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Molecular Characterization and Nematicidal Activity of some Soil Bacteria against Root-knot Nematodem, *Meloidogyne javanica* in Strawberry

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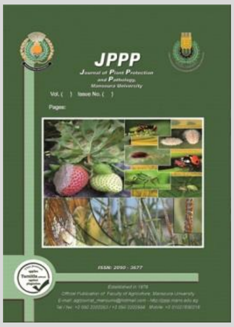

ABSTRACT

Meloidogyne species are the highest eradicative pest in most of yields causing huge losses in many crops. The use of rhizobacteria has gained attention in the control of *Meloidogyne javanica*. In our study, five rhizobacteria were isolated from sandy loamy soil samples in El Beheira governorate, and identified based on their molecular characteristics (16S rRNA sequences) and phylogenetic analysis. The selected isolates were *Staphylococcus pasteuri, Pseudomonas japonica, Bacillus cereus*, *B. altitudinis* and *B. safensis*. The five bacterial strains exhibited satisfactory nematicidal activity against *M. javanica in vitro*. Under field condition, the applied bacterial strains significantly increased the plant growth parameters and suppressed *M. javanica* reproductive factor but at different rates. However, *P. japonica* showed the best results as significantly suppressed root galling up to 63.73 to 82.08% during 2019 and 2020, respectively. As a result of the significant impact of the strain *S. pasteuri* DAM10, it could be utilized as a biocontrol factor against root-knot diseases caused by *M. javanica*, and has not been previously reported yet. Therefore, after further studies screened strains can be used as one of the biological control agents that lead to improving plant growth and reducing nematode infection and thus reducing the use of chemical nematicides and helping to develop safer sustainable agriculture.

Keywords: Root-Knot Nematodes; *Meloidogyne javanica;* Strawberry; Rhizobacteria

INTRODUCTION

Root-knot nematodes, *Meloidogyne* spp. are the most eradicative plants' parasitic nematodes that lead to severe yield and economic losses to wide range of plants (Collange et al., 2011; Onkendi et al., 2014). A severe crop decreases reach almost 15 to 25%, however, the decrease could be up to 75% leading to hundred billion dollar loss per year in whole world (Jianga et al., 2018; Kantor et al., 2022). Egypt is the fourth biggest producer of strawberries amongst whole universe (Essa, 2015) because of the weather and soil fertility, as well as its site that offers increased producing of this specific yield. Damage by plant-parasitic nematode to the strawberry yield is estimated annually in Egypt (Abd-Elgawad, 2014), where *Meloidogyne*, *Pratylenchus*, and *Aphelenchoides* species are considered severe dangerous plant parasitic nematodes (PPNs) in strawberry.

Application of chemical materials such as nematicides is the main protocol for controlling prevalent PPNs, but their significant harmful effects to humans and animals biological imbalance and higher outlay have generated a recent ecofriendly protocol to control nematodes. Biological control has received high incremental interest as an alternative method to control plant parasitic nematode; because they are environmental safe as well as outlay influence (Jiang et al., 2018). Fungi and bacteria in the form of biological agents were widely studied against nematodes in comparison to other organisms (El-Deriny, 2009; Ibrahim et al., 2020; Abdellatif et al., 2021; Ibrahim et al., 2021; Soliman et al., 2021). They suppress nematodes reproduction, eggs' hatch as well as juvenile survival and kill nematodes (Suryawanshi et al., 2014). In this respect, bacteria and their metabolites have been reported to affect both plant and microbial community (Berg et al., 2017). Direct antagonistic effect of soil bacteria could be done by parasites, antibiotics, or competing for nutrients and/or infections' sites. For example, rhizobacterial genera like *Serratia*, *Bacillus, Streptomyces* and *Pseudomonas* can use chitin as an energy source and infect phytopathogens which contain chitin (Abdelrazek and Yassen, 2020; Mohamed, 2020; Song et al., 2020). In another way, bacteria could influence host defense mechanisms enhancing the induced systemic resistance (ISR) (Raymaekers et al., 2020). Bacterial degradation products are enriched bioactive components which act as antimicrobial agents against many plant pathogens (Habash et al., 2020). For example, purification of prodigiosin pigments of *Serratia marcescens* were found having great effect on juveniles of the plants' parasitic nematodes i.e *Radopholus similis, M. incognita* and *M. javanica* in decreased concentration as well as inhibited nematode egg-hatching ability (Rahul et al., 2014; Mohamed et al., 2020). Application of families *Bacillaceae* and *Pseudomonadaceae* received greater attention in controlling *Meloidogyne* species. Bacilli and Pseudomonads took place in natural environment, particularly in plants' roots (Mandic-Mulec et al., 2015; Dehghanian et al., 2020).

Therefore, the aims of current investigation were: 1) to identify bacterial strains isolated from Egyptian soils using molecular technique. 2) to assess the potential of five novel bacterial strains as biological control agents against *Meloidogyne javanica* in strawberry: *in vitro* and *in vivo* studies.

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MATERIALS AND METHODS

Bacterial isolates

Sixty soil samples (250g) were taken under similar environmental conditions and tested in microbiology laboratory at Central Lab of Organic Agriculture, Agricultural Research Center. Fourteen bacterial strains were isolated according to Sharma and Shrivastava, (2017). A safety assessment (susceptibility to antibiotics, haemolytic activity and in vitro cytotoxicity) was performed. The refreshment of bacterial cultures was done by streaking a single colony of each bacterial isolate on fresh LB agar plates then incubating the plates at 35 °C. The plates were then stored at 4 °C for experimental use.

Molecular identification of isolated bacteria strains by polymerase chain reaction amplification and sequence of 16S rDNA

Extraction of genomic DNA from bacterial isolates

The genomic DNA (gDNA) was extracted from cultured bacterial isolates. This was achieved by culturing a colony of each bacterial isolates in conical flasks (Pyrex, USA) with 20ml LB medium. The mixture was then incubated on a rotary shaker at 180 rpm under room temperature for 18 h. The cultures were centrifuged at 13,000 rpm at 4 °C for 5 min and the recovered pellets were diluted in distilled water for gDNA extraction by means of QIAamp DNA Mini Kit, Qiagen, Germany. The purified DNA was subsequently used as a template for PCR amplification of 16S rDNA sequence. PCR was performed by using the universal primer sequence 5´AGAGTTTGATCCTGGCTCAG3´ and 5´CTACGGCTACCTTGTTACGA3´ as forward and reverse primer sequences respectively to amplify the bacterial 16S rDNA gene. The resulting reaction produced an amplicon of approximately 1500 bp. The amplifications were performed in 50 µl reaction according to the manufacturers´ handbook using a PCR master mix kit by Qiagen, Germany. The PCR program was run on a GeneAmp PCR system 2400 thermal cycler (Perkin-Elmer) at 94 °C for 3 min as denaturation initiation step, then 35 cycles of denaturation at 94 °C for 30 s, annealing step at 55 °C for 1 min, first extension step at 72 °C for 2 min, and final extension step at 72 °C for 10 min (El-sayed et al., 2018). Purification of the PCR products were performed with QIAquick Gel extraction kit from Qiagen, following the manufactures´ protocols. Resolutions were performed by electrophoresis on 1% agarose gel. The nucleotide sequence was also observed with a PRISM ready reaction dye terminator cycle sequencing kit (Perkin-Elmer Corp., Norwalk, CN and USA) using the aforementioned primers through dideoxy-chain termination technique. The acquired sequences were then analyzed and aligned against known bacterial sequences on the BLAST database program to identify their similarities.

Nematode inoculum

Root- knot nematode (RKN), Meloidogyne javanica was isolated from infected roots of strawberry obtained from a farm at Badr County, El Beheira Governorate, Egypt. The chosen species of RKN in this study was recognized by the female perineal patterns as detailed by the method of Taylor and Netscher (1974).

***In vitro* nematicidal activity of the selected bacterial strains against root-knot juveniles (J_2) mortality bioassay:**

The bacterial strains were screened for their antagonistic activity against eggs hatchability and juvenile mortality. Two milliliters of each bacterial culture were added at a concentration of 50% in addition to one hundred eggs and one hundred second stage juveniles (J2s) of M. javanica were separately placed in each well of a 48-well plates. Wells received media and free of any bacterial isolates were served as control. Treatments of each bacterial isolates were replicated three times. The numbers of hatched juveniles were recorded after 72 and 150 hours and percentages of egg hatching inhibition were then calculated and recorded for each bacterial isolates tested. As well as, juveniles exhibited no movement and attained the shape of straight line were considered as dead. Dead nematodes were counted and recorded after 48-72 hrs. Percentages of nematode mortality were then calculated and recorded for each bacterial isolates tested.

***In vivo* nematicidal activity of the selected bacterial strains against root-knot nematodes**

Bacterial inoculum preparation

A single colony from each 24 h old bacterial isolate was picked up with a sterile inoculation loop and transferred in 100 mL sterile LB medium in 250 mL Erlenmeyer flasks. The cultures were then grown at 30 °C for 2 days in an incubator with shaking at 180 rpm. The culture broth containing bacteria at a concentration of 1 × 109 colony forming units per mL (cfu/ mL) was used as inoculum in the field experiment.

Field experiment

A micro plot field experiment was conducted during 2019 and 2020 seasons, at Badr County, El Beheira Governorate, Egypt. respectively to test the antagonistic effects of selected bacterial isolates on M. javanica development as well as strawberry growth parameters and yield@ 20± 3, 17± 3 ºC. The plots were naturally infested with the root-knot nematodes, M. javanica. The soil was a typical alluvial soil with a sandy clay loam texture with good drainage, slightly acidic pH and moderate fertility. Seedlings of strawberry cv. Festival (35 days old) were transplanted in September 2019 and 2020 in M. javanica infested field. The field experiment covered a total area of 252 m2, were a randomized complete block design and treatments were replicated five times. Each block included untreated control and six treated plots. A plot consisted of one row, 60cm wide and 6m long. Every three weeks, all tested bacterial cultures were applied as soil drenches (one L/10 L water) at three intervals during the season. Oxamyl as conventional nematicide was used for the comparison at the rate of 3g/plant. At the end of experiment, plants were harvested 6 months after transplanting and roots were washed free from adhering soil. Data dealing with fresh shoot weight, dry shoot weight, fruit weight were recorded. From each plot, a composite soil (250g) was processed for nematode extraction by sieving and modified Baermann technique (Goodey, 1957). For each treatment, root hairs (3g) were stained in 0.01 acid fuchsin and lactic acid (Bybd et al., 1983) and examined for the developmental stages, females, galls and egg masses under stereomicroscope.

Data collection

At harvest, root and shoot fresh weights were recorded. Fruit weight per plant was also recorded. Root-galling and egg masses indices were calculated by using a 0–5 scale (Taylor and Sasser, 1978).

Statistical analysis:

Data were subjected to statistical analysis using computer based software “MS-Excel” and results were submitted to analysis of variance (Snedecor and Cochran, 1989). Differences among treatment means were determined by using the LSD test at a significance level of 0.05 (Waller and Duncan, 1969).

RESULTS AND DISSCUSSION

Results:

Safety assessment of bacterial isolates

For antibiotic susceptibility profiles, six tested antibiotics i.e. Vancomycin, Fusidic Acid, Erythromycine, Tetracycline, Kanamycin and Ampicillin were used. Out of the fourteen isolates only five strains showed safe characters for human being, animal plus the environment use and suitable to all of the examined antibiotics. Strains showed obvious inhibiting zone of diameter ranged from nine to thirty mm. As well as for hemolytic activity, there wasn't change in coloration surrounds colonies of the selected isolates when examined on blood agar. Absent beta hemolysis in any examined strains indicated their safe characters. Therefore, the five bacterial isolates were studied for their molecular characterization and nematicidal activity against *M. javanica in vitro* and *in vivo*.

Molecular characterization of the tested bacterial strains

Molecular characterization, phylogenetic trees were constructed for the 16S rRNA sequences (Fig.1). The sequences were deposited at the NCBI GenBank database with accession numbers (Table 1).

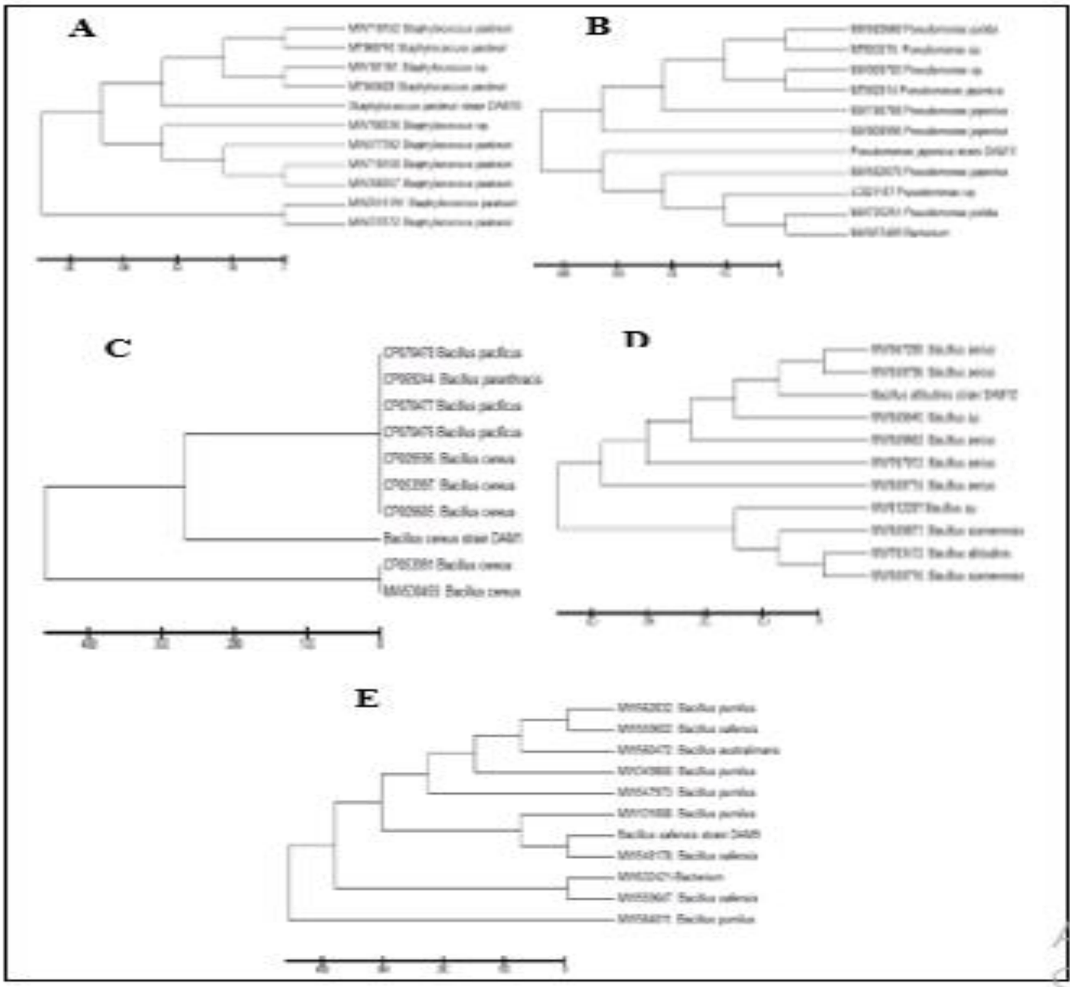

Fig. 1. Phylogenetic trees related to the tested bacterial strains: A) *Staphylococcus pasteuri*, B) *Pseudomonas japonica*, C) *Bacillus cereus*, D) *Bacillus altitudinis*, and E) *Bacillus safensis*

Table 1.The molecular characters of selected rhizobacterial strains based on 16s rRNA sequencing (submitted to NCBI, USA)

Rhizobacterial isolates	Accession number	Base pairs amplified No.	Identified isolates
DAM10	MW940794	450	*Staphylococcus pasteuri* strain DAM10
DAM11	MW940801	350	*Pseudomonas japonica* strain DAM11
DAM1	MW857199	515	*Bacillus cereus* strain DAM1
DAM12	MW940810	550	*Bacillus altitudinis* strain DAM12
DAM9	MW940785	350	*Bacillus safensis* strain DAM9

In vitro study

Data in Table (2) represent the impact of five bacterial isolates namely *Staphylococcus pasteuri*, *Pseudomonas japonica, Bacillus cereus, B. altitudinis* and *B. safensis* on eggs hatching inhibition and juveniles death of *M. javanica*. Irrespective to examined isolates all of them were found causing significant inhibition in hatching rate and juveniles mortality to various extents. Among all treatments, *P. japonica* (100.0 %) sustained the highest and significant inhibition in hatching rate followed by *B.altitudinis* (93.02, 91.85 %) at 72 and 150hrs exposure periods , respectively.

The previously mentioned isolates revealed nematicidal activity against hatched juveniles of *M. javanica* survival after 48h of exposure. A positive correlation among all isolates was revealed. Herein, *B. altitudinis* exceeded other isolates after 24 and 48h of exposure. The most increased percent of *M. javanica* juvenile's death (100%) was significantly in *B. altitudinis* and *P. japonica* after 48h of exposure. However, the least percentage of juvenile's mortality was recorded with *B. safensis* (74.33, 89.33 %) after 24 and 48h of exposure, respectively (Table 2).

Table 2. Nematicidal potentiality of five tested bacterial isolates against eggs hatching and juveniles' survival of *Meloidogyne javanica* under laboratory conditions.

Treatment	Reduction in egg hatching			
	72 h.		150 h.	
	No. of hatched eggs	Red. (%)	No. of hatched eggs	Red. (%)
Control (Nematode only)	43.00^{a}	0.0	90.00^{a}	0.0
Staphylococcus pasteuri	8.66^{b}	79.86	22.60^{b}	74.88
Pseudomonas japonica	0.00^{c}	100.00	0.00^{e}	100.00
Bacillus cereus	2.66^{c}	93.81	11.33^{cd}	87.41
B. altitudinis	3.00^{c}	93.02	7.33^{d}	91.85
B. safensis	9.00^{b}	79.06	17.00^{bc}	81.00
L.S.D at 0.05	3.533	---	6.85	---

Treatments	Juveniles Mortality (JM)			
	24 h.		48 h.	
	No. of immobile juveniles	JM (%)	No. of immobile juveniles	JM (%)
Control (Nematode only)	00.00^{d}	-	6.33^{d}	6.33
Staphylococcus pasteuri	82.00^{bc}	82.00	94.00^{b}	97.00
Pseudomonas japonica	84.33^{b}	84.33	100.00^{a}	100.00
Bacillus cereus	81.66^{bc}	81.66	95.66^{b}	95.66
B. altitudinis	99.33^{a}	99.33	100.00^{a}	100.00
B. safensis	74.33^{c}	74.33	89.33^{c}	89.33
L.S.D at 0.05	7.933	-	2.51	-

Every value is the mean of five replicates. Mean in every column followed by the same letter/s didn't differ at P ≤ 0.01 according to Duncan`s multiple range test.

In vivo study

Impact of the five isolates of rhizobactera as resistance inducers on plant growth parameters *Meloidogyne javanica* infecting strawberry

The effect of the five bio-control agents; *Staphylococcus pasteuri*, *Pseudomonas japonica, Bacillus cereus, B. altitudinis* and *B. safensis* on plant growth response of strawberry plant cv. Festival infested by *M. javanica* in optimum field conditions during two successful seasons (2019, 2020) is shown in Table (3). Result indicates that *M. javanica* infections cause a clear decrease in plants growth parameters with a significant decrease in fresh and dry shoot weights and fruit weight reach 38.30; 35.38 &46.0 and 3.63;3.17&43.33 during the mentioned seasons, respectively. Most isolates showed intermediate increase in shoots and roots weights exceeded than those of untreated inoculated plants with various degrees. Obviously, *P. japonica* showed better results than did other isolates. Meanwhile, the highest improvement in strawberry shoot weight was significantly recorded with *B. cereus* at 2019 and 2020 with percentage of increase reached 111.57 and 149.55%, respectively. Conversely, *B. safensis* showed the lowest percentage values of growth parameters expressed by fresh shoot weight (18.83; 41.41), dry shoot weight (76.31; 124.61) and fruit weight (31.15; 50.01) during 2019 and 2020, respectively.

Table 3. Effect of five bacterial strains on growth parameters of strawberry infested with *Meloidogyne javanica* under field conditions.

Season 2019			
Treatments	**Growth parameters**		
	Fresh Shoot weight	**Dry Shoot weight**	**Fruit weight/ plant**
Control (Nematode only)	38.30^{b}	3.63^{b}	46.00^{b}
Staphylococcus pasteuri	48.50^{b}	7.23^{a}	59.33^{c}
Pseudomonas japonica	73.64^{a}	8.06^{a}	107.66^{a}
Bacillus cereus	81.03^{a}	7.08^{a}	100.66^{ab}
B. altitudinis	54.54^{b}	6.83^{a}	90.66^{b}
B. safensis	45.51^{b}	6.4^{a}	60.33^{c}
Oxamyl	49.03^{b}	5.58^{ab}	71.33^{c}
L.S.D at 0.05	16.63	2.59	12.45

Season 2020			
Treatments	Growth parameters		
	Fresh shoot weight	Dry shoot weight	Fruit weight/ plant
Control (Nematode only)	35.38^{e}	3.17^{d}	43.33^{e}
Staphylococcus pasteuri	54.29^{cd}	8.78^{ab}	63.66^{d}
Pseudomonas japonica	80.40^{a}	9.83^{a}	118.00^{a}
Bacillus cereus	88.29^{a}	7.56^{abc}	108.00^{ab}
B. altitudinis	69.91^{bc}	7.52^{abc}	104.00^{b}
B. safensis	50.03^{de}	7.12^{bc}	65.00^{d}
Oxamyl	51.43^{de}	5.88^{c}	80.33^{c}
L.S.D at 0.05	15.63	1.92	10.14

Every value represented mean of 5 replicates.Means in every column followed by the same letter/s didn't differ at P ≤ 0.05 according to Duncan`s multiple range test.

It is worth to note that significant differences between *P. japonica* and *B. cereus* and other tested isolates in respect to strawberry shoot growth parameters during the two studied seasons were recorded. Oxamyl as a standard nematicide exceeded some isolates and improved fresh shoot, dry and fruit weights with percentage of increase reached 28.01; 45.36%, 53.72; 85.49%, 55.06; 85.39% during 2019 and 2020, respectively.

Influence of five isolates of rhizobacteria on reproduction of *Meloidogyne javanica* infecting strawberry under field conditions

The nematicidal properties of five isolates of rhizobacteria against *M. javanica* infecting strawberry are depicted in tables (4, 5). At all, evaluated isolates significantly suppressed nematodes population whether in soil or roots as compared to control. Meanwhile, the greater suppression in nematode population in soil was detected by *B. altitudinis*. Similar result was noticed with numbers of developmental stages, females and egg masses in roots of strawberry during the two successful seasons under this study. The pronounced suppression in nematode population in soil and roots was recorded with plots treated with *B. altitudinis* (71.79; 83.20%) followed by *P. japonica* (64.24; 80.27%) and *B. cereus* (59.76; 73.97%) with reproduction factor (Rf) =0.36, 0.20; 0.46, 0.23 and 0.52, 0.31 at 2019 and 2020, respectively. Even though, *B. safensis* suppressed nematodes population during 2020 (74.35%). Oxamyl as conventional nematicide surpassed some isolates and significantly suppressed nematode population in soil and roots with reproduction factor (Rf) = 0.45, 0.27compared to untreated infected plants (Table 4).

Table 4. Effect of five bacterial strains on the population density of *Meloidogyne javanica* infected strawberry under field conditions.

Treatments	No. of juveniles / 250 g soil	No. of females/ 5 g of root	No. of developmental stages/ 5 g of root	Final population	Red. %
Season 2019					
Control (Nematode only)	666.66^{a}	64.33^{a}	6.33^{a}	737.32	-
Staphylococcus pasteuri	280.00^{b}	22.00^{cd}	4.66^{ab}	306.66	58.40
Pseudomonas japonica	220.00^{bc}	16.00^{de}	0.66^{c}	263.66	64.24
Bacillus cereus	280.00^{b}	25.00^{bc}	2.33^{bc}	296.66	59.76
B. altitudinis	193.33^{c}	14.00^{e}	0.66^{c}	207.99	71.79
B. safensis	266.66^{bc}	30.66^{b}	2.00^{bc}	299.32	59.40
Oxamyl	233.33^{bc}	21.66^{cd}	0.00^{c}	254.99	65.41
L.S.D at 0.05	70.46	5.73	3.00	-	
Season 2020					
Treatments	No. of juveniles / 250 g soil	No. of females/ 5 g of root	No. of developmental stages/ 5 g of root	Final population	Red. %
Control (Nematode only)	963.33^{a}	73.33^{a}	11.00^{a}	1047.66	-
Staphylococcus pasteuri	233.33^{b}	19.00^{c}	2.00^{b}	254.33	75.72
Pseudomonas japonica	193.33^{b}	13.33^{c}	0.00^{b}	206.66	80.27
Bacillus cereus	240.00^{b}	30.66^{b}	2.00^{b}	272.66	73.97
B. altitudinis	163.33^{b}	11.00^{c}	1.66^{b}	175.99	83.20
B. safensis	246.66^{b}	21.66^{bc}	0.33^{b}	268.65	74.35
Oxamyl	226.66^{b}	17.33^{c}	0.00^{b}	243.99	76.71
L.S.D at 0.05	101.75	10.92	2.44	-	-

Final population = number of juveniles+ females + developmental stages. Red. (%) = (F.C-F.T)/F.C × 100 where, F.C: Final population in untreated control and F.T: Final population in treated plant. Rf=Reproduction factor=Final population/Initial population Each value presented the mean of five replicates. Initial population = 570; 890 juveniles/250 g soil during 2019 and 2020, respectively. Means in each column followed by the same letter(s) did not differ at P ≤ 0.05 according to Duncan`s multiple range test.

Root galls were obviously decreased in all treatments of rhizobacteria isolates (Table 5). Among different isolates, *P. japonica* significantly suppressed root galling with percentage of reduction amounted to 63.73 and 82.08% during 2019 and 2020, respectively followed by *B. cereus* (55.88%) at 2019. However, *B. altitudinis* (80.69%) revealed better performance in decreasing root galling than did *B. cereus* during 2020.

Table 5. Effect of five bacterial strains on the development and reproduction of *Meloidogyne javanica* in strawberry under field conditions.

Treatments	No. of galls / 3g root	Red. %	Root gall index (RGI)	No. of egg masses/ 3 g root	Red. %	Egg masses index (EI)
Season 2019						
Control (Nematode only)	34.00^{a}	0.0	4	39 .00^{a}	0.0	4
Staphylococcus pasteuri	15.33^{bc}	54.91	3	20.00^{bc}	48.71	3
Pseudomonas japonica	12.33^{c}	63.73	3	15.66^{cd}	59.84	3
Bacillus cereus	15.00^{bc}	55.88	3	21.66^{bc}	44.46	3
B. altitudinis	15.33^{bc}	54.91	3	13.00^{d}	66.66	3
B. safensis	20.00^{b}	41.17	3	22.66^{b}	41.89	3
Oxamyl	12.66^{c}	62.76	3	16.33^{bcd}	58.12	3
L.S.D at 0.05	4.64	---	---	6.11	---	---
Season 2020						
Treatments	No. of galls / 3g root	Red. %	Root gall index (RGI)	No. of egg masses/ 3 g root	Red. %	Egg masses index (EI)
Control (Nematode only)	48.33^{a}	0.0	4	41.66^{a}	0.0	4
Staphylococcus pasteuri	12.66^{bc}	73.62	3	16.00^{b}	61.59	3
Pseudomonas japonica	8.66^{c}	82.08	2	8.00^{c}	80.70	3
Bacillus cereus	11.66^{bc}	75.87	3	17.00^{b}	59.19	3
B. altitudinis	9.33^{c}	80.69	2	9.33^{c}	77.48	2
B. safensis	16.66^{b}	65.52	3	19.00^{b}	54.39	3
Oxamyl	11.66^{bc}	75.87	3	15.33^{b}	63.20	3
L.S.D at 0.05	6.04	---	---	5.04	---	---

Reduction; RGI: Root galls index and EI: Egg masses index were determined by the scale given by Taylor and Sasser, 1978 as follows: 0= no galls or egg masses, 1= 1-2; 2= 3-10; 3= 11-30; 4= 31-100 and 5= more than 100 galls or egg masses. Each value represented the mean of five replicates. Means in each column followed by the same letter/s didn't differ at P ≤ 0.05 according to Duncan`s multiple range test.

Similar result was noticed with number of egg masses (66.66 %) at 2019, but at 2020, *P. japonica* excessed all isolates with percentage of reduction reached 80.70%. Oxamyl (62.76, 75.87%) showed obvious decrease in root galls with RGI=3.0 at the above mentioned seasons. However, in most treatments of rhizobacteria isolates significant differences in root galls or egg masses have not noticed between examined isolates.

The present study also elucidated the impact of screened isolates on the peroxidase (PO) and polyphenol oxidase (PPO) activities and total phenol (TP) in fresh leaves of strawberry infected with *M. javanica* (Table 6). Strawberry plants treated with *B. safensis* recorded the highest PO activity (0.76%) showed in Fig.2. However, there is no treatment was recorded to increase PPO activity compared to nematode alone. Conversely, the highest decrease in PO and PPO activities were more pronounced with all bacterial isolates applications as well as oxamyl. Data also revealed that strawberry plants grown in soil naturally infested with *M. javanica* showed obvious increment in total phenol compared to screened isolates.

Table 6. Impact of five bacterial strains on total phenol, peroxidase and polyphenol oxidase in leaves of strawberry infected with root-knot nematode *Meloidogyne javanica* under field conditions.

Treatments	Total phenol mg/1g	PO	PPO
Control (Nematode only)	52.79	0.395	0.195
Staphylococcus pasteuri	52.29	0.392	0.191
Pseudomonas japonica	43.448	0.303	0.188
Bacillus cereus	42.420	0.376	0.185
B. altitudinis	42.550	0.293	0.179
B. safensis	43.960	0.398	0.190
Oxamyl	42.800	0.305	0.173

Each value represented the mean of five replicates. Means in each column followed by the same letter/s didn't differ at $P \leq 0.05$ according to Duncan`s multiple range test. PO= Peroxidase PPO=Polyphenol oxidase

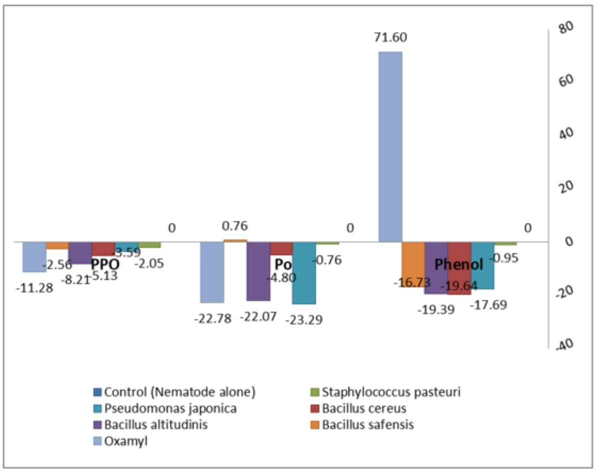

Fig.2. Reduction and increscent values of peroxidase (PO), polyphenol oxidase (PPO) activities and total phenol (TP) in fresh leaves of strawberry infected with *Meloidogyne javanica*.

Discussion

In the current study although chemical nematicide, oxamyl, showed high suppressive effect against root-knot nematode, *M. javanica*, biological control of root-knot nematodes by novel bacterial strains like *Pseudomonas japonica* and *B. altitudinis* is considered accepted to the environment as an alternatives to chemicals. Microorganisms which inhabit the rhizosphere provide the front line of defense against root pathogens and perfect for usage as biocontrol agents (Migunova and Sasanelli, 2021). Thus, the overall goal of such biocontrol agents is the identification and development of effective microbial strain(s) against such aggressive pytonematodes. Inefficient bacterial colonization is the main obstacle of application of bio control agents for management of nematodes. Zhou et al. (2016) performed a comparative study between both application methods, soil drenching and seeds inoculation, and concluded that soil drenching appeared to have a great effect on control of root-knot nematode disease on tomato. In our current study, best management was achieved by application of different bacterial treatments as soil drench for three times in each season.

The current *in vitro* experiment revealed that *P. japonica* strain DAM11 as well as *B. altitudinis strain* DAM12 caused 100% mortality to J_2s of *M. javanica* after 48 h of exposure. Various researches cleared that *Bacillus* and *Paenibacillus* species can cause high mortality to second juveniles by 80 – 100 per cent *in vitro* when exposed to bacterial culture filtrates at least 24 hours (Cheng et al., 2017 ; Bui et al., 2019).

In present study *P. japonica* strain DAM11 recorded the maximum nematicidal activity against *M. javanica* infected strawberry with reduction in gall formation by 82.08% during the second season of application. Our study agreed with Sharma et al. (2021) as the author cleared that the applicated *P. fluorescens* decreased the development and reproduction of *M. javanica* in eggplant. *Pseudomonas* genus' members are physiologically and metabolically multifunctioning. Several strains were proved for positive production of siderophores, hydrogen cyanide and proteases which have strong nematicidal and antimicrobial activities (Siddiqui et al., 2005).

Several studies have been done on *Bacillus* spp. *B. amyloliquefaciens*, *B. subtilis* and *B. aryabhattai* proved their biocontrol potentiality against root-knot nematodes (Rao et al., 2017; AbdElSalam et al., 2018; Zhao et al., 2020). *Bacillus* species could reproduce antimicrobial materials which provide defense against nematode infection (Földes et al., 2000). These materials are subtilin, bacilysin, mycobacillin, bacillomycin, mycosubtilin, iturins, fengycins, and surfactins. These materials have been exerting antibacterial and, or antifungal activities against pathogenic microorganisms (Ntushelo et al., 2019).Our study agreed with other similar studies as it is globaly reported that *Bacillus* species secret a wide variety of toxic compounds which have inhibitory action on the reproduction and activities of nematodes. In the current study *B. altitudinis strain* DAM12was the most effective *Bacillus* strain against *M. javanica* infected strawberry under field conditions. Our results agreed with Wang *et al.* (2021) who revealed the remarkable potentiality of *B. altitudinis* AMCC1040 in decreasing nematode reproduction, root galling and severity of ginger bark cracking disease. Further, five PGPR strains, namely *B. firmus*T11, *B. aryabhattai* A08, *Paenibacillus barcinonensis* A10, *P. alvei* T30, and *B. cereus* N10w, caused 86.0, 85.2, 84.6, 81.5 and 82.1% inhibition in galls formation (Viljoen et al., 2019). *B. cereus* reduced the penetration of *M. javanica* into roots and suppressed nematodes reproduction

(Oka et al., 1993). *B. cereus* produced nematicidal sphingosine, a kind of lipids, which could be used as an antiinflammatory agent and antiseptics. Sphingosine is safe for environment, humans and animal, however highly toxic to nematodes with a nematicidal LC_{50} value of 0.64 µg/ml (Gao et al., 2016).

Mechanisms of nematode population decrement could be explained because of competition for space and nutrients as well as production of antibiotics and hydrogen cyanide by *Pseudomonas* spp. and non-cellular extract and toxic metabolites like bacillopeptidase, subtillin E and β lactamase from *Bacillus* species (Vetrivelkalai, 2019). Lian et al. (2007) cleared that *Bacillus* species produce nematicidal and cuticle-degrading extracellular molecules, such as serine alkaline protease, that decreased nematode action. Additionally, *B. altitudinis* strains are well known producers for serine alkaline protease and acido-thermostable endo-chitinase which can act as nematicidal compounds (Asmani et al., 2020).

Staphylococcus pasteuri was reported as good phosphorous and potassium solubilizing bacteria (Sukmadewi et al., 2021). However, in the current study *S. pasteuri* strain DAM10 has been reported for first time as bio-control agent as the tested strain was able to reduce root galling up to 73.62 %. Rhizobacteria induce systemic resistance by activation of various defense related enzymes peroxidase (PO) and polyphenol oxidase (PPO). Increased activity of PO or PPO has been elicited by biocontrol agent strains in different plants (Govindappa et al., 2010; Nogueira de Moura Guerra et al., 2013; Joni et al., 2020). Research work has reported that the bio-agents *P. fluorescens* and *Bacillus* species might stimulate the production of biochemical compounds associated with the host defense (Kavino et al., 2007; Raj et al., 2016; Rais et al., 2017). Of these, early induction of peroxidase (PO) is more important as it is the first enzyme in the phenylpropanoid pathway, which leads to production of phytoalexin and phenolic substances leading the formation of lignin (Bruce and West, 1989). Conspicuously, the current investigation recorded the higher activity of (PO) and (PPO) in strawberry plants treated with *B. safensis*. Peroxidase activity is vital in the reinforcement of cell walls at the border of infection in resistant plants and are considered as important components of active defense response of nematode invaded tissue (Zacheo et al., 1995).

CONCLUSION

In conclusion, the five bacterial strains, *Staphylococcus pasteuri* strain DAM10, *Pseudomonas japonica* strain DAM11, *B. cereus* strain DAM1, *B. altitudinis* strain DAM12 and *B. safensis* strain DAM9 belonging to three genera, could act as non-chemical alternatives to control root-knot nematode, *M.javanica*. *P. japonica* strain DAM11 and *B. altitudinis* strain DAM12 showed the highest nematicidal activity in both laboratory and field. Furthermore, *S. pasteuri* strain DAM10 showed an effort for controlling *M. javanica* and wasn't announced as bio control agent.

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التوصيف الجزيئي والنشاط الإبادى لبعض بكتيريا التربة ضد نيماتودا تعقد الجذور في الفراولة

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الملخص

تعد نيماتودا تعقد الجذور (.*Meloidogyne* spp) من أكثرالآفات تهديدا للمحاصيل الزراعية وتتسبب في خسائر اقتصادية كبيرة لكثير من المحاصيل الهامة ومن اهمها محاصيل الخضر. لفت استخدام بكتريا العقد الجذرية الكثير من الانتباه كأحد العوامل لمكافحة نيماتودا تعقد الجذور. في هذه الدراسة تم عزل خمس سلالات بكتيرية من ارض رملية طينية في محافظه البحيره وتم تعريفها بناء على خصائصها الجزيئية وتحليلها الوراثي فكانت العزلات هي *Staphylococcus pasteuri, Pseudomonas japonica, Bacillus cereus, B. altitudinis ,B. safensis*. حيث أظهرت العزلات الخمسة تأثيرأ ابادياً للنيماتودا *M. javanica* تحت ظروف المعمل والحقل ، وادت الى زيادة ملحوظه في نمو نباتات الفراولة بدرجات متفاوتة. أدت العزله *P. japonica* الى حدوث خفض ملحوظ في معدل تكوين العقد على الجذور بنسب 63,73 و82,08% خلال عامين 2019 و2020 على التوالي. ومن الدراسة لوحظ انه كنتيجة للتأثير الإيجابي للعزلة *S. pasteuri* DAM10 فانه يمكن التوصية باستخدامها كأحد عوامل المكافحة الحيوية ضد نيماتودا تعقد الجذور حيث لم يتم تسجيلها سابقا. كذلك يمكن استخدام السلالات المختبرة لتقليل التأثير الضار من النيماتودا بالإضافة الي تحسين نمو النبات وتقليل الضرر الناتج عن استخدام المبيدات للمساعدة في تطوير زراعة مستدامة أكثر امانا بعد اجراء المزيد من الدراسات.

الكلمات الدالة : نيماتودا تعقد الجذور- الفراولة – بكتيريا