Potency Evaluation of *Serratia marcescens* and *Pseudomonas fluorescens* as Biocontrol Agents for Root-knot Nematodes in Egypt

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**Abstract:** A number of bacterial species, isolated from either root-knot nematodes conductive or suppressive soil of different localities in Egypt, were evaluated for suppression of *Meloidogyne incognita*, the causal agent of root-knot of faba bean. Nine isolates of 50 bacterial isolates significantly reduced nematode larvae population in soil belonging to *Meloidogyne* spp. Death percentage of nematode larvae ranged from 54.7% to 96.25%. Two potent bacterial isolates with higher nematocidal activity were selected and identified as *Serratia marcescens* and *Pseudomonas fluorescens* using morphological and biochemical diagnosis tests. The identification was confirmed by genetic characterization, applying molecular finger printing of DNA of both isolates. RAPD sequencing and PCR sequencing analysis revealed genetic variation among the two isolates. The effect of bacterial treatment as bio-control agent on the development of *Meloidogyne incognita* infecting faba bean was evaluated under greenhouse conditions. Both *Serratia marcescens* and *Pseudomonas fluorescens* were effective as bio-control agent and significantly reduced the incidence of root-knot disease in soil artificially infested with *Meloidogyne incognita*. All bacterial treatments significantly increased all growth parameters in the presence or absence of the pathogen. The application of the bio-control agents increases shoot and root dry weight, number of nodules and number of pods. The study indicated that *Serratia marcescens* and *Pseudomonas fluorescens* were potent as bio-control agents for root-knot nematodes, the production of local Egyptian inoculums of both bacterial species as a safe bio-control agents for the root-knot disease is possible.

**Key words:** Biocontrol agents, root-knot nematodes, *Serratia marcescens* and *Pseudomonas fluorescens*

**INTRODUCTION**

The concept of PGPR is now well established and so some consideration of the relationship of PGPRs to biocontrol is worthwhile. PGPR increase plant growth indirectly either by the suppression of well-known diseases caused by major pathogens or by reducing the deleterious effects of minor pathogens (micro-organisms which reduce plant growth but without obvious symptoms). Alternatively, PGPR may increase plant growth in other ways, for example, *Pseudomonas* promote plant growth and reduce the population of deleterious microorganisms; by associative N2 fixation; solubilizing nutrients such as P; promoting mycorrhizal function, regulating ethylene production in roots; releasing phytohormones; decreasing heavy metal toxicity; production of plant growth regulators, vitamins and enhancement the uptake of plant nutrients as well as suppression of pathogenic and deleterious organisms. *Serratia* sp. jointed with other PGPRs in a multi-strain inoculation enhanced growth and yield of peanut crop in sandy soil. It has been suggested that the two groups should be reclassified into biocontrol plant growth-promoting bacteria (biocontrol PGPB) and PGPB.

Root-knot nematodes are widespread and important pests of both annual and perennial crops in Egypt and other countries, where they cause extensive damage to fruit and vegetable crops. Management of the disease has relied largely upon pesticides application. The frequent and extensive use of chemical insecticides has resulted in developing a wide spread resistance of certain pathogens to a range of pesticides. The divesting implication of pesticides resistance developing in insect populations combined with the increased global interest to reduce the input of harmful pesticides has encouraged research into the development of environmentally benign strategies for pest control including the use of microbial species. Bacteria, yeast and filamentous fungi are common inhabitants of soil and plant.
The predominant nematodes in soils and roots of crops were of the genus Meloidogyne[33]. The biological control of root-knot nematode Meloidogyne java was investigated using several strains including Trichoderma harzianum, Pochonia chlamydosporia, Metarizium anisopliae and Baeuvaria sp.[16,8,6,7,32,37,38]. Limited numbers of bacterial species have been reported as biological control agents for root-knot nematode disease. Some bacterial species with nemacidal activity has been used with some success for controlling against root-knot diseases including Streptomyces spp., Serratia spp., Bacillus spp., Azotobacter chroococcum, Rhizobium, Corynebacterium and Pseudomonas[4,32,19,47,61,25,26]. The objectives of the present study were: isolation of local bacterial strains that suppress root-knot nematodes, identification of the most potent isolates and evaluation of the potent antagonistic isolates for controlling of Meloidogyne on faba bean under greenhouse conditions.

MATERIALS AND METHODS

Isolation of Antagonistic Bacterial Strains: A number of bacterial species were isolated from root-knot nematode conductive or suppressive soil. Fifty bacterial isolates were isolated from soil samples collected from rhizosphere of tomato, okra, bean nut, grapes and banana plants by using dilution-plate method. From each sample, one gram soil was mixed aseptically with 100 ml of sterilized distilled water. The soil homogenous suspensions and subsequent serial dilutions (up to 1x10⁷) were made. One ml of each suspension was poured aseptically on solidified nutrient agar medium with sterilized pipette and incubated at 30±1ºC. Selection of bacterial colonies and purification took place by using streak plate method. The purified bacterial isolates, were then stored at 4 Cº for experimental use.

Screening of Bacterial Isolates Against Root-knot Nematodes In vitro: Bacterial isolates (numbered from Ba to Baₙ) were screened for their antagonistic activities against nematode larvae of Meloidogyne. One ml of each bacterial isolate suspension grown on nutrient broth medium, was mixed with 10 ml of water suspension containing certain number of root-knot nematodes larvae per ml dispensed in sterilized bottle (25 ml capacity) and incubated at 28-30º C for 48 h. After incubation period, one ml of mixture suspension was pipetted onto Hawksesley counting slide. Examination under stearo-microscope was done. Control and each treatment were replicated thrice. The reduction percentage of nematode larvae was calculated for each antagonist comparing with the control.

Nematode Larvae Extraction: Soil samples (150 g) were collected, then processed for nematode larvae extraction by using Oosten brink's elutritor method. The resulting suspension was collected and concentrated to 25ml in glass vial by using 400-mesh sieve. One ml of the suspension was pipetted into Hawsksley counting slide and examined under stearo-microscope. Nematode larvae were identified to generic level and were counted.

Identification of the Bacterial Isolates: Selected isolates with nematocidal activity were characterized and identified according to Bergey's Manual of Determinative Bacteriology[11]. The morphological and cultural characteristics of selected bacterial isolates were examined on nutrient agar medium. The morphological characters included shape, size, Gram-reaction, endospore and pigmentation. Motility in nutrient broth was also tested. Growth on nutrient agar at 4º C and 40º C was investigated. Biochemical characteristics (nitrate reaction, casein hydrolysis, V.P. test, M.R. test, indole production, fermentation and assimilation of carbohydrates, gelatin liquefaction, arginine dihydrolase, starch hydrolysis) were also studied.

Molecular finger printing of DNA of Serratia marcescens (Ba₋) and Pseudomonas fluorescens (Ba₋₁): Extraction and Purification of DNA: DNA was extracted according to Tompson et al.,[55] with some modification. 1.5 ml aliquot of culture was pelleted by centrifugation. The medium was decanted. The pellets were resuspended in 500µl of TES buffer (50mM tris, 5mM EDTA pH (8) and 50 mM NaCl). Lysozyme was added to obtain a final concentration of 1 mg/ml. The solution was incubated at 55 C for 30 min. 4µ l RNase was added, vigorously shacked and incubated at 37°C for 10 min. After the addition of 10 µl 1 protease K (10 mg/ml) and 20 µl 1 of SDS (1%) the mixture was incubated at 55°C for 10 min. The solution was chilled on ice and extracted with equal volumes of ethanol: chloroform : isoamyl alcohol (25: 24: 1). The organic was repeated and equal volume of 4 M ammonium acetate was added. Total genomic DNA was precipitated by the addition of 2 volumes of isopropanol. DNA extracts were resuspended in TES buffer (0.001M EDTA). DNA quantity was determined using Becman-spectrophotometer DU650-series at 260 nm and 280 nm absorbance. DNA quality was controlled by agarose gel electrophoresis.
RAPD Analysis: Seventeen primers (obtained from the University of British Columbia Nucleic acid-Protein Service Unit, Canada) were used for polymerase chain reaction (PCR) based on the protocol of Gussow and Clackson[31] and Tompson et al.[55]. The successful primers are listed in Table 1. Reaction similar to that described by Tompson et al.[55] was performed with some minor modification. Reaction volume (25µl) contains 25 ng genomic DNA, 0.2 µM primer, 0.2M of each dNTP, (From Boehringer Mannheim), 1.5 units of Taq DNA polymerase (from advanced Biotechnologies Ltd. Blenheim Road, Surry KT 199QQ, UK), 2.5 µL 10x buffer and 1.25 µl MgCl2 and sterile bidistilled water up to 25µl. The reaction mixture was overlaid with minute drops of light mineral oil (sigma). Amplification was performed in a thermal cycler (Perkin Elmer GeneAmp PCR system 2400) programmed for 1 cycle at 95°C of 1 min., and 40 cycles of 40 sec. at 94°C, 1 min. at 37°C of 1 min., a final extension at 72°C for 12 min. Amplification products were analyzed by electrophoresis in 1.5 % agarose gels with 0.5x TBE buffer and detected by staining with an ethidium bromide solution for 30 min. Gels were then photographed under UV light.

Data sharing analysis was carried out for RAPD DNA profiles using pair wise comparison among samples. The formula used to determine the coefficient of similarity is according to Sambrook et al. this formula is F=2n_y / (n_x + n_y ), where n_x is the total number of DNA fragments from sample X, n_y is the total number of DNA fragments from sample Y and n_y is the total number of DNA fragments that were identical in the two samples.

Potential Antagonism of Serratia marcescens and Pseudomonas fluorescens Against Meloidogyne incognita Under Green House Experiment: A Pot experiment was conducted to explore effectiveness of both Serratia marcescens and Pseudomonas fluorescens to reduce the population density of root-knot nematodes larvae, under greenhouse conditions. Seeds of faba bean (Giza, 714) were sown in 30 cm pots containing autoclaved sandy loam soil (1:1). Five seeds were sown in each pot, then thinned to two plants/pot just 10 days after germination. Pots were divided into nine groups each contained four replicates. Treatments were bio-agents of Serratia marcescens and Pseudomonas fluorescens, which were individually incorporated into the soil at dose rate of 20 ml/pot (10^7 cells/ml) every 10 days for four applications. Rhizobium leguminosarum bv. vaceae was used to inoculate faba bean seeds before planting using seed coating technique. Pots were arranged in a complete randomized block design, watered and received the normal agricultural practices. Pots nematodes infested were received newly hatched second stage larvae of Meloidogyne incognita at dose rate of 20 ml/pot (50 larvae/ml) after 10 days of planting. Two months later, the plants in each pot were uprooted and the roots were gently separated from the soil, washed with flow water and dried by pressing lightly between blotting paper. Average numbers of galls and rhizobia nodules were counted. Nematode larvae population density after harvest (pf) were extracted from soil and counted using Oosten Brink’s elutritor technique. Reduction of nematodes population density in soil sample was calculated according to Tilton formula, as follows:

\[
\frac{\text{Population density in the treated pot before application} - \text{Population density in the treated pot after application}}{\text{Population density in the control pot before application} - \text{Population density in the control pot after application}} = \frac{X}{(1 - X)}
\]

The growth responses of faba bean (roots, shoot dry weight and number of pods/plant) were also recorded. Statistical analysis were achieved according to Gomez and Gomez[30].

RESULTS AND DISCUSSION

Table 1: The sequence of successful primers amplifying DNA fragments of the tested isolates.

<table>
<thead>
<tr>
<th>Code</th>
<th>5-Sequences-3</th>
<th>(G+C)%</th>
</tr>
</thead>
<tbody>
<tr>
<td>UBC 17</td>
<td>CCT GGG CCTC</td>
<td>80</td>
</tr>
<tr>
<td>UBC 25</td>
<td>CCC GCC TCCC</td>
<td>80</td>
</tr>
<tr>
<td>UBC 35</td>
<td>ACA GGG GTGA</td>
<td>80</td>
</tr>
</tbody>
</table>

Results:

Isolation and Identification of Bacteria Antagonistic for Root-knot Nematodes: Bacteria were isolated from nematodes conductive and suppressive soils, more than fifty bacterial isolates were screened for their potentialities to reduce nematode larvae population density in vitro. Based on the preliminary screening, nine isolates possessed a pronounced activity, were selected for their efficacious to reduce nematodes larvae belonging to Meloidogyne spp. The selected bacterial isolates showed variation in their potentialities to reduce population of nematodes larvae In vitro. Death percentage of nematodes larvae ranged from 54.70% to 96.25% (Table 2).

Identification of Bacterial Isolates: Nine selected isolates with nematocidal activity were characterized and identified according to Bergey's Manual of Determinative Bacteriology[31]. From the morphological and biochemical characteristics, the selected isolates could be arranged into two major groups:
The first group included isolates number: Ba-2, Ba-9, Ba-18 and Ba-40. They are short rods, Gram negative, motile and non-spore forming organisms. Catalase and V. P test are positive. Isolates of this group are not indole producers and are capable of utilizing citrate and acetate as a sole carbon source. Arginine dihydrolase and methyl red test are negative. They can assimilate starch, glycerol, inositol and cellobiose, they produce acid from glucose, glycerol, cellobiose, inositol and sucrose. Isolates of this group succeeded to produce red pigments (prodigiosin) on starch containing medium. According to Bergey's Manual of Determinative Bacteriology\(^{[11]}\), isolates could be characterized as genus Serratia and showed affinities to be considered as Serratia marcescens.

The second group included isolates number: Ba-11, Ba-14, Ba-13 and Ba-44. They are short or long rods, Gram negative, motile and non-spore forming organisms. Catalase positive and methyl red test negative. Isolates that do not produce neither acetyl methyl carbinol nor indole. Arginine dihydrolase test positive. Isolates could not assimilate arabinose, lactose, starch and cellobiose. They could not produce gas from carbohydrates. Pyoverdin pigment was distinguished under source of UV light of short wave when isolates of this group were cultivated on King's medium. Therefore, these isolates could be identified as Pseudomonas fluorescens.

The identification of both isolates was confirmed by genetic analysis, thus, molecular finger printing of DNA of Serratia marcescens (Ba-) and Pseudomonas fluorescens (Ba-11) was analyzed for detection of genetic similarity among the two isolates. Molecular finger printing using random amplified polymorphic DNA (RAPD) and polymerase chain reaction (PCR) was carried out (Fig. 1, Table 3). There primers out of the tested seventeen different random primers were succeeded to amplify DNA fragment to make fingerprints. The successful primers were UBC 17, 25 and 35: result of agarose gel electrophoresis of PCR products (Fig. 1, Table 3) revealed that, DNA of Pseudomonas fluorescens (Ba-11) amplified nine fragments were obtained by using primer UBC 35, while the lowest number of synthesized DNA fragments (four bands) was observed with Serratia marcescens (Ba-2), using primer UBC 25.

The resulted finger prints using the three primers that able to amplify DNA fragments showed that, primer UBC 35 could amplify nine fragments bands with DNA of Pseudomonas fluorescens (Ba-11) and six fragments with DNA of Serratia marcescens (Ba-2). Four fragments were similar or identical in both isolates, where as they had molecular weight 1246, 1038, 950 and 838 bp. According to the formula of similarity coefficient as mentioned in materials and methods, the similarity equal to 4/11. The obtained results presented in Table 3, also indicated that primer UBC 25 succeeded to amplify six fragments of DNA of Pseudomonas fluorescens (Ba-11) and four fragments of DNA of Serratia marcescens (Ba-2). Three bands were similar and the degree of similarity coefficient was 3/8. Primer UBC 17 amplified five fragments of DNA of Pseudomonas fluorescens (Ba-11) and seven fragments of DNA of Serratia marcescens (Ba-2).

**Table 2:** Effect of the selected isolates on the population density of root-knot larvae of Meloidogyne spp. (in vitro).

<table>
<thead>
<tr>
<th>Isolate No.</th>
<th>Number of larvae / ml</th>
<th>Reduction %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pi</td>
<td>Pf</td>
<td></td>
</tr>
<tr>
<td>Ba - 2</td>
<td>80</td>
<td>3</td>
</tr>
<tr>
<td>Ba - 9</td>
<td>75</td>
<td>16</td>
</tr>
<tr>
<td>Ba - 18</td>
<td>60</td>
<td>10</td>
</tr>
<tr>
<td>Ba - 40</td>
<td>70</td>
<td>20</td>
</tr>
<tr>
<td>Ba - 11</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Ba - 20</td>
<td>69</td>
<td>19</td>
</tr>
<tr>
<td>Ba - 49</td>
<td>70</td>
<td>15</td>
</tr>
<tr>
<td>Ba - 3</td>
<td>75</td>
<td>34</td>
</tr>
<tr>
<td>Ba - 8</td>
<td>53</td>
<td>23</td>
</tr>
<tr>
<td>Check</td>
<td>50</td>
<td>45</td>
</tr>
</tbody>
</table>

According to efficacious of the selected isolates to reduce the population density of root-knot larvae (Table 2), both of Serratia marcescens (Ba-2) and Pseudomonas fluorescens (Ba-11), were selected for further studies.

**Table 3:** Molecular weight of PCR fragments amplified using different RAPD primers UBC.

<table>
<thead>
<tr>
<th>Primer</th>
<th>RF MW(bp)</th>
<th>Primer</th>
<th>RF MW(bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UBC 35</td>
<td>0.45 1410</td>
<td>UBC 25</td>
<td>0.52 1262</td>
</tr>
<tr>
<td>0.53</td>
<td>1246 1309</td>
<td>0.61</td>
<td>1098 1157</td>
</tr>
<tr>
<td>0.66</td>
<td>1016 1101</td>
<td>0.661</td>
<td>1016 1101</td>
</tr>
<tr>
<td>0.71</td>
<td>947 0.721</td>
<td>0.71 1098</td>
<td></td>
</tr>
<tr>
<td>0.789</td>
<td>833 0.789</td>
<td>0.789 833</td>
<td></td>
</tr>
<tr>
<td>UBC 17</td>
<td>0.4 1317</td>
<td>UBC 25</td>
<td>0.52 1262</td>
</tr>
<tr>
<td>0.55</td>
<td>1122 925</td>
<td>0.55</td>
<td>1122 925</td>
</tr>
<tr>
<td>0.649</td>
<td>1035 954</td>
<td>0.649 1035</td>
<td></td>
</tr>
<tr>
<td>0.709</td>
<td>943 0.79</td>
<td>0.709 943</td>
<td></td>
</tr>
<tr>
<td>0.79</td>
<td>838 0.79</td>
<td>0.79 838</td>
<td></td>
</tr>
<tr>
<td>0.79</td>
<td>838 0.79</td>
<td>0.79 838</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3:** Molecular weight of PCR fragments amplified using different RAPD primers UBC.
Fig. 1: Ethidium bromide-stained agarose gel resolving RAPD-PCR profile of the two bacterial isolates (Ba-2 and Ba-11). M₁ and M₂ are DNA markers.

Table 4: Extended effect of Serratia marcescens or Pseudomonas fluorescens on the root-knot nematodes *Meloidogyne incognita* infecting faba bean (Giza, 714) under greenhouse conditions

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Nematode populations density in soil</th>
<th>No. of galls/root system</th>
<th>No. of rhizobial nodules/root</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pi reduction %</td>
<td>Pf reduction %</td>
<td>reduction %</td>
</tr>
<tr>
<td>M₁+Se+Rh</td>
<td>1000</td>
<td>950</td>
<td>77.2</td>
</tr>
<tr>
<td>M₁+Ps+Rh</td>
<td>1000</td>
<td>830</td>
<td>80.1</td>
</tr>
<tr>
<td>M₁+Se</td>
<td>1000</td>
<td>750</td>
<td>82</td>
</tr>
<tr>
<td>M₁+Ps</td>
<td>1000</td>
<td>650</td>
<td>84.4</td>
</tr>
<tr>
<td>M₁+Rh</td>
<td>1000</td>
<td>3900</td>
<td>0</td>
</tr>
<tr>
<td>Se+Rh</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ps+Rh</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M₁</td>
<td>1000</td>
<td>4100</td>
<td>0</td>
</tr>
<tr>
<td>Rh</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LSD</td>
<td>0.01</td>
<td>16.6</td>
<td>13.65</td>
</tr>
</tbody>
</table>


Only four bands were similar in both isolates with similarity coefficient of 4/8. In conclusion, although there is genetic diversity between the two isolates demonstrate a high degree of genetic heterogeneity and the two isolates were not closely related.

**Evaluation of Serratia Marcescens and Pseudomonas Fluorescens as Biocontrol Agents for Meloidogyne Incognita in Situ:** Data presented in Table (4), showed the effect of microbial treatments on the development of *Meloidogyne incognita* infecting faba bean (Giza, 714) under greenhouse conditions. Results revealed that, both *S. marcescens* and *P. fluorescens* had the potentiality to reduce the root–knot nematodes infectivity and reproduction to great extends along the growing season of faba bean plants. Thus, high percentage reduction in the number of the juveniles in the soil ranged from 77.20% to 84.40% during the growing season was recorded. Data also revealed that, the infectivity of the nematode was tremendously...
affected. Therefore, the number of galls per root system were significantly (P<0.01) decreased from 104 (control treatment) to 27.0 and 23.0 with reduction percentage of 74.04 % and 77.90% when the bioagent of S. marcescens or P. fluorescens was added to nematodes infested pots inoculated with Rhizobium leguminosarum, respectively. The corresponding data of the pots non-inoculated with R. leguminosarum were 18.0 and 10.0 with reduction percentages of 82.70% and 90.40%, respectively. The result showed an increase in number of root galls in the pots treated with meloidogyne + rhizobia in comparison with the control treatment.

Regarding the effect of bacterial agents on faba bean growth, Results of Table 5 showed that all bacterial treatment significantly (p< 0.05 and 0.01) increased plant growth parameters as compared with control (Mi, treatment) while, Mi + Rh treatment failed to cause significant increase. Also, results indicated that, there was a marked effect of biocontrol agents (S. marcescens (Ba-)) or P. fluorescens (Ba-1) and rhizobia on the growth response of faba bean in either nematodes free pots or nematodes infested pots compared with that of control (Rh treatment). Thus, the application of S. marcescens (Ba-) resulted in increasing shoot, root dry weights and number of pods up to 28.40, 43.64 and 2.14% over Rh-treatment, respectively. The corresponding data of P. fluorescens (Ba-1) were 46.80, 77.27 and 45.24%, respectively.

Discussion: In the present study, more than fifty bacterial isolates were isolated. Out of this number, nine isolates possessed a pronounced nematocidal activity. These active isolates showed variation in their potency to reduce nematodes larvae belonging to Meloidogyne spp. ranged from 54.7 to 96.25 %. Similarly bacterial isolates with nematotoxic effect have been also isolated from rhizosphere and soil samples in Egypt and other countries[21,52,28,30,31,53]. Several bacteria were isolated from Meloidogyne incognita egg masses[29]. Screening the isolates for nematotoxic effect resulted in 5 species with antagonism to plant parasitic nematodes. Enterobacter cloaceae and P. mendocina were isolated from the rhizosphere of tomato plants growing in a soil heavily infested with root-knot nematode[21]. Both strains inhibited reproduction of Meloidogyne incognita.

The selected potent isolates were identified isolates Ba-,, Ba-5, Ba-14, and Ba-49 have great similarities in diagnostic properties with Serratia marcescens, whereas isolates Ba-3, Ba-5, Ba-14, Ba-20 and Ba-49 were identified as Pseudomonas fluorescens. The identification of both isolates was confirmed by genetic analysis. Molecular finger printing of DNA using RAPD and PCR of Serratia marcescens (Ba-3) and Pseudomonas fluorescens was analyzed for detection of genetic similarity among the two isolates. The results indicated that there is genetic diversity between the two isolates. The difference among banding patterns of the two isolates demonstrates a high degree of genetic heterogeneity and the two isolates were not closely related.

Studies were conducted under greenhouse conditions to evaluate the biological control of S. marcescens leave space before the bracket (Ba-) and P. fluorescens (Ba-1) as a soil treatment against M.
incognita infesting faba bean. Results indicated that population of the nematode M. incognita were affected by application of both bacterial isolates. The suppressive effect on the number of juveniles ranged between 77.2% and 84.4% during the growing season. Nematodes infectivity was greatly affected in the presence of any of the bio agents, the number of galls per root system were significantly decreased, more prominently in pots inoculated with Rhizobium leguminosarum. These results are in a line with those reported by Zavaleta and Vand Gundy[61], who reported potentiality of S. marcescens to suppress root-knot larvae of Meloidogyne incognita. They attributed this effect to the volatile substances produced during its metabolic activity. Eklund[27] Confirmed that Pseudomonads, are natural inhibitors on the root surface and primary consumers of root exudates rich in amino acids which are converted to ammonia along the root to maintain a micro-zone around the growing roots that would be suppressive to pathogens. The reduction of root galls number may be due to the failure of majority of the encumbered juveniles to penetrate the host root. It was of interest to notice that the tested biocontrol agents not only reduce the infectivity of nematodes, but also increased the number of rhizobial nodules on the root system. These results agree with those reported by Stirling and Sharma[44] and El-Nagar et al.,[25], who reported that Bacillus penetrans not only prevents reproduction of root-knot nematodes but also reduce the infectivity of the juveniles. In the agreement with the present results, some investigators have reported the nematicidal activity of S. marcescens[41,26,4] and P. fluorescens[12,22,49,57,47,13] against M. incognita.

Most researches on biocontrol of nematodes in soil has been concerned mainly on fungal antagonists[48,43,3,54,25,42,8,50,7,6,46,38,52]. Whereas, there are relatively few reports on bacterial antagonists that bring protective effects against phyto-nematodes diseases and limited number of bacterial species have been reported as biocontrol agents for nematode diseases. Bacillus penetrans[44,3,25] and Pasteura penetrans[44,49,38] were as antagonistic to phyto-nematodes including root knot nematodes. Entomopathogenic strains of Serratia marcescens were used as a bio-control in New Zealand[83]. In recent years, some reports have shown that Azotobacter chroococcum, Bacillus megatherium[61], Arthrobacterium sp., Serratia sp., Streptomyces sp. and Corynebacterium sp.[26,4] have nematicotoxic effect and cause reduction in nematode population.

The present results showed an increase in number of root galls in the pots of Meloidogyne + Rhizobia treatment in comparison with the control treatment. Coinciding with this result, El-Bahrawy & Salem[24] concluded that, Rhizobia had stimulatory effect on M. incognita infecting broad beam. On the other hand, 54.35 % reduction in rhizobial nodules in comparison with control was recorded. This reduction could be attributed to deleterious effect of M. incognita on the development of rhizobial nodules, or due to the interference of root-knot nematodes with nitrogen fixation in legume hosts inoculated with Rhizobium[31,32]. There are reported that P. fluorescens applied to seed or soil bring protective effect against nematodes infection[49]. Eapen et al.[22] reported that treating pepper seedlings with isolates of P. fluorescens reduced the nematode damage caused by M. incognita. Similarly, inoculation of wheat plants with P. fluorescens resulted in significant lower nematode population[13].

The present results also agreed with the findings of Shanthi et al.[49] who tested soil with P. fluorescens as a biocontrol agent of M. incognita on Vitis sinifera in greenhouse studies. They reported that, application of P. fluorescens has a suppressing effect on nematodes multiplication as well as giving higher plant yield. Also, Ramakrishnan et al.[27] showed that application of P. fluorescens as a nematode biocontrol on rice (seed treatment) gave significant seedling protection against the nematodes and promoted rice growth. Application of P. fluorescens reduced, as well nematode parasitization of potato roots[49]. Enterobacter cloacae, Pasteura penetrans and Pseudomonas sp. stimulated tomato plants growth and inhibited the reproduction of M. incognita[21]. Serratia marcescens had significant suppressive effect on M. incognita on sunflower[4]. Mercer et al.[41] examined the effect of chitinase from Serratia marcescens and Streptomyces griseus on eggs and juveniles of Meloidogyne helpa. They suggested that chitinase of both strains cause premature hatch of nematode eggs and could be used as an aid in the control of nematodes.

In addition to the suppressive effect on nematode disease, the two biocontrol agents used in this study, enhanced the growth of faba bean plants. Similar results on the effect of P. fluorescens were obtained by Santhi and Sivakumar,[48] who found that P. fluorescens treatment protects tomato against root-knot diseases caused by M. incognita and increases plant growth. Similarly, application of B. thuringiensis and Streptomyces sp reduced root galling and increased lettuce head weight[77]. The growth promotion of faba bean observed in the present study may be attributed to that, these bio-agents may benefit plant growth by providing growth regulators or by producing toxic metabolites which may inhibit nematodes and exclude other deleterious microorganisms. The ability of S. marcescens and P. fluorescens isolated from soil and rhizosphere to produce biologically active compounds have been reported by several investigators[62].
According to the aforementioned results obtained in this study, we concluded that, the local isolates of both Serratia marcescens and Pseudomonas fluorescens could be mass produced as a safe biological control agent of root-knot diseases in Egypt. Consequently, the introduction of such bacteria in soils, or cultural practices aimed to increase the activity of native strains of these bacteria could greatly contribute to the efficiency of nematode biocontrol with Serratia marcescens and Pseudomonas fluorescens.

REFERENCES


