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Nematode-Antagonistic Compounds from Certain Bacterial Species

Abdelnabby[*], H. M.; H. A. Mohamed[**] and H. E. Abo Aly[***]
[*]Department of Plant Protection, Faculty of Agriculture, Benha University, Egypt
[**]Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt
[***]Department of Botany, Faculty of Agriculture, Benha University, Egypt
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ABSTRACT

The present study was outlined to investigate the role of some bacterial species as biocontrol agents against *Meloidogyne incognita* and *Tylenchulus semipenetrans*. Three bacterial filtrations, *Bacillus thuringiensis* (*Bt*), *Serratia* marcescens (*Sm*) and *Pseudomonas fluorescence* (*Pf*) were evaluated. The results exhibited that, *Sm* and *Bt* achieved high significant effect for suppressing nematode juveniles with immobilization rate of 100% against the concerned nematode species, while *Pf* caused 81.7 and 53.2% immobilization of *M. incognita* and *T. semipenetrans*, respectively. Egg-hatching test was conducted using 24-well tissue culture plates with the bacterial filtrations against *M. incognita* and *T. semipenetrans* eggs. *Sm* and *Pf* showed significant inhibition rates of egg hatch against tested nematode species. *Sm* achieved the highest effect against *M. incognita* eggs resulting 41.9% hatchability after 15 days, while the egg hatch of *T. semipenetrans* was not affected with the filtration of *Bt*. Filtrate mixtures of bacterial species varied in their effects with respect to the presence of the genus *Serratia* in the mixture that showed an obvious effect against juveniles and egg hatch of both nematode species. Thin Layer Chromatography (TLC) was used for separating the active volatile organic compounds (VOCs) of the bacterial filtrates with different organic solvents. Fractions of the bacterial extracted culture broth were assayed for their activities against *T. semipenetrans*. Bioassay-guided fractionations of the extracted broth were undertaken to identify the compounds using Gas Chromatography-Mass Spectrometry (GC-MS).

Key words: Nematoxic compounds, *Bacillus thuringiensis*, *Serratia marcescens*, *Pseudomonas fluorescence*, *Meloidogyne incognita*, *Tylenchulus semipenetrans*, TLC, GC-MS, VOCs.

INTRODUCTION

Root-knot nematodes are capable of harshly damaging a broad range of crops, in particular vegetables, causing dramatic yield losses mainly in tropical and sub-tropical agriculture (Sikora and Fernandez, 2005). Citrus nematode is one of the most important root nematodes of plant trees that have worldwide distribution and cause reduction of crop production and vegetative growth. In addition, this nematode causes slow decline of citrus trees (Ayazpour *et al*, 2010).

Concerns about public health and environmental safety have led to restrictions on chemical nematicide applications for the control of plant-parasitic nematodes. Cultural practices are also used for nematode management, but extensive annual losses in crop yields and quality demonstrate a crucial need for new, environmentally friendly methods to enhance current management systems (Meyer, 2003). Application of nemato-antagonestic microbes is one area being investigated to fill this need. At present, microorganisms and their metabolites have attracted the most attention as potential nematode biocontrol agents. Several fungi and bacteria have been developed into commercial formulations and are successfully used to control nematodes in agricultural fields (Butt *et al.*, 2001). Limited numbers of bacterial species have been reported as biological control agents for root-knot nematode disease including *Streptomyces* spp., *Serratia* spp., *Bacillus* spp., *Azotobacter chroococcum*, *Rhizobium*, *Corynebacterium* and *Pseudomonas* (Verma *et al.*, 1999).

Identification of nematode-antagonistic compounds from bacterial species is important for at least three reasons. First, natural products from fungi and bacteria are being sought as alternatives to the use of fumigant and non-fumigant nematicides (Nitao *et al*, 2001). Second, it would help determine if such compounds contribute to the detrimental effects of these antagonists (Viaene and Abawi, 1998). Third, although it is unknown if compounds produced by concerned bacteria *in-vitro* play a role in natural interactions between bacteria and plant parasitic nematodes, identification of nematode-antagonistic compounds can be the first step towards examining such interactions in future studies.

Previously, many researches testified that volatile and nonvolatile compounds of some bacteria made considerable contributions to the biocontrol of plant diseases (Hou *et al.* 2006). Little is known about nematicidal volatile organic compounds (VOCs) and their potential use as biological control agents against plant parasitic nematodes (Ying *et al.*, 2007).

The objectives of the present study are to: (1) determine nematicidal activities (NA%) of *Bacillus thuringiensis*, *Pseudomonas fluorescens* and *Serratia marcescens* culture filtrates against juveniles and eggs of *M. incognita* and *T. semipenetrans*; (2) detect the anti-nematode activity of VOCs in the cell-free culture filtrate of the bacterial strains, and (3) identify the nematicidal VOCs.

MATERIALS AND METHODS

Nematode stock culture

Meloidogyne incognita race 3 (Kafoid and White) stock culture was initiated from well identified single egg- masses which were collected from galled tomato roots. The fresh egg- masses were then propagated on tomato seedlings (cv. Supper Marmand) cultivated in sterilized soil. The pure culture of *M. incognita* was maintained in greenhouse.

Citrus nematode, *Tylenchulus semipenetrans* juveniles were originally extracted from the infected citrus orchards of the Experimental Farm of the Faculty of Agriculture at Moshtohor, Benha University, Egypt (30° 21' N, 31° 13' E, 46 ft above sea level) and propagated on sour orange seedlings grown in 50 cm pots filled with sandy loam soil.

Nematode preparation

Eggs and second stage juveniles (J2) of *M. incognita* and *T. semipenetrans* were prepared for the assays as described by Meyer *et al.* (2004). Egg masses were picked from plant roots, collected in tap water, and rinsed three times with sterile deionized water (DIW). Egg masses were broken apart and eggs were surface-sterilized by agitating for about 3 min in 0.05% sodium hypochlorite. The sterilized eggs were collected, concentrated, rinsed on a 25 μm sieve, and then refrigerated overnight at 7 °C in DIW and used next day for the assays. J2 were allowed to hatch from eggs for 3 days and were collected using 25 μm sieves and used immediately for assays.

Bacterial strains

Bacterial isolates of *Bacillus thuringiensis* (*Bt*), *Pseudomonas fluorescence* (*Pf*) and *Serratia marcescens* (*Sm*) were firstly identified by their morphological and biochemical features at Department of Agricultural Botany, Faculty of Agriculture, Moshtohor, Benha University, Egypt.

Preparation of cell-free bacterial suspensions

The identified bacteria were grown in Trypticas Soya Broth (TSB) (Kajimura *et al.*, 1996) incubated at 37°C for 72h. The bacterial suspension was centrifuged twice at 3,000 g for 20 min. The cell pellets were discarded and cell-free culture filtrate was collected in a sterile baker before use in the bioassay tests (Khan *et al.*, 2010).

Microwell assay procedures

Microwell assays were conducted to determine the activity of free-cell bacterial culture of *Bt, Pf, Sm* and their equal mixtures against eggs or J2 of *M. incognita* and *T. semepenetrans* using procedures similar to those described by Nitao *et al.* (1999). The nematode eggs or J2 were then placed into the free-cell bacterial culture filtrates, sterile water or control medium (not inoculated with bacteria) in 24-well tissue culture plates. The water controls were used to monitor egg hatch and J2 viability. As the bacteria had been cultured in (TSB) medium, egg hatch and J2 viability in the media controls were used as a comparison with egg hatching and J2 viability in the filtrates. Each bioassay trial consisted of five replicate wells per treatment, *ca* 50 eggs or J2 in a volume of 0.5 ml were added to the same volume of each bacterial filtrates or mixture in each well, resulting 50% concentration of bacterial filtrations. To determine the effects of filtrates on egg hatch, numbers of motile and non-motile J2 were counted after 3, 6, 9, 12 and 15 days in the treatments, while viability of J2 was determined by counting spontaneously moving J2 after 1, 6, 12, 24 and 48 h. in each treatments.

Extraction of anti-nematode volatile organic compounds (VOCs) from cultural filtrates of *Bt, Sm and Pf*

Different solvents were used for extraction of anti-nematode compounds from cell free supernatant; the solvents used were n-hexane, ethyl acetate, and chloroform to determine the best solvent for extraction of anti-nematode VOCs. These solvents were added separately to the supernatant by 1:1 ratio, and then separated by separating funnel (Weiwei *et al.*, 2008).

Separation and purification of anti-nematode VOCs

Each solvent containing VOCs was evaporated under vacuum at 40°C and tested for number of components present using TLC (E. Merk, AG, and Darmstadt, Germany), by spotting about 40 - 50 μl of the organic solvent extract on the TLC plate. A system consisted of chloroform: methanol: petroleum ether (60: 20: 20) was used as a mobile phase. The plates were developed and bands were visualized using UV light at 365 nm (Kumar *et al.*, 2009).

Detecting the anti-nematode activity of VOCs

Anti-nematode activity of the active VOCs extracted by previous organic solvents were bio-assayed directly using J2 of *T. semipenetrans* as indicator. The fractionated compounds of each organic solvent was scrapped from the plate and re-extracted using mobile phase to exclude the silica gel as solid phase and evaporate the solvent to make a thin film in the testing vials, then adding 0.2 ml of nematode suspensions. The active fractions against J2 in each organic solvent were tested for identifying chemical constituents using GC-MS chromatography.

Identification of chemical constituents of active anti-nematode VOCs by using GC-MS chromatography

The GC-MS analysis for derivative extracted VOCs using BSTFA [N,O-bis-(trimethylsilyl trifluoroacetamide) + TMCS (trimethylchloro-silane)] as derivative kit were performed using Agilent 6890 N gas chromatography instrument coupled with an Agilent MS-5975 inert XL mass selective detector and an Agilent auto sampler 7683-B injector (Agilent Technologies, Little Fall, NY, USA) with capillary column HP-5MS (5% phenyl methylsiloxane) with dimension of 30 m x 0.25 mm Id x 0.25 µm film thickness (Agilent Technologies, Palo Alto, CA, USA). The initial oven temperature was 40°C, held for 2 min, ramped at 6°C min^{-1} to 180°C and ramped at 10°C min^{-1} to 250°C and held for 3 min. The ions were detected in the range 30-350 m/z. The mass spectra of the unknown compounds were compared with Chem Station 6890 Scale Mode software with two libraries (NIST & Wily) which provide best information about the identification of active compound separated from TLC (Liu *et al.*, 2008).

Statistical analysis

Nematicidal activity (NA) values were calculated as the means of five replicates. Data were analyzed using analysis of variance (ANOVA), and the means were compared by the least significant differences (LSD) at $P \geq 0.05$ described by Snedecor and Cochran (1980), the significant mean differences between treatment means were separated according to Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Efficiency of cell-free culture filtrates of *Bt, Pf, Sm* and their mixtures on egg hatching of *M. incognita* and *T. semipenetrans*

Bacterial filtrate of *Pf; Sm; Bt+Sm; Bt + Pf; Pf + Sm* and *Bt + Pf + Sm* treatments against *M. incognita* did not show any egg hatch even after 6 days of exposure and exhibited high reduction in the egg hatch as compared to untreated control. The greatest effect was achieved when using the mixture of *Pf + Sm* filtrates with egg hatch of 36.7%, followed by *Sm* filtrate (41.9%) after 15 days exposure period. Filtrate of *Bt* was the least effective in reducing the egg hatching rate of *M. incognita.* It was observed that all mixtures of the studied bacterial isolates did not show any antagonistic effect to each other as a result of their mixture. On the other hand, synergistic effect occurred when *Pf* was mixed with *Sm* recording the lowest hatching% of 36.7% compared to their separately effect with 60.6 and 41.9%, respectively (Fig. 1).

Bacterial filtrate of *Pf* and *Pf + Sm* treatments with *T. semipenetrans* did not show any egg hatching even after 6 days of exposure. These exhibited significant reduction of the egg hatch as compared to untreated control after 15 days of exposure recording 64.8 and 70.2% egg hatch, respectively. The single free-cell culture filtrates of *Sm* followed the effect of *Pf + Sm* with no clear difference recording egg hatch of 70.3% at the end of the experiment. Filtrate of *Bt* was not found to be effective in reducing the egg hatching of *T. semipenetrans* (95.4%) compared to the untreated controls. The mixture of the studied bacterial isolates did not show any antagonistic nor synergistic effect (Fig. 2).

Efficiency of cell-free culture filtrates of *Bt, Pf, Sm* and their mixtures on juveniles of *M. incognita* and *T. semipenetrans*

In vitro studies revealed that cell-free culture filtrates of *Bt, Pf, Sm* and their mixtures at 50% concentration, caused significantly ($P < 0.05$) antagonistic impact on the juveniles of both root-knot and citrus nematodes compared to the untreated control. The suppressive activity of all bacterial sets against the tested nematodes increased gradually with the elongation of the exposure period.

Sm and *Bt* exhibited the highest nematicidal activity against second stage juveniles (J2) of *M. incognita* as 100% after 24 and 48 h, respectively. However, Pf exhibited considerable effect and recorded 81.7% of J2 mortality after 48h. As for the bacterial filtrate mixtures, *Bt + Sm* manifested the highest nematicidal effect recording 100% after 48h. Concerning the filtrate mixtures of *Bt + Pf + Sm* and *Bt + Pf,* high nematicidal effects were achieved with 89.2 and 86.4%, respectively. The mixture of *Pf + Sm* exhibited an antagonistic effect since the J2 mortality decreased to 71.4% which was less than the effect of each isolate as its single filtrate (Table 1).

The reduction percentages in population density of citrus nematode larvae ranged from 53.2 to 100% at the end of the experiment (48h). Complete mortality (100%) of *T. Semipenetrans* was achieved as exposure to filtrates of *Sm* and *Bt* after 24 and 48h, respectively. However, *Pf* exhibited the least success after 48h recording J2 mortality of 53.2%. Regarding to bacterial filtrate mixtures, the most effective mixture filtrate was *Bt + Sm* at all exposure times. A descending arrangement for these lethal mixtures can be illustrated as follows:

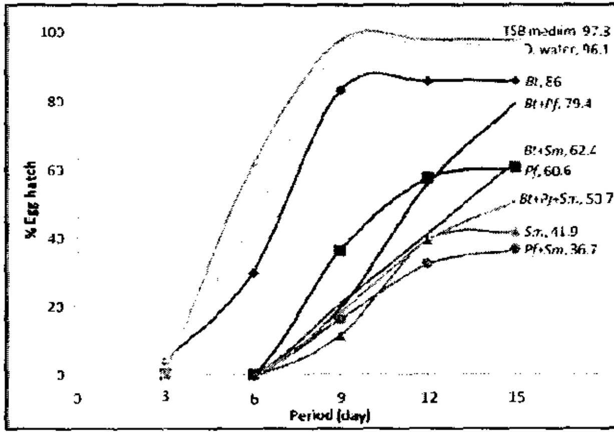

Fig. (1): Effect of certain bacterial filtrates and their mixtures on the egg-hatch of *Meloidogyne incognita*.

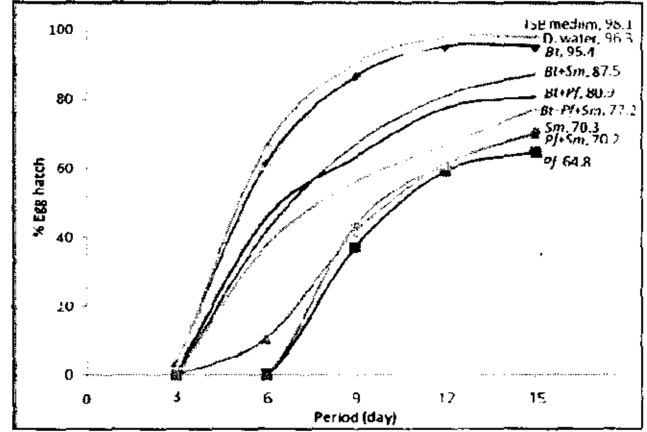

Fig. (2): Effect of certain bacterial filtrates and their mixtures on the egg-hatch of *Tylenchulus semipenetrans*.

Fig. (3): GC-MS profiles of bacterial volatile analysis from *Bacillus thuringiensis* (A), *Pseudomonas fluorescence* (B), and *Serratia* marcescens (C).

Table (1): Nematicidal activity of certain bacterial filtrates and their mixtures against *Meloidogyne incognita* juveniles (J2) at different exposure periods

Bacterial stain	% inactive juveniles (J2) at different exposure periods (h)				
	1	6	12	24	48
Bacillus thuringensis (Bt)	16.7 cd	33.3 b	50.0 c	77.8 bc	100.0 a
Pseudomonas fluorescence (Pf)	14.3 d	28.7 b	53.0 c	77.4 bc	81.7 b
Serratia marcescens (Sm)	31.6 a	47.4 a	78.9 a	100.0 a	100.0 a
Bt + Pf	19.7 c	30.0 b	39.6 d	61.4 d	86.4 b
Bt + Sm	20.1 c	41.6 a	66.3 b	87.6 ab	100.0 a
Pf + Sm	20.6 c	30.9 b	36.4 d	50.8 d	71.4 c
Bt + Pf + Sm	25.6 b	34.3 b	45.7 cd	72.6 cd	89.2 b
Control 1	0.0 e	0.0 c	0.0 e	0.0 e	0.0 d
Control 2	0.0 e	0.0 c	0.0 e	0.0 e	0.0 d
L.S.D. $_{(0.05)}$	4.04	8.39	9.52	12.52	8.54

- J2: Second stage juveniles; h: hour; Control 1: TSB Media; Control 2: Distilled water
- Each value represents the mean of five replicates;
- Mean values with the same letter in column are not significantly different by Duncan's Multiple Range Test at ($P \leq 0.05$).

Table (2): Nematicidal activity of certain bacterial filtrates and their mixtures against *Tylenchulus semipenetrans* juveniles (J2) at different exposure periods

Bacterial stain	% inactive juveniles (J2) at different exposure periods (h)				
	1	6	12	24	48
Bacillus thuringensis (Bt)	12.4 d	43.6 c	76.9 a	97.3 ab	100.0 a
Pseudomonas fluorescence (Pf)	6.2 e	12.3 e	21.3 c	32.7 e	53.2 d
Serratia marcescens (Sm)	42.3 a	66.4 a	83.6 a	100.0 a	100.0 a
Bt + Pf	11.2 d	39.2 cd	57.9 b	71.7 d	86.4 c
Bt + Sm	15.6 b	51.2 b	79.3 a	93.4 b	100.0 a
Pf + Sm	15.2 bc	42.6 cd	62.9 b	86.4 c	94.9 b
Bt + Pf + Sm	12.9 cd	37.4 d	61.4 b	83.8 c	92.1 b
Control 1	0.0 f	0.0 f	0.0 d	0.0 f	0.0 e
Control 2	0.0 f	0.0 f	0.0 d	0.0 f	0.0 e
L.S.D. $_{(0.05)}$	2.53	5.74	7.70	5.46	4.60

- J2: Second stage juveniles; h: hour; Control 1: TSB Media; Control 2: Distilled water
- Each value represents the mean five replicates;
- Mean values with the same letter in column are not significantly different by Duncan's Multiple Range Test at ($P \leq 0.05$).

Table (3): Mortality rates of citrus nematodes influenced by the application of bacterial secreted compounds

Bacterial stain	Solvent	J2 in 0.2 ml water	Inactive J2 (after 4 h of exposure)	% inactive J2	
Bacillus thuringensis	Ethyl acetate 1	31	23	74.2	Moderate
	Ethyl acetate 2	26	8	30.8	Slight
	n-Hexane 1	28	23	82.1	Strong
	n-Hexane 2	26	10	38.5	Slight
	n-Hexane 3	31	12	38.7	Slight
	Chloroform 1	27	16	59.3	Moderate
	Chloroform 2	29	5	17.2	Not active
Serratia marcescens	Ethyl acetate 1	25	18	72.0	Moderate
	Ethyl acetate 2	27	21	77.8	Moderate
	Ethyl acetate 3	31	8	25.8	Slight
	n-Hexane 1	34	22	64.7	Moderate
	n-Hexane 2	30	18	60.0	Moderate
	Chloroform 1	29	17	58.6	Moderate
	Chloroform 2	28	17	60.7	Moderate
Pseudomonas fluorescens	Ethyl acetate 1	33	19	57.6	Moderate
	Ethyl acetate 2	28	20	71.4	Moderate
	Ethyl acetate 3	32	8	25.0	Slight
	Ethyl acetate 4	34	14	41.2	Slight
	n-Hexane	28	18	64.3	Moderate
	Chloroform	27	5	18.5	Not active
Control (Water only)		32	3	9.4	Not active

- J2: Second stage juveniles; Control: 0.2 ml of water nematode suspension
- VOCs with Strong = NA ≥ 80%, Moderate = 50% ≤ NA < 80%, Slight = 20% ≤ NA <50% or Not active = NA < 20% (Ying-Qi *et al.*, 2007).

Bt + *Sm* > *Pf* + *Sm* > *Bt* + *Pf* + *Sm* > *Bt* + *Pf* with mortality percentages of 100, 94.9, 92.1and 86.4, respectively. The mixture of the concerned bacterial filtrates did not show any antagonistic or synergistic effect to each other (Table 2).

Many of the secondary metabolites of microorganisms and plants showed nematicidal activity (NA) and therefore these substances may become potential substitutes for the highly toxic chemical nematicides. More than 90 nematicidal toxins have been intensively investigated (Dong *et al.*, 2001). They include a wide range of compounds, such as quinines (Hayashi *et al.*, 1981), alkaloids (Blanchflower *et al.*, 1991), terpenoids (Stadler *et al.*, 1995a), pyrans (Stadler *et al.*, 1995b), furans (Shan *et al.*, 1996) and naphthalenes (Anke *et al.*, 1995).

Data in the present study agree with Dhawan *et al.* (2004) who demonstrated that the mobility of *M. incognita* juveniles completely ceased after 24-hr *of B. thuringiensis* exposure in S and S/10 dilutions. However, all dilutions above S/25 were ineffective. The cell-free culture filtrates of *B. cereus* reduced egg hatch (90%) and caused 100% mortality of juveniles (Naghesh *et al.*, 2005). Fluorescent Pseudomonads has been recognized as a potential biocontrol agent against cyst and root knot nematode (Oostendorp and Sikora, 1989). *Pseudomonas* isolates caused great inhibitory effect on hatching and penetration of *M. incognita* (Siddiqui *et al.*, 2009). Entomopathogenic strains of *S. marcescens* were used as a bio-control in New Zealand (O'Callaghan M and Jackson, 1993). *S. marcescens* had significant suppressive effect on *M. incognita* on sunflower and faba plants (Ali, 1996 and Mohamed *et al.*, 2010).

Analyzing the nematicidal activity of VOCs fractionated on TLC plates

In the sealed vials containing thin films of scraped fractionated compounds resulted from TLCs plate, nematodes gradually reduced their movement within 15 min to 4 h of incubation (Table, 3). When these immobile nematodes were transferred to fresh tap water, they could not be revived. The tested bacteria showed high variations in their nematicidal activity (NA) against nematodes.

Of the 20 bacterial fractionated compounds tested, a total of 18 fractions (accounting to 90%) displayed more than 20% NA against *T. semipenetrans*. Those included 6, 7 and 5 fractions of *B. thuringensis*, *S. marcescens* and *P. fluorescens*, respectively. Among the 18 fractions, one of *B. thuringensis* showed strong NA (≥ 80%). Among the remaining fractions, 11 (61.1% of the total) and 6 (33.3% of the total) showed NA as moderate (50% ≤ NA < 80%) and slight (20% ≤ NA < 50%), respectively. The 11 fractions with moderate NA included 2 fractions of *B. thuringensis*, 6 of *S. marcescens* and 3 of *P. fluorescens* (Table 3).

These results were consistent with the hypothesis that the VOCs from these bacteria were responsible for the NA. Among the 18 fractions, VOCs of 3 representatives that showed highest NA were further identified by GC/MS.

Identification of chemical constituents for selected nematicidal VOCs fractions using GC-MS chromatography

Three VOCs fractions with highest NA released from ethyl acetate extract of *S. marcescens* and *P. fluorescens,* & n-hexane extract of *B. thuringensis,* were selected for identification using GC/MS (Table 4 and Fig. 3). 16–26 peaks were detected from the fractions of the highest nematicidal activity. In total, 61 distinct VOCs were detected from the three fractions of the bacterial organo-solvent extract using GC/MS based on a comparison with the mass spectrum of the substance with GC/MS system data banks (Wiley and NIST library), (Table 4). The 61 VOCs covered a wide range of ketones (2), alkanes (6), alcohol (1), alkenes (18), esters (10), aromatic dicarboxylic acids (2), aromatic ester (1), aromatic hydrocarbons (7), oxygenated hydrocarbon (1), Epoxy (1), Pyrones (1), phenyl alcohol (1), heterocyclics (5) and phenol (5).

Of the 61 VOCs detected, the presented data show some identified VOCs belong to groups posses anti-microbial and/or nematicidal effect. For instance, the ethyl acetate extract from *P. fluorescens* was characterized by presence of [2, 6-di-tert butyl –4-cresol], [2,4-di-tert-butylphenol] and [n-Nonylphenol] as phenol compounds; [7,9-di-tert–butyl–1-oxaspiro (4,5) deca–6,9-diene–2,8-dione] as ketone compound; [Podocarpa-8,11,13-trien-15-oic acid, 13 isopropyl, methyl ester], [Cyclotetracosane] & [Pyrrolo (1,2-a pyrazine-1,4-dione, hexahydro-3-(phenyl methyl)] as heterocyclic compounds and [1, 2 – benzenedicarboxylic acid] as aromatic dicarboxylic acid compound. The ethyl acetate extract from *S. marcescens* was characterized with presence of [2,4-di-tert-butylphenol] as phenol compound; [7,9-di-tert–butyl–1-oxaspiro(4,5) deca–6,9-diene–2,8-dione] as ketone compound; [Dehydroabietic acid] as pyrones compound and [1, 2 – benzenedicarboxylic acid] as aromatic dicarboxylic acid compound. While, the n-hexan extract from *B. thuringensis* was characterized with presence of [2, 6-bis (1,1-dimethylethyl)-4-methyl Phenol] as phenol compound;[Heptadecane] & [Icosane] as alkane compounds; [Cobalt (II) bis (O, O'-diethyl dithiophosphate)] & [Phthlic acid, mono (2-ethylhexyl) ester] as ester compounds; [Benzene-O-dicarboxylic acid di-N-butyl ester] as aromatic ester compound, (Table 4).

Table (4): Selected VOCs released from ethyl acetate extract of *S. marcescens* and *P. fluorescens*, & n-hexane extract of *B. thuringensis*, with high identification quality ratings (>90) to the NIST & Wily mass spectral library

Pk	IUBC Name	RT	*Ps*	*Bt*	*S*	Molecular Structure	Class	Quality rating
1	Methyl benzene	3.96	+		+	C_7H_8	Aromatic hydrocarbon	91
2	O-Xylene	5.29	+	+	+	C_8H_{10}	Aromatic hydrocarbon	97
3	1,2,3-trimethyl benzene	8.19		+		C_9H_{12}	Aromatic hydrocarbon	95
4	2,6-ditert butyl benzo 1, 4-quinone	19.11	+			$C_{14}H_{20}O_2$	Aromatic hydrocarbon	98
5	2, 6-di- tert-butyl-4-cresol	20.03			+	$C_{15}H_{24}O$	Phenyl Alcohol	98
6	2,4-di-tert-butyl-phenol	20.14	+		+	$C_{14}H_{22}O$	Phenol	95
7	2, 6-bis (1,1-dimethylethyl)-4-methyl Phenol	20.16	+	+		$C_{15}H_{24}O$	Phenol	95
8	1-Hexadecene	21.39	+	+		$C_{16}H_{32}$	Alkene hydrocarbon	99
9	Diethyl phthalate	21.79	+	+	+	$C_{12}H_{14}O_4$	Ester	98
10	Heptadecane	23.34		+		$C_{17}H_{36}$	Alkane	98
11	n-Nonylphenol	23.99	+			$C_{15}H_{26}O$	Phenol	90
12	1-Octadecene	24.97	+	+	+	$C_{18}H_{36}$	Alkene	99
13	Octdecane	25.07		+		$C_{18}H_{38}$	Alkane	98
14	1-Octadecene	25.19		+		$C_{18}H_{36}$	Alkene	98
15	3-Octadecene	25.40		+		C18 H36	Alkene	97
16	1-Propyl tetradecanoate	25.62		+		$C_{17}H_{34}O_2$	Oxygenated hydrocarbon	95
17	1,2-benzenedicarboxylic acid, bis (2-methylpropyl) ester	26.42		+		$C_{16}H_{22}O_4$	Ester	94
18	Hexadecanoic acid methyl ester	27.09	+	+		$C_{17}H_{34}O_2$	Ester	98
19	7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	27.15	+		+	$C_{17}H_{24}O_3$	Ketone	99
20	Benzene-O-dicarboxylic acid di-N-butyl ester	27.59		+		$C_{16}H_{22}O_4$	Aromatic ester	91
21	1-Octedcene	27.88		+	+	$C_{18}H_{36}$	Alkene	96
22	Icosane	27.93		+		$C_{20}H_{42}$	Alkane	92
23	5-Icosene	28.18		+	+	$C_{20}H_{40}$	Alkene	99
24	Oxirane tetradecyl	28.25		+		$C_{16}H_{32}O$	Epoxy	95
25	1-Octadecene	28.99	+	+	+	$C_{18}H_{36}$	Alkene	99
26	1-Docosane	30.15		+		$C_{22}H_{46}$	Alkane	99
27	1-Docosanol	30.03			+	$C_{22}H_{46}O$	Alcohol	94
28	1-Octadecene	30.25	+	+	+	$C_{18}H_{36}$	Alkene	99
29	Cobalt (II)bis (O, O'-diethyl dithiophosphate)	30.84		+	+	$C_4H_{14}NO_2P$ S_2Co_2	Ester	91
30	(Trans)-2-nonadecene	31.06	+	+		$C_{19}H_{38}$	Alkane	91
31	Podocarpa-8,11,13-trien-15-oic acid, 13 isopropyl, methyl ester	31.64	+			$C_{20}H_{26}O_3$	Heterocyclic	97
32	Dehydroabietic acid	31.64			+	$C_{20}H_{28}O_2$	Pyrones	94
33	Cyclotetracosane	31.85	+	+	+	$C_{24}H_{48}$	Heterocyclic	99
34	Adipic acid bis (2-ethylhexyl) ester	31.99		+		$C_{22}H_{42}O_4$	Ester	93
35	Pyrrolo (1,2-a pyrazine-1,4-dione, hexahydro-3-(phenyl methyl)	32.14	+			$C_{14}H_{16}N_2O_2$	Heterocyclic	99
36	1, 2 – benzenedicarboxylic acid	33.35	+		+	$C_8H_6O_4$	Aromatic dicarboxylic acid	91
37	Phthlic acid, mono (2-ethylhexyl) ester	33.37		+		$C_{16}H_{22}O_4$	Ester	91
38	1-Nonadecene	33.62	+			$C_{19}H_{38}$	Alkene	99

If a compound was found in multiple samples, the highest ID quality rating was reported.

However, little is known about nematicidal volatile organic compounds (VOCs) and their potential use as biological control agents against plant parasitic nematodes (Lewis *et al.*, 2006). Such volatile organic compounds (VOCs) are ideal infochemicals, because they can act over a wide range of distances and their severe of activity will extend from proximal interactions to greater distances via diffusion in air, including in soil pores (Wheatley, 2002). Thus, non-chemical methods that can effectively control plant diseases are highly desirable. Bacterial volatiles and traditional fumigation agents may be similar in their chemical structures and mechanisms of action (Gamliel *et al.*, 2000).

The volatile organic compounds as long chain Hexadecanoic acid (C_{16}) and Octadecanoic acid (C_{18}), which is considered to be more biologically active than shorter hydrocarbon side chains Dodecanoic acid (C_{12}), Myco-subtilins with C_{16} or C_{17} fatty acid chains are considered to be more biologically active than the iturins, which have shorter hydrocarbon side chains (C_{14} and C_{15}).

Meanwhile, toxicity increases with the number of carbon atoms in chain; *i.e.*, C_{17} homologues are 20-fold more active than the C_{14} forms (Weiwei *et al.*, 2008).

Results of the present work agree with Ying-Qi *et al.* (2007) who tested the NA of certain commercial VOCs against nematodes, in comparison with VOCs secreted naturally by isolates of *Bacillus simplex*, *Bacillus subtilis*, *Serratia marcescens* and *Streptomyces maltophilia*. They identified 14 bacterial VOCs with nematicidal effects: phenol, 2-octanol, benzaldehyde, benzeneacetaldehyde, decanal, 2-nonanone, 2-undecanone, cyclohexene, phenyl ethanone, dimethyl disulfide, terpineol, nonane, propanone and benzeneethanol. VOCs with 100% NA belonged to six categories: alcohols, aldehydes, ketones, alkenes, ethers and phenolic compounds. In addition, different volatile compounds of the same category can show very different NAs. Compounds of alcohols, aldehydes and ketones were the most frequently observed compounds showing high NA to both tested nematode species.

However, results of this study showed the detection of about 61 VOCs, from *S. marcescens, P. fluorescens*, and *B. thuringensis*. The volatiles of the tested bacteria usually contained more than one kind of nematicidal compounds. The mixture of compounds produced by soil bacteria may be more effective to control nematodes and less likely to select for resistance than treatment by synthetic nematicides composed of a single compound. This finding should be considered when formulating bicontrol compounds or establishing a biocontrol strategy. This suggested that some of the bacterial VOCs could be used as main skeletons for developing novel nematicidal agents by further chemical modifications.

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