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Effects of *Serratia marcescens* and Prodigiosin Pigment on the Root-Knot Nematode *Meloidogyne incognita*

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ABSTRACT

The effect of *Serratia marcescens* culture and culture filtrate on juvenile mortality of the root-knot nematode *Meloidogyne incognita* was studied *in vitro* and *in vivo* on tomato seedlings. The percent of mortality was proportional to the concentration of culture filtrate and exposure period. Prodigiosin from *S. marcescens* was extracted with actone and ethyl acetate then purified using puriflash column and characterized by TLC, mass spectrometry, FTIR. Prodigiosin was screened for controlling root-knot nematode at juvenile stage. The obtained results revealed that the molecular weight of the pigment was 324.7 m/z, this confirms that the pigment extracted from *S. marcescens* is prodigiosin. In FTIR analysis red pigment from *S. marcescens* revealed absorption at 3315.3 per cm (N-H) and 2941.6 per cm (aromatic C-H), 1450 (C-H) alkenes, 1020, 1141 (C-O, C-N) carboxylic groups. Bacterial cultures showed better results than the control of nematode alone with a value of 58.5 % for juveniles mortality. The culture filtrate of *S. marcescens* at the four tested concentrations showed nematicidal activities against newly hatched juveniles of *M. incognita* after 72 h of exposure. The pigment was found effective against juvenile stage of *M. incognita* at low concentrations (EC_{50} value after 72 h was 31.9 mg ml^{-1}). It is proved that the use of microbial secondary metabolites can be effective for nematode control rather than using whole organism.

***Keywords*:** *Serratia marcescens*, prodigiosin, *Meloidogyne incognita*, secondary metabolites.

Introduction

The root-knot nematode *Meloidogyne incognita* is a worm-shaped microscopic animal,that causes negligible injury or constraints in crop production. Present strategies for nematode management depend on cultural practices such as crop rotations, use of resistant varieties and use of nematicides which have several disadvantages such as environmental pollution (Gitanjali and Bora, 2019). A number of bacterial species has been used as biocontrol against *Meloidogyne* spp. such as *Enterobacter* (Duponnois *et al*., 1999), actinomycetes (Yavuzaslanoglu *et al.*, 2011), *Pseudomonas* (Gitanjali and Bora, 2019) and *Serratia marcescens* (Suryawanshi, 2014). Microorganisms can inhibit plant parasitic nematodes by producing secondary metabolites, enzymes and toxins. Their modes of action include suppression of nematode reproduction, egg hatching and juvenile or direct killing of nematodes (Suryawanshi, 2014). *S. marcescens* is a Gram negative bacterium, reported to produce cell associated red-colored pigment called prodigiosin (Carbonell *et al.*, 2000). Prodigiosin produced by *S. marcescens* is a promising drug owing to its characteristics of having antifungal, immunosuppressive and antiproliferative activities. It was screened against the juveniles of plant-parasitic nematodes associated with banana and eggplant. Another study has also shown the role of different metabolites synthesized by *Pseudomonas* sp. in nematode infected plants. This study isolated different metabolites, such as alkaline metalloproteinase, cyclo (L-Pro-L-Ile) and many other volatile organic compounds (VOCs) that shown nematicidal activities (Gao *et al*., 2016). Also several studies showed the effect of different dilutions of cell-free culture filtrate on *Meloidogyne incognita* under laboratory and in greenhouse conditions. In laboratory, toxicity effect of filtrate was recorded on second stage juveniles (J2), 100 % mortality was recorded in all treatment above the 75 % concentration (Sharma and Sharma 2017). Many PGPR, such as *Pseudomonas* and *Burkholderia*,

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have been shown to induce suppressive activities against *M. incognita* by reducing gall formation, controlling nematode reproduction, and hatching and killing juveniles through the release of toxins (Khanna *et al*., 2019).

Material and Methods

Bacterial strain

The bacterial strain (*S. marcescens*) used in the present study was obtained from Soil, Water and Environment Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

Preparation of *S. marcescens* culture and culture filtrate

The bacterial strain was inoculated in nutrient broth medium and incubated at 30 °C on a shaker for 48 hrs. The liquid culture was centrifuged at 10000 rpm for 30 min and the supernatant was passed through a 0.045 µm Millipore filter. The culture filtrates were kept at 4 °C for further use.

Preparation of *Meloidogyne incognita* juveniles (J2) inocula

Heavily infected root systems of coleus plants were carefully washed by running tap water. Roots were cut into pieces and egg masses were allowed to hatch using modified Baerman technique (Goodey, 1957). Hatched juveniles were collected daily for a week. Numbers of second stage juveniles were counted for *in vivo* and *in vitro* experiments according to the design of each experiment.

Extraction of prodigiosin pigment

A loopful of *Serratia marcescens* grown on nutrient agar plate for 24 h was inoculated into 250 ml flask containing 50 ml NB (Giri *et al.,* 2004) and incubated in a shaking incubator at 150 rpm and 28 °C for 24 h. Then, 10 ml of the culture broth was inoculated in powdered peanut broth (2 % powdered peanut seeds, NB medium, pH 7) and incubated in a shaking incubator at 150 rpm and 28 °C for 96 h. Culture broth was centrifuged at 10.000 rpm for 15 minutes and the supernatant was extracted with ethyl acetate. The pigment from the cell pellet was extracted with acetone and the extract was centrifuged at 10.000 for 15 minutes. The white pellet was discarded, extracts were evaporated and the wave length scan was done from 200-700 nm (Srimathi *et al.,* 2017).

Purification of the prodigiosin

The extracted prodigiosin was applied to thin layer chromatography (TLC) plate for further purification using the mixture of chloroform and methanol (9:1) as a solvent system (Casullo de Araujo *et al*., 2010).. Then, prodigiosin was separated by puriflash column 30 silica HP -25.0 g (22 bar). The dried concentrated sample was dissolved in 5 ml of n-hexane and loaded onto the silica gel column and solvent system comprising of n-hexane and ethyl acetate at a flow rate 15 ml min^{-1}. The eluted red colored fractions were collected and assayed for the presence of prodigiosin. The fractions containing prodigiosin were pooled and concentrated by evaporation at 40 °C using rotary evaporator.

Characterization of prodigiosin

Thin-Layer Chromatography (TLC)

Thin layer chromatography (TLC) was performed on silica gel TLC- cards for purification using a mixture of chloroform: methanol (9:1) as a solvent system.

Mass spectroscopy

The molecular weight of the prodigiosin was determined using Quick mass determination (Probe / TLC-MS) at Nawah Scientific Research, Cairo, Egypt.

Fourier-Transform Infrared Spectroscopy (FTIR Spectroscopy)

The purified prodigision sample was subjected to FTIR spectroscopic analysis. FTIR was carried out by mixing the sample with finely grounded KBr. The parameters used in FTIR analysis were; spectral range between 4000 and 400 cm^{-1}. Upon pressing under 2000 kPa, pellet disc obtained was analyzed using JASCO FTIR -3600 infrared spectrometer by employing KBr pellet technique,

equipped with KBr beam splitter with DTCS (Deuterated triglycine sulfate detector (7800-350 cm^{-1}) at the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Egypt.

Nematicidal effect of bacterial culture and culture filtrate of *S. marcescens* on *M. incognita* juvenile's mortality

One milliliter of bacterial culture was added to one hundred second stage juveniles (J_2) of *M. incognita* were separately placed in Petri dishes (5 cm-dia). Petri dishes receiving distilled water and free of bacterial culture were served as control. Treatments of bacterial culture were replicated three times. Juveniles exhibited no movement and attained the shape of straight line were considered dead. Dead nematodes were counted and recorded after 24 hrs. Meanwhile, one milliliter of culture filtrate in four concentrations (200 x 10^3, 400 x 10^3, 800 x 10^3 and 1000 x 10^3 µl) in addition to one hundred second stage juveniles (J_2) of *M. incognita* were separately placed in Petri dishes. Treatments of each concentration were replicated three times. Dead nematodes were counted and recorded after 72 hrs. Percentages of nematode mortality were calculated and recorded for each concentration tested. EC_{50} was also estimated for each concentration. Concentrations of culture filtrate that gave the highest suppression in nematode population were chosen for *in vivo* experiment.

Nematicidal effect of prodigiosin pigment on *M. incognita* juvenile's mortality

Meloidogyne incognita was treated with prodigiosin dissolved in DEMSO at the concentrations 25, 50, 75, 100 mg ml^{-1} with four wells per treatment in each trial. Juveniles exhibited no movement and attained the shape of straight line were considered dead. Dead nematodes were counted and recorded after 72 hrs. Percentages of nematode mortality were calculated and recorded for each concentration tested.

***In vivo* experiment**

Seedlings of tomato cv. 010 (25-day old) were separately transplanted in plastic pots (15 cm-dia) filled with 850 g steam sterilized clay sandy soil (v / v. 1:1). Simultaneously, culture and culture filtrate of *Serratia marcescens* were separately applied @ 60 ml $plant^{-1}$ pot^{-1}. Five days later, seedlings were inoculated with 1000 juveniles of *M. incognita*. The conventional nematicide oxamyl was applied at the recommended dose (0.3 g $plant^{-1}$ pot^{-1}) two days after nematode inoculation for comparison. Pots free of nematode inoculum were served as CK1, however those received nematode inoculum served as CK2. Each treatment was replicated four times. All pots received water when needed and arranged in a complete block design and kept under greenhouse conditions at 27±3 ºC. Forty two days after nematode inoculation, plants were harvested. Lengths and fresh weights of shoots and roots as well as shoot dry weights for each replicate were recorded. Second stage juveniles of *M. incognita* were extracted from soil using modified Baermann-technique (Goodey, 1957) and counted. Roots were stained in acid fuschin lactic acid (Byrd *et al*., 1983), for the numbers of galls, egg masses, females, and development stages. The nematode reproduction (Rf), root gall index (RGI) and egg mass index (EI) were determined as well.

Defense related compounds

Total phenols (TP) in fresh leaves of tomato were measured according to the method of Javaheri *et al*. (2012).

Enzymes Activities

Enzyme extracts were prepared following the method described by Maxwel and Bateman (1967). Fresh leaves (0.5 g) of each treatment were ground in 3 ml Na-phosphate buffer at pH 6.8 in a mortar then centrifuged at 1500 rpm for 20 min at 6 °C. The resultant supernatant fluids were processed for enzyme assays.

Peroxidase activity (PO)

Peroxidase activity was assayed using photochemical method as described by Amako et al. (1994). The reaction mixture was added as the following sequences, 15 ml phosphate buffer, 4.8 ml hydrogen peroxidase, 10 ml pyrogallol and 0.2 ml sample extract. Increasing the absorbance at 430

nm was recorded against blank with phosphate buffer instead of enzyme extract. One unit of enzyme activity was defined as the amount of the enzyme, which changing the optical density at 430 nm per min. at 25 °C under standard assay conditions. Specific activity was expressed in units by dividing it to mg protein.

Polyphenol oxidase (PPO)

Polyphenol oxidase activity was assayed using photochemical method as described by Coseteng and Lee (1987). The reaction mixture was added as the following sequences: 2.7 ml potassium phosphate buffer 90.05 M, pH 6.2, 0.25 ml of 0.25 M catechol, 0.05 ml of enzyme extract. The increasing in absorbance at 420 nm was measured. One unit of enzyme activity is defined as the amount of the enzyme that causes an increase of 0.001 absorbance unit per minute at 25 °C.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) (Gomez and Gomez, 1984), followed by Duncan's multiple range tests to compare means (Duncan, 1955).

Results and Discussion

Purification of prodigision

Twenty µl containing 10 mg of the purified pigment were spotted on TLC and separated using chloroform : methanol (9:1) solvent system. A single band with Rf 0.9 was obtained (Fig.1).

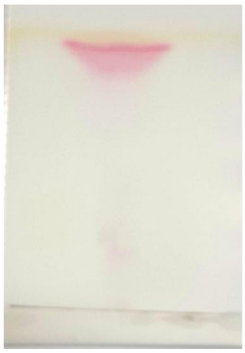

Fig.1: Detection of prodigiosin from *S. marcescens* on TLC.

Characterization and identification of prodigiosin

Mass spectroscopy

Characterization of pigment was carried out by TLC mass spectroscopy, the obtained results revealed that the molecular weight of the pigment was 324.7 m/z (Fig. 2) This confirms that the pigment extracted from *S. marcescens* is prodigiosin.

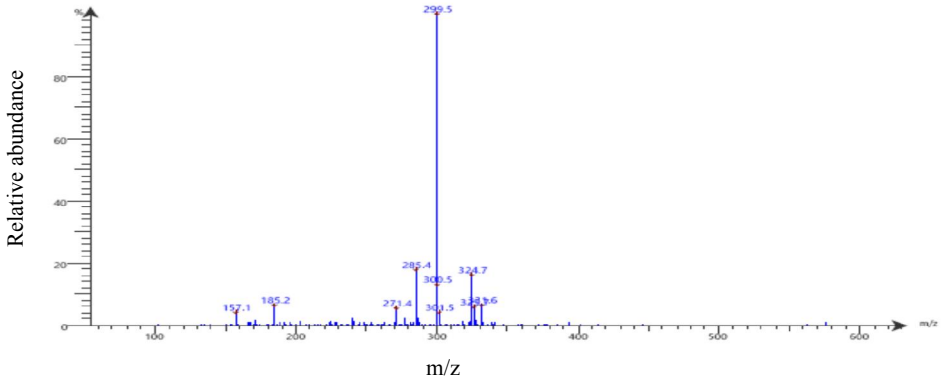

Fig. 2: TLC mass spectroscopy of prodigiosin produced by *S. marcescens*

Fourier Transform Infrared (FTIR) spectrum of prodigiosin pigment

In FTIR analysis red pigment from *S. marcescens* revealed absorption at 3315.3 per cm (N-H) and 2941. 6 per cm (aromatic C-H) , 1450 (C-H) alkenes, 1020, 1141 (C-O, C-N) carboxylic groups, as represented in (Fig. 3) These results are in accordance with Song *et al*. (2006).

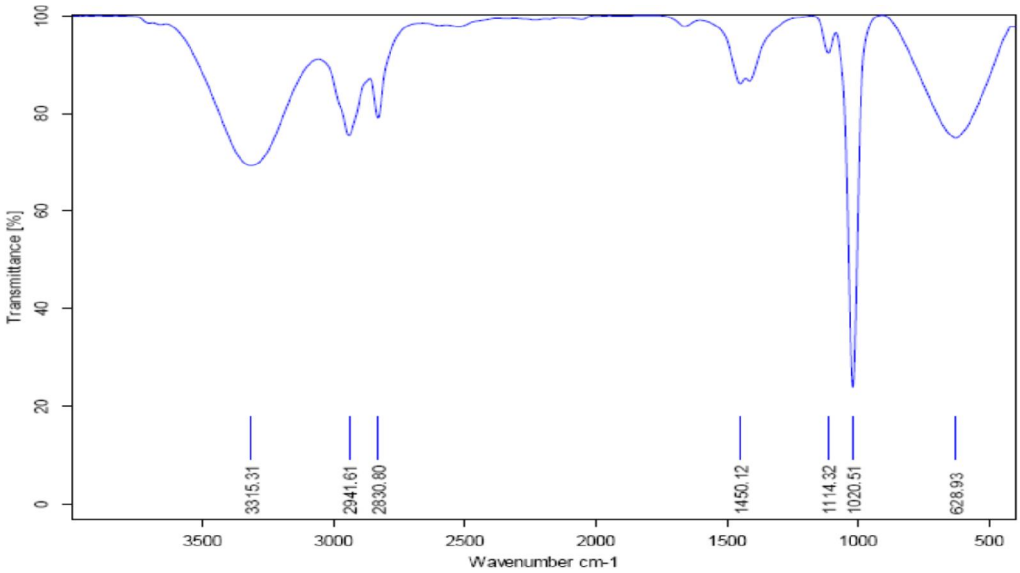

Fig. 3: FTIR analysis for prodigision.

Nematicidal effects of bacterial culture and culture filtrate of *S. marcescens* on juveniles survival of *M. incognita*

Data in Table (1) represent the impact of bacterial culture as well as culture filtrate of *Serratia marcescens* at different concentrations (200 x 10^3, 400 x 10^3, 800 x 10^3 and 1000 x10^3 µl) on juveniles mortality of *Meloidogyne incognita*. Bacterial cultures showed better results than the control of nematode alone with a value of 58.5 % for juveniles mortality. The culture filtrate of *S. marcescens* at the four tested concentrations showed nematicidal activities against newly hatched juveniles of *M. incognita* after 72 h of exposure. Irrespective of tested concentrations, all treatments were found to cause significant inhibition in juveniles mortality to various extents. However, a positive correlation was achieved among tested concentrations, the higher concentrations the greater juveniles mortality. The highest percentage of *M. incognita* juveniles mortality (82.0 %) was recorded with the highest concentration of culture filtrate.

Table 1: Nematicidal effect of bacterial culture and culture filtrate of *S. marcescens* on *M. incognita* juveniles mortality

Bacterial culture	**% of mortality after 24 h**		
S. marcescens	58.5		
Nematode alone	0.0		
Concentration of culture filtrate (µl)	**Period of exposure (h)**		
	24 h	48 h	72 h
200	15.0	25.0	33.0
400	28.0	35.0	41.0
800	36.0	42.0	64.0
1000	45.0	56.0	82.0
Nematode alone	0.0	0.0	3.0
EC_{50}	1037	892.85	421

Moderate results in nematode survival were recorded with other concentrations. These results are in the same line with El-Hadad *et al*. (2010) who reported that, the filtrates of all bacterial cultures were less effective on nematode mortality compared with whole bacterial cultures. In addition Zaghloul *et*

al. (2015) showed that, the bacterial culture of *S. marcescens* at dilutions of 10^{-1}, 10^{-2} and 10^{-3} recorded the highest values of *M. incognita* mortality being 92.9, 82.9 and 54.3 %, respectively. Ketabchi *et al.* (2016) studied the effect of *Serratia* sp. on second stage juvenile mortality of *M. incognita* under laboratory conditions after 24, 48 and 72 hrs which caused the higher mortality of second stage juvenile. These results are also conformity with the findings of previous reports by Mokbel and Alharbi (2014) who found that *S. marcescens* showed moderate inhibition of 50.5 - 62.0 % in egg-hatch and J_2 activity.

Nematicidal effect of prodigiosin pigment on *M. incognita* juvenile's mortality

The purified pigment of *S. marcescens* was evaluated as well for nematicidal activity against *M. incognita in vitro* at different concentrations after 72 h of exposure (Table 2). The pigment exhibited high toxic action against juveniles. The higher the concentrations the greater juveniles mortality. The highest percentage of *M. incognita* juveniles mortality (84.0 %) was recorded with the highest concentration of 100 mg ml^{-1}. Herein, the least percentage (44.6 %.) of juveniles mortality was recorded with 25 mg ml^{-1} of purified pigment, with EC_{50} = 31.9. Results indicated that the pigment of *S. marcescens* had strong nematicidal candidature compared with synthetic pesticides. These results are in accordance with Rahul *et al.* (2014). The mode of action of prodigiosin may be due to it possesses proton sequestering ability **(**Roser *et al*., 2007) which affects intracellular pH gradient. Prodigiosin also affects mitochondrial ATP synthesis; it causes reduction in ATP production without decreasing the oxygen consumption.

Table 2: *In vitro* nematicidal effect of *S. marcescens* pigment on root-knot nematode *M. incognita* juveniles mortality *in vitro*

Concentration mg ml^{-1}	% of mortality Period of exposure (h)		
	24 h	**48 h**	**72 h**
25	25.2	33.32	44.6
50	31.3	48.3	62
75	37.3	48.6	67.3
100	66.6	67.2	84
EC_{50}	85	57.8	31.9

Impact of bacterial culture and culture filtrate of *S. marcescens* on growth of tomato infected with *M. incognita*

Data in Table (3) summarize the effect of bacterial culture and culture filtrate of *S. marcescens* on growth of tomato cv. 010 infected with *M. incognita* under greenhouse conditions. Results indicated that *M. incognita* infection caused an obvious reduction in plant growth parameters with reduction percentage in total plant fresh weight and plant length reached 77.3 and 22.3 %, respectively.

Table 3: Impact of bacterial culture and culture filtrate of *S. marcescens* on growth of tomato infected with *M. incognita*

Treatments	Length (cm)		Fresh weight (g)		Shoot D.W (g)
	Shoot	**Root**	**Shoot**	**Root**	
***S. marcescens* culture**	32.6 ^{a}	9.5 ^{a}	3.65 ^{b}	0.6 ^{cd}	0.0310 ^{b}
***S. marcescens* culture filtrate**	28 ^{c}	8 ^{b}	3.2 ^{b}	0.5 ^{cd}	0.0293 ^{d}
Oxamyl	30 ^{b}	8 ^{b}	4.5 ^{a}	0.8 ^{ab}	0.0411 ^{a}
Plant free of N and Untreated	26 ^{d}	8.6 $^{a-b}$	3.3 ^{b}	0.83 ^{a}	0.0302 ^{c}
Nematode alone	22.6 ^{e}	5.6 ^{c}	1.866 ^{c}	0.46 ^{d}	0.0125 ^{e}

Means in each column followed by the same letter (s) did not differ at $p < 0.05$ according to Duncan's multiple range test.

Irrespective of treatments, both application, showed moderate increase in shoot and root lengths and plant biomass exceeded that of untreated plants. Apparently, *S. marcescens* culture showed better results than *S. marcescens* culture filtrate. The highest significant improvement in tomato plant length

was recorded with *S. marcescens* culture with percentage increase reached (48.8). On the other hand, the best increase in total plant fresh weight was recorded with *S. marcescens* culture (82.4 %) followed by *S. marcescens* culture filtrate (58.7%). Oxamyl as a standard nematicide exceeded other treatments and improved fresh shoot and root weights. Some authors proposed that endophytic bacteria can offer several benefits to the host plant, particularly growth promotion and protection from pathogens; and that under diverse environmental conditions endophytes are able to communicate and interact with the plant more efficiently than rhizospheric bacteria (Ali *et al*., 2012; Coutinho *et al*., 2015; Mokbel and Alharbi, 2014; Santoyo *et al*., 2016).

The nematicidal properties of culture and culture filtrate of *S. marcescens* against *M. incognita* infecting tomato are depicted in Table (4). Both bacterial treatments significantly suppressed nematode population whether in soil or root as compared to control. Culture of *S. marcescens* detected greater suppression in nematode population in soil. Similar trend was noticed with number of developmental stages, females and eggs /egg mass within tomato roots. Oxamyl as conventional nematicide significantly suppressed nematode population in soil and roots with reproduction factor (Rf) = 0.34 compared to untreated inoculated plants. Root galling was significantly reduced by both treatments of bacterial extracts (Table 5). Bacterial culture treatments significantly suppressed root galling with reduction percentage of 73.5 % and showed better performance in suppressing root galling than culture filtrate. Similar trend was noticed with number of egg masses. Oxamyl (97.1 %) showed significant reduction in root galling with RGI=1.0. These results are in harmony with those of (Mohamed *et al*. (2009), Abd-Elgawad and Kabeil, (2012), Ketabchi *et al*., (2016) and Sharaf *et al*. (2016).

Table 4: Development and reproduction of *M. incognita* as influenced by the addition of bacterial culture and culture filtrate of *S. marcescens*

Treatments	Nematode population in			Total nematode population	Rf*
	Soil	Root Developmental stages	Females		
***S. marcescens* culture**	616.0 $^{c-e}$	1.0^{b}	2.0 ^{c}	626	0.63
***S. marcescens* culture filtrate**	718.0 $^{b-d}$	5.0 ^{a}	9.0 ^{b}	738	0.74
Oxamyl	335.0 ^{e}	5.0 ^{a}	1.0^{c}	342	0.34
Nematode alone	2120.0 ^{a}	0.0 ^{b}	32.0 ^{a}	2171	2.17

Nematode(Pi) = 1000 juveniles (J2) of M. incognita.
Each value is the mean of four replicates.
Rf = Reproduction factor = Final population (Pf)/Initial population (Pi)
Pf = Nematode population in soil+ N. developmental stages +No. females+ No. egg mass
Means in each column followed by the same letter (s) did not differ at $p < 0.05$ according to Duncan multiple range test.

Table 5: Reduction percentages in root galling and number of egg masses of *M. incognita* in tomato plant as influenced by the addition of bacterial culture and culture filtrate of *S. marcescens*

Treatments	No. of galls	Red. (%)	RGI*	No. of egg masses	Red. (%)	E.I.*
***S. marcescens* culture**	9.0 ^{c}	73.5	2.0	6.0 ^{b}	68.4	2.0
***S. marcescens* culture filtrate**	11.0 ^{b}	67.6	3.0	7.0 ^{b}	63.2	2.0
Oxamyl	1.0 ^{d}	97.1	1.0	1.0 ^{c}	94.7	1.0
Nematode alone	34.0 ^{a}	0.0	3.0	19.0^{a}	0.0	3.0

Nematode = 1000 juveniles (J_2) of *M. incognita*. Each value is the mean of four replicates.
* Root gall index (RGI) or egg-masses index (EI) was determined according to the scale given by Taylor & Sasser (1978) as follows : 0= no galls or egg masses, 1= 1-2 galls or egg masses ; 2= 3-10 ; 3= 11-30 ;4= 31-100 ; and 5= more than 100 galls or egg masses.

Biochemical activities

The influence of bacterial culture and culture filtrate of *Serratia marcescens* on chemical components viz., total phenol, PO and PPO activities in leaves of tomato infected with *M. incognita* is presented in Table (6).

Total phenol

As a result of root-knot nematode *M. incognita* infection, total phenol increased (48.0 %) in fresh tomato leaves. The highest reduction in total phenol (17.3 %) was recorded with the conventional nematicide, oxamyl. Among tested bacterial extracts, the highest reduction in total phenol was recorded with *S. marcescens* culture (15.7 %) than *S. marcescens* culture filtrate (7.2 %).

Enzyme activities

As a result of *M. incognita* infection, Po activity obviously raised in tomato leaves with percentage increase of 45.7 . Among tested bacterial extracts, the highest reduction in PO enzyme (20.7 %) was recorded with *S. marcescens* culture followed by *S. marcescens* culture filtrate (10 %). Oxamyl exceeded both bacterial treatments giving the highest reduction in PO enzyme (27 %). Similar trend was noticed with PPO activity. Herein, PPO activity (38.4 %) was also increased in fresh tomato leaves as a result of *M. incognita* infection. However, application of tested bacterial extracts as soil drench showed PPO activity reduction in tomato leaves. Tomato treated with *S. marcescens* culture (19.8 %) exhibited the highest percentage of decrease in PPO activity followed by *S. marcescens* culture filtrate (7.9 %). Oxamyl recorded that the highest percentage of decrease (24.5 %) in PPO activity exceeding both bacterial treatments. Some authors showed that nematode infestation induces an increase in the activity of PO and PPO enzymes. In this respect, Abd-Elgawad and Kabeil, (2012) indicted that PPO activity was enhanced in inoculated roots treated with commercial suspension of *S. marcescens* (Nemaless) with respect to uninoculated roots. In tomato roots infected with root-knot nematodes, genes with homology to several known plant defense genes such as peroxidase (PO) and chitinase are induced locally within 12 h of inoculation (Williamson and Hussey, 1996).

Table 6: Impact of bio-agents on resistance compounds in leaves of tomato infected with *Meloidogyne incognita*

Treatments	Chemical parmeters					
	PO	Dec %	PPO	Dec %	T. phenol mg/1g	Dec %
***S. marcescens* culture**	0.563 ^{c}	20.7	0.121 ^{c}	19.8	4.059 ^{c}	15.7
***S. marcescens* culture filtrate**	0.639 ^{b}	10	0.139 ^{b}	7.9	4.465 ^{b}	7.2
Oxamyl	0.518 ^{d}	27	0.114 ^{d}	24.5	3.978 ^{d}	17.3
Plant free of N and untreated	0.385 ^{e}	45.7	0.093 ^{e}	38.4	3.252 ^{e}	32.4
Nematode alone	0.71 ^{a}	0.0	0.151 ^{a}	0.0	4.813 ^{a}	0.0

Means in each column followed by the same letter (s) did not differ at $p < 0.05$ according to Duncan's multiple range test.
PO = peroxidase. PPO = polyphenol oxidase

Conclusion

The present study proved that the culture and culture filtrate of *Serratia marcescens* have potentialities for controlling the root-knot nematode *Meloidogyne incognita* under *in vitro* and *in vivo* studies. Also, prodigision pigment extracted from *S. marcescens* was very effective for nematode inhibition, low concentrations of prodigision was found to inhibit the juvenile stage of *M. incognita*. The LC_{50} value of prodigision was 31.9 mg ml^{-1} after 72 h. In order to recommends the nematicidal activity of prodigision, field studies are required.

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