameters than positive control treatment (*A. tumefaciens* and *M. incognita*), except the root length and root weight. Meanwhile, *Pseudomonas* sp. Gamma-81 decreased all tomato plant growth parameters than the positive control treatment. Also, *P. chlororaphis* decreased shoot weight, root length, root weight and total fresh weight and increased shoot length and total dry weight compared to the positive control treatment.

In addition to its ability to promote the growth of plants indirectly by inhibiting the growth of pathogens, it is well established that some *Pseudomonas* spp. can produce substances that promote the growth of plants directly as indole-3-acetic acid (IAA), siderophores that chelate iron, and phosphatases that solubilize phosphorus (Rai et al. 2017). Moreover, *Bacillus* spp. beside its ability to act as a biological agent by producing HCN and antibiotics, they also produce phytohormones like IAA and GA, increases uptake of nutrients like phosphate and iron by siderophore production, produces ammonia, protects cell from oxidative damage by producing catalase enzyme and exhibited tolerance

against heavy metal and salinity (Rayavarapu and Padmavathi 2016). More interesting, it has been recently found that *A. tumefaciens* isolate, isolated from nodules of plants growing in zinc-lead mine tailings, showed high metal resistance and enhanced the growth of plants in a metal-contaminated environment (Hao et al. 2012). On the other hand, *M. incognita* decreased the plants growth, causing loss in plant parameters that may be directly correlated with the multiplication of the nematodes (Anver and Alam 1989). The results herein showed that *A. tumefaciens* caused an increment in all plant parameters compared to the negative control and healthy plants, while *M. incognita* decreased all plant parameters than the healthy plants.

Finally, although all obtained isolates were recovered locally from rhizosphere soils of some plants and had a role in biocontrol of *A. tumefaciens* and *M. incognita*, but *P. japonica* and *P. tolaasii* cannot be used in this field, until being sure that these strains are not pathogenic to humans and animals. In this regard, it has been reported that *P. japonica* is a novel species in the sub-cluster of *P. putida* group, isolated from an activated sludge and more recently reported as a novel cause of skin bacteremia and soft tissue infection (Coomes et al. 2018). *P. tolaasii* is a species of gram-negative bacteria causing bacterial blotch on cultivated mushrooms (*Agaricus bisporus*) under some environmental conditions (Soler-Rivas et al. 1999). Thus, further field trials and pilot experimentation are suggested.

Conclusion

Six isolates of HCN-producing rhizobacteria were recovered locally from rhizosphere soils of some plants in Egypt. These HCN-producing rhizobacteria were able to inhibit growth of *Agrobacterium tumefaciens* and affect viability of *Meloidogyne incognita* juveniles in vitro. The isolates decreased the weight and number of galls produced by *A. tumefaciens* on tomato plants, regardless to the presence of *M. incognita*. The 6 HCN producers decreased the population of *M. incognita* and the number of nematode galls produced on tomato when used against *M. incognita*, and a similar effect was achieved against mixed infection with *M. incognita* and *A. tumefaciens.* The HCN-producing rhizobacteria in the presence of *A. tumefaciens* and/or *M. incognita* caused obvious increment in all growth parameters of tomato plant. Further investigations are still needed.

Acknowledgements

The authors would like to thank Dr. Nabil S. Farag (Department of Plant Bacterial Disease, Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt) and Dr. Fatma Abdel Mohsen Mostafa (Agric. Zoology Dept., Fac. of Agric., Mansoura University, Egypt) for reviewing the manuscript.

Authors' contributions

AFA conceived the idea. All of the authors of this manuscript contributed equally to the design and/or execution of the experiments described in the manuscript. All authors read and approved the final manuscript.

Funding

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 12 February 2019 Accepted: 7 June 2019
Published online: 26 June 2019

References

Abd El-Ghany H, Moussa Z, Salem EA, Abd El-Rahman AF (2017) Management of potato soft rot by gamma irradiation. Arab J Nucl Sci Appl 50(3):159–173

Abd El-Rahman AF (2012) Biological control of crown gall disease of stone fruit trees. Ph.D. Thesis, Faculty of Agriculture, Ain Shams University, Egypt, 123 pp

Abd El-Rahman AF, Shaheen HA (2016) Biological control of the brown rot of potato, *Ralstonia solanacearum* and effect of bacterization with antagonists on promotion of potato growth. Egyptian J Biol Pest Control 26(4):733–739

Abdel-Salam MS, Ameen HH, Soliman GM, Elkelany US, Asar AM (2018) Improving the nematicidal potential of *Bacillus amyloliquefaciens* and *Lysinibacillus sphaericus* against the root-knot nematode *Meloidogyne incognita* using protoplast fusion technique. Egyptian J Biol Pest Control 28:31

Alemu F (2016) Isolation of *Pseudomonas flurescens* from rhizosphere of faba bean and screen their hydrogen cyanide production under *in vitro* study, Ethiopia. Am J Life Sci 4(2):13–19

Anver S, Alam MM (1989) Effect of root-knot and reniform nematodes on plant growth and bulk-density of plant residues of pigeonpea. Biol Wastes 30(4):245–250

Coomes E, Silverstein WK, Zipursky JS, Shojania K (2018) *Pseudomonas japonica*: a novel cause of bacteremia and skin and soft tissue infection. Infect Dis Clin Pract 26(5):e43–e44

Eden PA, Schmidt TM, Blakemore RP, Pace NR (1991) Phylogenetic analysis of *Aquaspirillum magnetotacticum* using polymerase chain reaction-amplified 16S rRNA-specific DNA. Int J Syst Bacteriol 41(2):324–325

Farag SMA, Elhalag KMA, Hagag MH, Khairy AM, Ibrahim HM, Saker MT, Messiha NAS (2017) Potato bacterial wilt suppression and plant health improvement after application of different antioxidants. J Phytopathol 165:522–537

Hao X, Xie P, Johnstone L, Miller SJ, Rensing C, Wei G (2012) Genome sequence and mutational analysis of plant-growth-promoting bacterium agrobacterium tumefaciens CCNWGS0286 isolated from a zinc-lead mine tailing. Appl Environ Microbiol 78(15):5384–5394

Ibrahim DSS, Nour El-Deen AH, Khalil AE, Mostafa FAM (2013) Induction of systemic resistance in sugar-beet infected with *Meloidogyne incognita* by humic acid, hydrogen peroxide, thiamine and two amino acids. Egypt J Agronematol 12(1):22–41

Kifle MH, Laing MD (2016) Isolation and screening of bacteria for their diazotrophic potential and their influence on growth promotion of maize seedlings in greenhouses. Front Plant Sci 6:1225

Lanteigne C, Gadkar VJ, Wallon T, Novinscak A, Filion M (2012) Production of DAPG and HCN by Pseudomonas sp. LBUM300 contributes to the biological control of bacterial canker of tomato. Phytopathology 102(10):967–973

Lukkani NJ, Reddy ECS (2014) Evaluation of plant growth promoting attributes and biocontrol potential of native fluorescent *Pseudomonas* sp. against *Aspergillus niger* causing collar rot of ground nut. Int J Plant, Animal Environ Sci 4:256–262

Mostafa FAM, Khalil AE, Nour El Deen AH, Ibrahim DS (2014) Induction of systemic resistance in sugar- beet against root-knot nematode with commercial products. J Plant Pathol Microb 5:236

Pulawska J (2010) Crown gall of stone fruits and nuts, economic significance and diversity of its causal agents: tumorigenic *Agrobacterium* spp. J Plant Pathol 92:S87–S98

Rai A, Rai PK, Singh S (2017) Exploiting beneficial traits of plant-associated fluorescent pseudomonads for plant health. In: Singh JS, Seneviratne G (eds) Agro – environmental sustainability Vol. (1). Springer, Cham, pp 19–41

Rayavarapu VGB, Padmavathi T (2016) *Bacillus* sp as potential plant growth promoting rhizobacteria. Int J Adv Life Sci 9(1):29–36

Reetha AK, Pavani SL, Mohan S (2014) Hydrogen cyanide production ability by bacterial antagonist and their antibiotics inhibition potential on *Macrophomina Phaseolina* (Tassi.) Goid. Int J Curr Microbiol App Sci 3(5):172–178

Rijavec T, Lapanje A (2016) Hydrogen cyanide in the rhizosphere: not suppressing plant pathogens, but rather regulating availability of phosphate. Front Microbiol 7:1785

Rubio-Cabetas M, Minot J, Voisin R, Esmenjaud D (2001) Interaction of root-knot nematodes (RKN) and the bacterium *Agrobacterium tumefaciens* in roots of *Prunus cerasifera*: evidence of the protective effect of the Ma RKN resistance genes against expression of crown gall symptoms. Eur J Plant Path 107:433–441

Siddiqui IA, Shaukat SS, Sheikh IH, Khan A (2006) Role of cyanide production by *Pseudomonas fluorescens* CHAo in the suppression of root-knot nematode, *Meloidogyne javanica* in tomato. World J Microbiol Biotechnol 22:641–650

Soler-Rivas C, Jolivet S, Arpin N, Olivier JM, Wichers HJ (1999) Biochemical and physiological aspects of brown blotch disease of Agaricus bisporus. FEMS Microbiol Rev 23(5):591–614

Taylor AL, Sasser JN (1978) Biology, identification and control of root-knot nematodes (*Meloidogyne* species). Department of Plant Pathology, North Carolina State University, United States Agency for International Development. Raleigh, North Carolina, USA, vii + 111 pp

Tolba IH, Soliman MA (2013) Efficacy of native antagonistic bacterial isolates in biological control of crown gall disease in Egypt. Ann Agric Sci 58(1):43–49

Publisher's Note