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PROVIDENCIA VERMICOLA MEDIATED GROWTH ALTERATION AND INHIBITED GALL FORMATION ON TOMATO PLANTS INFECTED WITH THE ROOT KNOT NEMATODE *MELOIDOGYNE JAVANCIA*

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Abstract

The root knot nematode (RKN) (*Meloidogyne javanica*) is a widespread deleterious pathogen affecting plant growth and yields; thus limiting the agricultural activity worldwide. Therefore, an effective safe control method against the pathogen is urgently needed. The objectives of this research were to isolate an effective biocontrol agent against RKN and to examine its effect on tomato growth. In the present study, only 7 bacterial isolates out of 142 exhibited antagonistic activities against RKN and one isolate showed almost 100% egg hatching suppression. The strain was identified as *Providencia vermicola* using 16s rRNA and was chosen for further study. In dual culture bioassay, examination under the light microscope showed distinguishable intake black egg masses compared to control. Furthermore, when the black-color egg masses were crushed, a similar alteration of the egg color was observed. In addition, the treated egg masses were unable to infect tomato roots in pots. Under the greenhouse conditions, application of *P. vermicola* at a rate of 50 ml/pot ($1x10^8$ CFU/ml) significantly reduced disease symptoms and galls number. Moreover, bacterial application enhanced tomato growth related parameters considerably compared to untreated plants. Similarly, the bacterial filtrate exhibited a substantially nematicidal activity against RKN. Further analysis of the bacterial filtrates showed production of an active substance(s) that was heat stable. Determination of the strain characterization revealed a strong ability to solubilize phosphate and production of Indole Acetic Acid (IAA) whereas siderophore and hydrogen cyanide (HCN) production were not detected. The results indicated that *P. vermicola* represents a novel bacterial strain protecting tomato plants against RKN.

Key words: Nematicide; PGPR; *Meloidogyne javanica*; *Providencia vermicola*

Introduction

Tomato (*Lycopersicon esculentum* Miller) crop is one of the most consumed vegetables worldwide either fresh, cooked or processed. The fruit is rich in nutrients and its health benefits are well documented (Olaniyi *et al*., 2010; Bhowmik *et al*., 2012). In general, the crop is infected with a wide range of pathogens such as fungi, bacteria, viruses and nematodes. Of all parasites, the root-knot nematodes (RKNs) are the most economically destructive pathogens (Oka *et al*., 2000; Trudgill and Blok 2001) especially in sandy soil. In Egypt, tomato is a major crop cultivated in three consecutive cycles per year and severely affected by RKNs (Ibrahim *et al*., 2000). The main two species that are frequently reported to cause damage in North East, Egypt is *Meloidogyne javanica* and *Meloidogyne incognita* (Ibrahim *et al*., 2000; Mahgoob and El-Tayeb 2010). Unfortunately, control of RKN is a challenge due to the life cycle of the pathogen, its adaptation capability to the environment, and the limiting availability of effective and environmentally safe chemical nematicides. Therefore, new strategies for RKN management are urgently needed to control the pathogen with emphasizing on alleviating the environmental stresses. Of all the alternatives, biological control using soil microorganisms provide an acceptable method to manage RKN.

Soil microbiota is a conventional source for new and effective biocotrol agents. Recently, many promising antagonistic organisms have been reported as efficient

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bioagents and bacteria were a predominant group of the antagonistic agents. The commonly bacterial genera that have been reported previously include *Bacillus*, *Pseudomonas* and *Pasteuria* (Ali *et al*., 2002; Chinheya *et al*., 2017; Padgham and Sikora, 2007; Tian *et al*., 2007; Oliveira *et al*., 2009; Siddiqui and Mahmood 1999; Hallmann *et al.,* 2009) that were reported to use various modes of action against RKN. In the literatures, the reported mechanisms in controlling the pathogen varied from direct parasitism, secretion of secondary metabolites, and activation of the host resistance machinery. Earlier, *Pasteuria penetrans* was described to attach its spores to the second stage juveniles (J2), penetrate the cuticle, proliferate and damage the reproductive system (Davies *et al*., 2001; Sayre and Wergin 1977). Nevertheless, the two other genera *Bacillus* and *Pseudomonas* were reported to act indirectly against RKN via releasing secondary metabolites in soil, including enzymes, antibiotics and toxins, that suppresses the nematode reproduction, eggs hatching and J2 survives (Siddiqui and Mahmood 1999; Zuckerman and Jasson 1984) .Recently, Batool *et al*., (2013) recognized *P. aeruginosa* capability to cause 90% mortality of J2 *in vitro*. Furthermore, Siddiqui and Shaukat (2004) and Waseem *et al*., (2014) reported induction of the plant defence mechanism by several biocontrol agents against RKN host penetration or reproduction as a possible indirect mechanism.

Providencia vermicola was reported previously as a soil inhabiting and root colonizing bacteria that enhanced rapeseed growth (Hussain *et al*., 2015), a plant growth promoting bacteria protected lentil seedlings from copper toxicity (Islam *et al*., 2016) and a fluoride remedy agent of a contaminated groundwater (Mukherjee *et al*., 2017). The genus *Providencia* was first isolated by Ewing (1962) and characterized to contain eight different species. Lately, *P. vermicola* was isolated from infective juveniles of the entomopathogenic nematode *Steinernema thermophilum* collected from different larvae of the greater wax moth (Somvanshi *et al*., 2006) followed by a report of bacterial isolation from infected Indian major Carp *Labeo rohita* (Ramkumar *et al*., 2014). Up to now, little is known about the capability of *P. vermicola* to antagonize RKN.

The aims of the current investigation were to isolate an effective biocontrol agent against RKN and determine the isolate efficacy in protecting tomato plants.

Materials and Methods

Nematode isolation, identification and inoculum preparation

Meloidogyne javanica were originally isolated from infected tomato roots collected from a naturally infested field in Ismailia province, North east Egypt and identified using the perineal pattern technique. Pure culture was maintained on tomato plants (GS cv.) grown on pots under the greenhouse condition. Species identification was confirmed using the specific Forward primer (5-GGTGCGCGATTGAACTGAGC-3) and reverse primer (5-CAGGCCCTTCAGTGGAACTATAC-3) (Fjav/Rjav) that amplifies the sequence characterized amplified regions (SCAR) markers as described by Meng *et al*., (2004) and Zijlstra *et al*., (2000). For the greenhouse experiments, matured egg masses were collected, surface sterilized and incubated in sterilized water at room temperature for 2-3 days. The hatched J2 were examined under stereoscope to ensure survival. The J2 total number was adjusted to 250 J2 m^{-1} and used immediately for inoculation.

Bacterial isolation and identification

The antagonistic bacteria were isolated from healthy pepper plants grown in naturally nematode- infested soil at the Experimental Field Station, Suez Canal University. Intact plant roots were shaken gently to get rid of the non-adhering soil. One gram of roots was washed, cut into pieces and placed in 9 ml sterilized water, shaken vigorously for 10 min, and then a serial dilution was made. About 100 µl of each dilution (10^3- 10^5) were spread onto nutrient agar (NA) medium. Single colonies were selected and subjected to bioassay examination.

The active selected bacterial strain was identified based on gram stain reaction, colony morphological and physiological characteristics and 16s rRNA at the Microbiology Lab, Faculty of Science, Suez Canal Univ. as follows: DNA was extracted using Qiagen kit following the manufacturer's instructions. The 16S rRNA was amplified as described by Rainey *et al.,* (1996). The amplification was carried out using the following protocol: (i) 30s at 94ºC, (ii) 30 reaction cycles with each cycle consisting of treatment at 94ºC for 30s, 54ºC for 30s and 72ºC for 1:30 min and; (iii) a final extension step of 5 min at 72ºC. The forward primer: 27F (5'-AGAGTTTGATY MTGGCTCAG-3'); and Reverse primer: 1492R (5'-ATCCTTGTTACGACTT-3') were used. PCR products were purified with the QIAquick PCR purification kit (Qiagen) and directly sequenced using the CEQ Dye Terminator Cycle Sequencing kit. Products were separated on a CEQ 8000 Genetic Analysis System. The 16S rRNA sequences were aligned with corresponding sequences from the DSMZ database using the ae2 editor (Maidak *et al*., 1997).

Bacteria nematicidal activity examination

A preliminary dual culture plate method was carried out to examine the antagonistic capability of all the bacterial isolates against RKN egg masses hatching. Briefly, about 15 ml of 3 days old bacterial culture (10^4 CFU ml^{-1}) were poured into a sterilized petri dish supplemented with three surface sterilized (0.5% NaOCl for 2min) egg masses. The plates were incubated at 25°C with four replicates per each bacterial isolates. The plates were examined after three, six and nine days to count the number of hatched juveniles. Positive control plates containing 15 ml sterilized media and negative control plates containing 15 ml sterilized water were used. The number of hatched J2 in each plate was recorded.

To examine the nematicidal activity of bacterial cell-free culture, *P. vermicola* were grown on nutrient broth (NB) for 3 days, centrifuged at 10000 rpm for 20 min and pellets were discarded. Culture filtrates were sterilized using 0.45 and 0.22 µm filters (Lian *et al.*, 2007). About 15 ml of culture filtrate were placed in a sterilized petri dish and inoculated with either three egg masses or 3ml of freshly hatched juvenile suspension (40 J2 ml^{-1}) using four replicates per treatment. The number of hatched J2 was counted after three and six days on plates supplemented with egg masses and the number of dead (immobile) J2 was counted in plates supplemented with active J2. To ensure survival, the J2 were nudged with a needle and considered dead when they did not move. Control plates were examined continually. The crude cell-free filtrates were further investigated for active substance(s) heat stability by boiling the filtrate for 6 min as described by Ali *et al*., (2002).

All nematicidal experiments were repeated four times to ensure the bacterial nematicidal activities with at least six replications each time.

Assessment of bacterial nematicidal activity in pots

Examination of the treated egg masses vitality

To insure un-recovery of treated egg masses, a greenhouse experiment was conducted to examine the pathogensis capability of the bacteria-treated egg masses resulted from the bioassay experiment. Tomato seedlings (*Lycopersion esclantum* Mill cv. Rutgers) susceptible to *Meloidogyne* spp. at 3-4 true leaves stage were transplanted into pots (10X10 cm containing 500 g soil) and inoculated with 5 intact bacteria –treated egg masses in holes around the roots. Control pots were inoculated with untreated egg masses and all treatments were replicated six times. Pots were kept for 60 days and roots were examined for galls development.

Assessment of bacterial efficacy against RKN in the pots

To examine the nematicidal activity of *P. vermicola*, an experiment was conducted under the greenhouse condition in a completely randomized block design. The treatments were as follows: (T1) control plants; (T2) *P. vermicola*; (T3) *M. javanica*; (T4) *P. vermicola* + *M. javanica*. Pots (15X15 cm) were filled with 1 Kg steamed sandy loam soil. To ensure that each plant received enough nutrients, seedlings were sprayed with Hoagland's nutrient solution as instructed. Each tomato seedling was inoculated with approximately 500 freshly hatched J2. *P. vermicola* suspensions (10^8 CFU/ ml) were added at the rate of 50 ml/seedling and control plants were treated with 50 ml sterilized water. Each treatment consists of 6 replicates and each replicate consisted of 2 tomato seedlings giving a total of 12 seedlings per treatment. The experiment was terminated after 45 days of inoculation. The galls number on tomato roots and egg masses index were recorded to evaluate bacterial efficacy.

Plant growth parameters (plant length and shoot and root fresh weight) were recorded after 45 days post inoculation. All pots experiments were repeated three times to ensure results and all values were displayed as the means of 12 replicates ± standard deviations (SD). Data were analyzed by (ANOVA) followed by least significant difference (LSD) test at 5 % probability level using COSTAT software program (CoHort Computer Software, Berkeley, CA, USA).

Bacterial characterization

Indole Acetic Acid production (IAA)

Auxin like Indole Acetic Acid (IAA) production was assayed using the method described by Gordon and Weber (1950) on Luria-Bertani (LB) medium. LB medium (50ml) was inoculated with one ml of bacterial culture containing 10^8 cfu with or without tryptophan at concentrations of 0.0, 1 and 3 mg ml^{-1} and incubated at 25°C for three days. IAA concentration was estimated using a standard curve and all measurements were made in three replicates. The experiment was repeated twice.

Siderophores production

The assay was based on the competition for iron between the ferric complexes of an indicator dye chrome azurol S (CAS) and the siderophores producing bacteria which has a higher affinity for chelating Fe^{+3} on CAS using the method described by Schwyn and Neilands (1987). Briefly, bacteria were spread onto NA medium plates and incubated for 24 h at 25°C, supplemented with 500 µl 0.7% agar containing CAS reagent spread over bacterial growth and re-incubated. Plates were observed for a yellow-orange halo formation as an indicator of siderophores production. Control plates were not inoculated with bacteria. The experiment repeated twice with six replicates.

Hydrogen Cyanide (HCN) production

The bacterial strain was examined for hydrogen cyanide production by spotting *P. vermicola* on LB agar medium supplemented with 4.4 g glycin/L and incubated for 24 h at 25°C. A saturated filter paper with 0.5% picric acid + 2% sodium carbonate was placed on the surface of each plate and the plates were incubated for 5 days. The filter papers were observed for color change from yellow to orange- brown as an indicator of positive reaction. Control plates were not inoculated with bacteria. There were six replicates per treatment and the experiment was repeated twice, (Pathma *et al*., 2011).

Phosphate solubilization

To examine the bacterial capabilities to solubilize phosphate, 15 µl of bacterial suspension (1×10^4 CFU/ml) were spotted on Sperber medium plate as mentioned by Malboobi *et al*., (2009). The plates were incubated at 25°C for 3 days and observed for development of clear zones.

Results

Organisms identification

A total of 7 bacterial isolates out of 142 showed nematicidal activity against RKN egg masses in dual culture plates with varied percentage. The bacterial isolate exhibited the lowest number of hatched J2 was chosen for further assessment. The chosen strain produced a creamy, circular, smooth, slimy and convex colony on NA media. A brown pigment was observed around the colonies associated with a notable smell. Under the light microscope, bacteria were rod shaped and gram negative. Identification using 16S rRNA resulted in production of 1500 pb DNA fragment. The sequencing of the strain revealed a homology of >99% with *Providencia vermicola*.

For RKN identification, PCR generated a fragment of 720 bp using the Fjav/Rjav primers confirming the nematode identification as *Meloidogyne javanica*.

Efficacy of *P. varmicola* against RKN egg masses hatching

Data showed a substantial nematicidal activity of *P. varmicola* against egg masses hatching. The treated egg masses showed almost no hatched juvenile (J2) with a mean number reached only 2.25% per plate in the third day and by the sixth day all the hatched J2 were immotile. In addition, at the six day, bacterial treatment caused reduction in J2 hatching reached 97% compared to media control whereas media treatment caused reduction reached 32% compared to water control. Furthermore, the treated egg masses showed a significant color change,

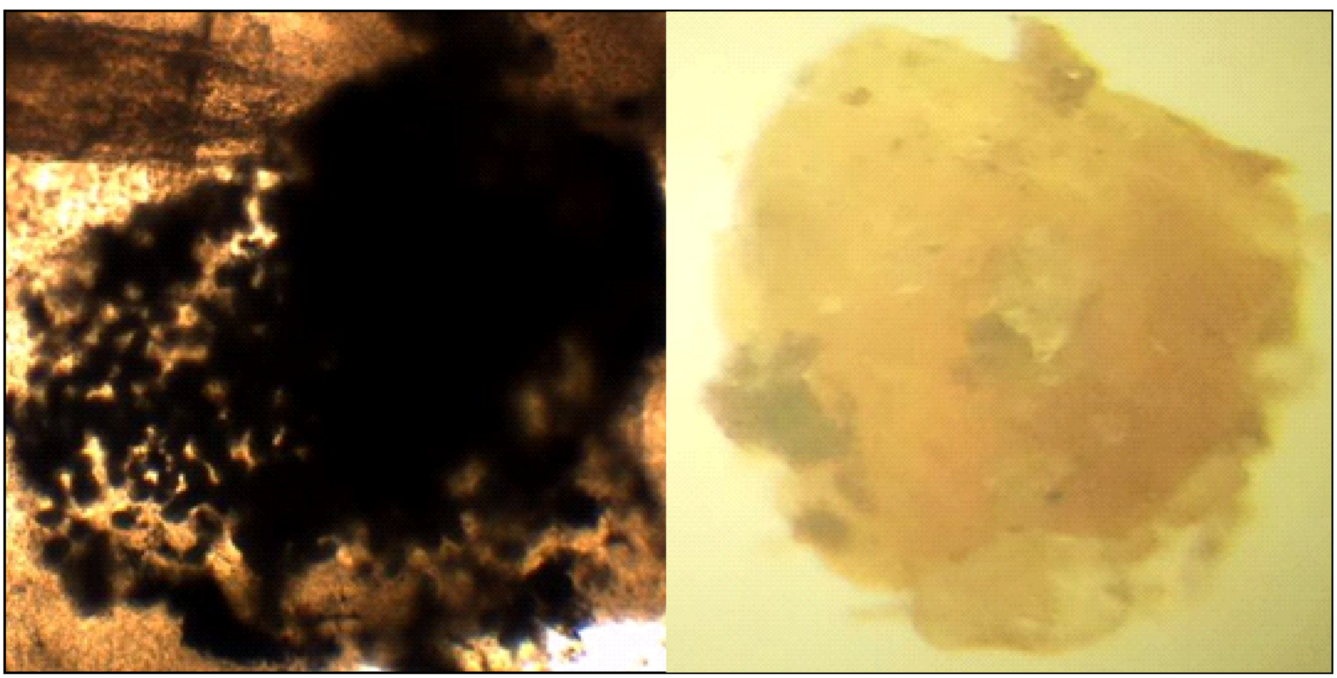

Fig. 1: The nematicidal effect of *P. vermicola* against *M. javanica* egg masses hatching *in vitro*. Treated egg masses failed to hatch and turned to be black color including eggs and matrix. (A) untreated yellow egg masses, (B) crushed treated egg masses with *P. vermicola*.

dark brown to black color, of both the matrix and eggs compared to media and water control Fig. 1.

The bacterial cultural filtrate treatment showed a significant nematicidal activity against egg masses hatching with a mean number of 2% J2 per plate as shown in table 1 and the same dark colored egg masses were observed. Heating of culture filtrate proved ineffective in eliminating the suppression factor(s).

Evaluation of *P. vermicola* treatment against RKN

Efficacy of *P. vermicola* on egg masses

Dark egg masses resulted from bioassay experiment failed to recover or infect tomato roots in pots. All Tomato seedlings treated with dark colored egg masses did not develop foliar symptoms or root galls but remained healthy when examined under dissector microscope whereas the control plants showed typical disease symptoms.

Efficacy of *P. vermicola* in controlling RKN in pots

Tomato seedlings grown in nematode infested soil amended or not with *P. vermicola* showed typical symptoms of RKN infection. Early disease symptoms of leaves yellowing, wilting and stunting of plant growth were observed on seedlings grown in pots infested with nematode (T3), however plants treated with both nematode and bacteria (T4) showed a reduced disease symptoms compared to control plants (T1) and bacteria treated plants (T2). Root examination revealed development of galls on plants treated or not with bacteria; however the number of root galls on T4 was significantly reduced by 84.4% compared to T3. Furthermore, the number of developed egg masses on tomato seedlings treated with *P. vermicola* alone were reduced by 86% in T4 compared to T3 whereas the reduction of J2 number/ g soil reached 80.6 % Fig. 2.

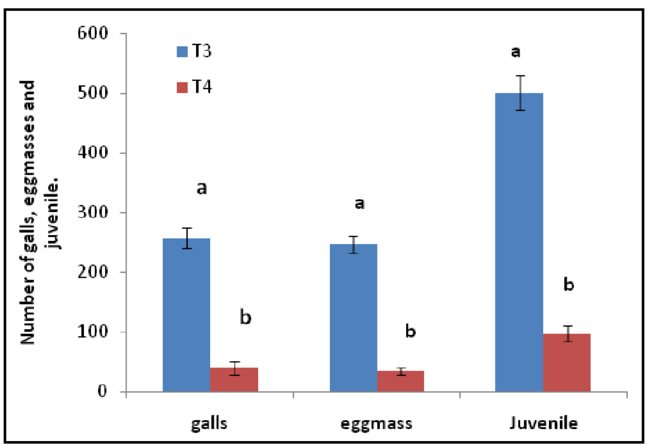

Fig. 2: The number of galls, egg-masses and J2 on infected tomato seedlings grown on pots under greenhouse condition. (T1) control plants; (T2) *P. vermicola* treated plants; (T3) seedlings grown on *M. javanica* infested soil; (T4) seedlings grown on *M. javanica* infested soil and treated with *P. vermicola*. Values are means of n = 12.Vertical bars indicate ±SD and columns with different letters are significantly different (P< 0.05). LSD for galls = 17.923; LSD for egg-masses = 13.256; LSD for Juveniles = 29.120.

Efficacy of *P. vermicola* on tomato growth

Plant length and fresh weight were used as a measuring parameter of *P. vermicola* growth promoting action. Tomato seedlings growth significantly increased when treated with bacteria in the presence or absence of *M. javanica*. The highest plant length and fresh weight were recorded on seedlings treated with *P. vermicola* alone (T2) with increase in plant length reached 24 % and increase of shoot and root fresh weight reached 57% and 59% respectively compared to T1. *P. vermicola* application significantly enhanced plant length of T4 by 29% compared to T3 but was less than T1 by 21.3%. Tomato seedlings shoot fresh weight treated with bacteria alone (T2) increased by 57% compared to control plants (T1) whereas seedlings infected with *M. javanica* and treated with *P. vermicola* (T4) increased the shoot FW by 52% compared to infected plants (T3). The same trend was observed with root FW (Fig. 3 & 4).

Bacterial characterization

IAA, HCN and Siderophore production and phosphate solubilization

IAA production by the bacteria *P. vermicola* was estimated in the presence and absente of L. Tryptophan.

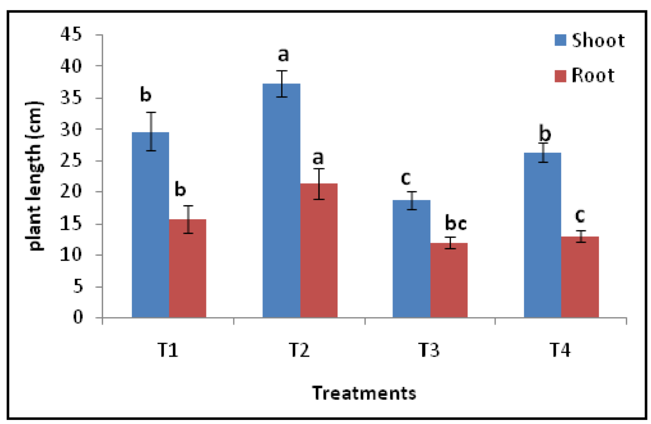

Fig. 3: Effect of *P. vermicola* on plant length infected by *M. javancia.* (T1) control; (T2) seedlings treated with *P. vermicola*; (T3) seedlings treated with *M. javanica*; (T4) seedlings treated with *P. vermicola* + *M. javanica*. Values are means of n= 12. Vertical bars indicate ±SD and columns with different letters are significantly different (P< 0.05). LSD of shoot= 4.030; LSD of root = 4.210.

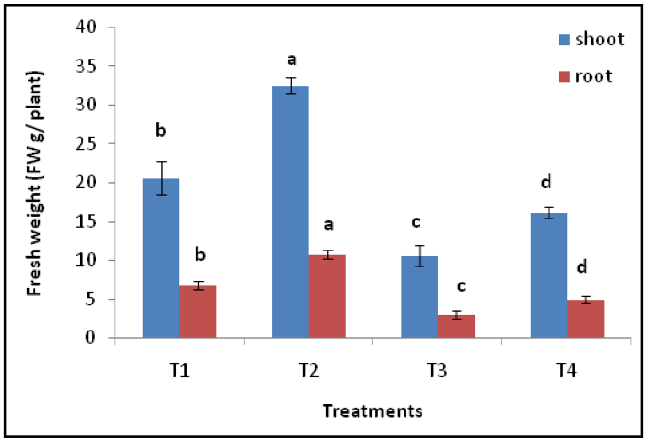

Fig. 4: Effect of *P. vermicola* treatment on tomato seedlings shoot and root FW. (T1) control; (T2) seedlings treated with *P. vermicola*; (T3) seedlings treated with *M. javanica*; (T4) seedlings treated with *P. vermicola* + *M. javanica*. Values are means of n= 12. Vertical bars indicate ±SD and columns with different letters are significantly different ($P< 0.05$). Shoot FW LSD= 1.87 and root FW LSD= 0.686.

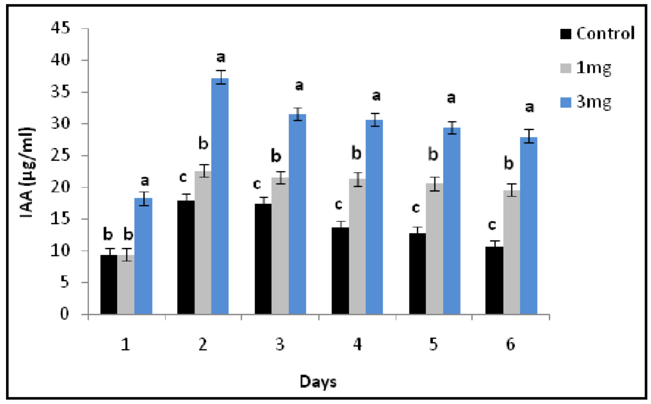

Fig. 5: IAA production by *P. vermicola* isolate grown on Lauria Bertani medium containing L-Tryptophan in two concentration (1µg/ml) and (3µg/ml).

LB medium supplemented with tryptophan significantly enhanced IAA production as shown in Fig. 5. The bacterial isolate produced the highest level of IAA at concentration of 3 mg ml^{-1} tryptophan on day 2 reached 37.41± 4.56, whereas IAA production reached only 22.71±0.39 at concentration of 1 mg ml^{-1} tryptophan on the same day compared to control 18.1±0.41.

Visual examination of the plates showed that *P. vermicola* neither produced an orange halo around bacterial growth on CAS agar media nor changed the filter paper color indicating a non siderophore and HCN producing strain. However, the strain was able to solubilize phosphate by showing a clear zone around the bacterial colonies.

Discussion

Numerous attempts have been described to isolate, identify and apply effective antagonistic microorganisms to manage RKN, nevertheless the success is still limited and the use of chemical nematicides proved to be more effective than biocontrol agents. According to the literature, several bacterial strains showed a significant antagonistic activity against RKN (Zhou *et al*., 2016; Vagelas and Gowen 2012; Moussa and Zawam 2010; Mendoza *et al*., 2008) using different modes of actions such as inhibiting egg masses hatching or causing J2 mortality. In the present study, a novel bacterial strain showed a strong inhibitory action against the RKN *M. javanica*. In dual culture bioassays, both the whole bacterial cell and cell-free culture exhibited a potent nematicidal activity reached almost 100%. Molecular identification of the bacterial strain proved a homologous genome sequence to Providencia *vermicola* reached >99%.

The genus *Providenica* belongs to Phylum: Proteobacteria; Class: Gamma proteobacteria; family: Enterobacteriaceae. The genus consists of 8 different species isolated from diverse environments. Nevertheless, *P. vermicola* was not isolated from any environments other than fish, soil and the entomopathogenic nematodes *Steinernema thermophilum* (Park *et al*., 2011; Somvanshi *et al*., 2006; Akhtar and Ali 2011). Furthermore, *P. vermicola* was not described as a biocontrol agent although it was previously reported as a plant growth promoting rhizobacteria (Hussain *et al*., 2015; Islam *et al*., 2016).

Examination of the treated egg masses under the light microscope revealed a remarkable color change from yellow to black and almost no hatched J2 was observed. In addition, it was noted that NB medium affected negatively egg masses hatching indicating an inhibitory effect of media components which may be attributed to the high concentrations of chemicals. However, no color change of the gelatinous matrix (GM) or eggs was observed on water media or control plates, suggesting presence of an inhibitory substance(s) produced by the bacterial cells, such as antibiotics (Haas and keel 2003) and/or enzymes (Lian *et al*., 2007) that could be responsible for egg masses color change and lack of vitality. Generally, the RKNs lay eggs into GM that is secreted via six rectal glands located around the female anal which consists of protein, carbohydrate and certain enzymes (Bird & Rogers, 1965; Bird & Soeffky, 1972); the laid eggs become enveloped in the GM completely. In addition, the egg shell consists of three distinctive layers in which the outermost layer is a protein (vitelline) followed by a chitinous layer and finally the innermost layer is lipid.

Since proteins and carbohydrates are apparently the common components of the egg shell and GM, it was hypothesized that the factor(s) that altered the egg shell and GM color was mainly affecting proteins which might be denatured and lost their functions. However, the color change and protein denaturation could be only indirect consequences of the inhibitory factors. Accordingly, the nature of the inhibitory substance(s) and nature of the morphological and physiological changes of eggs remain to be identified.

In this regard, nematode inhibition by bacterial supernatants and culture filtrates was reported previously (Oliveira *et al*., 2007; Vagelas and Gowen 2012). For example, Siddiqui *et al*., (2000) illustrated that ethyl acetate extracted from *Pseudomonas aeruginosa* culture caused 64% *M. javanica* juveniles' immobility within 24 h and consequently hypothesized that the nature of the active compound might be proteinaceous or glycoproteinaceous substance. Further investigation by Ali *et al*., (2002) characterized the active compound as heat and extreme pH sensitive, polar and its molecular weight was smaller than 8,000 Da. Other studies by Ahman *et al.,* (2002) and Mendoza *et al.,* (2008) demonstrated that bacterial proteases were able to digest nematode cuticle, degrade juveniles and reduce eggs hatching. In the present study, bacterial culture filtrate maintained its suppressiveness activity after heating at 100^{o}C for 6 min and the treated egg masses neither hatched nor infected tomato roots suggesting occurrence of permanent damage to eggs components.

Results showed that *P. vermicola* could not produce siderophore or HCN, but exhibited a strong capability to produce IAA and solubilize phosphate. IAA is a common secondary metabolite produced auxin by soil microorganisms that colonize the plant roots and enhance plant growth (Mendes *et al.,* 2007; Patten and Glick, 1996). Such organisms are called plant growth promoting rhizobacteria (PGPR) and can live freely in rhizosphere or as endophytic bacteria. The physiological effect of IAA is promoting the plant cell division and elongation that enhances root escalation whereas Phosphorus (Pi) is the second essential macronutrient after Nitrogen, plays a significant role for plant health and growth (Ali *et al*., 2012). Generally, agricultural soil commonly expresses lack of phosphorus availability to plants due to its insoluble forms as calcium phosphates in the alkali soil or insoluble iron and aluminum phosphates in acidic soils. Therefore, Pi is added regularly since a significant part of the added synthetic fertilizer is immobilized to the insoluble forms. Consequently, the availability of Pi is substantially depends mainly on the biological processes occurring specifically in the rhizospher (Deubel and Merbach, 2005); primarily via the phosphate-solubilizing gram negative bacteria through secretion of low molecular weight organic acids and the enzymes phosphatases. The secretions diffuse onto soil elevating considerably the amounts of available soluble Pi to plant roots (Sashidhar and Podile 2010). In the present study, *P. vermicola* application caused a significant increase of root and shoot length and fresh weight. The significant increase of tomato seedling growth may be attributed to IAA production and phosphate solubilization activities of the bacteria. Synergistic interaction between the two traits could cause the substantial observed proliferation in seedlings growth reached 24, 57 and 59% in plant length and shoot and root fresh weight respectively. In this regard, several rhizosphere bacterial genera have illustrated a significant capability to enhance plant growth via different modes of action (Deubel and Merbach, 2005, Ali *et al*., 2012; Malboobi *et al*., 2009; Youssef *et al*., 2016) and PGPR activity of *P. vermicola* was observed previously (Hussain *et al*., 2015; Islam *et al*., 2016).

Conclusion

The obtained results suggest that, *P. vermicola* is a potent biocontrol agent against RKN showing a strong nematicidal effect on egg masses hatching and J2 survival. Alteration on egg masses morphology could initiate interest of the nature of the inhibitory substance(s) produced in the microbial culture. In addition, results of the pot experiment suggesting production of such substances on soil. Further physiological and biochemical studies could reveal a novel mode of action in controlling RKN.

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