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Isolation and characterization of chemical constituents from *B. amyloliquefaciens* and their nematicidal activity

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Abstract
Secondary metabolites from cell free culture filtrate of *B. amyloliquefaciens* (Ba-14.5) showed strong nematicidal activity against root-knot nematode in preliminary tests. In the present study, chemical constituents present in secondary metabolites of *B. amyloliquefaciens* were investigated. Methanol fractions obtained from silica gel chromatography were analysed by using GC-MS. Thirteen compounds were revealed by GC-MS analysis and these constituents include stigmasta-3,5-dien-7-one (9.82%), Pregna-5, 16-dien-20-one, 3-(acetyloxy)-,(3.beta)-(5.12%), Benzeneacetaldehyde (3.96%), N-acetyl-3-methyl-1, 4-diazabicyclo[4.3.0]nonan-2,5-dione (3.65%), Pyrrolo (1,2-1) pyrazine-1,4-dione,hexahydro-(39.57%), 2,4,6,8-Tetramethyldecan-1-ol (0.54%), Pyrrolo (1,2-1) pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)- (4.96%), Hexadecanoic acid, methyl ester (0.35%), 5, 10-Diethoxy-2,3,7,8-tetrahydro-1H, 6H-dipyrrolo(1,2-a (1.35%), n-Octadecanoic acid methyl ester (0.39%), 3,6-Disobutyl-2,5-Piperazinedione (4.21%), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (9.7%), and Octadecanoic acid, 2,3-dihydroxypropyl ester (21.8%). Among thirteen compounds, three compounds like Benzeneacetaldehyde, 2-hydroxy-1-(hydroxymethyl) ethyl ester and Octadecanoic acid, 2,3-dihydroxypropyl ester possess nematicidal activity based on previous studies. Other compounds possess antimicrobial, antifungal, antioxidant, anticancer and insecticidal properties and are yet to be confirmed for their nematicidal activity. Our findings help to find potential compounds/metabolites from microbial source to develop nematicides for the management of root-knot nematode in horticultural crops.

Keywords: *B. amyloliquefaciens*, culture filtrate, secondary metabolites, GC-MS, nematicidal activity

1. Introduction
Plant parasitic nematodes are hidden enemies of crops and are recognised as one of the greatest threats to crops throughout the world [29]. Among the plant parasitic nematodes, root knot nematode is the major nematode causing damage to most of the economically important crops [24]. They are obligate parasites and have a wide host range including vegetables, cereals and fruit crops [1, 15]. It causes an estimated $118b annual losses to world crops [5]. Nematode infected plants exhibit symptoms like stunted growth, chlorosis, wilting and presence of galls on roots. The management of nematodes is more difficult than that of other pests because nematodes mostly inhabit the soil and usually attack the underground parts of the plants [44]. Although the application of chemical nematicides are effective but in long term usage and wide scale caused environmental pollution and enhancement of resistance in nematodes [16, 40]. Therefore, the search for novel, environmentally friendly alternative means instead of chemicals becomes emergency demand for management of root- knot nematodes [22, 49].
Nowadays, microorganisms and their metabolites have attracted the most attention as potential nematode biocontrol agents. Several antagonistic microbial strains such as *Bacillus*, *Pasteuria* and *Pseudomonas* have been developed into commercial formulations and are successfully used to control nematodes in agricultural fields [8, 19, 26, 33, 43]. Among bacterial bioagents, *Bacillus* spp. are dominant within the rhizosphere and are directly associated with plants and the soil environment, the inhibition of phytopathogens is a fundamental function of the bioactive molecules they produce [35, 47]. Number of investigations showed that nematode pathogenic bacteria like *Bacillus* kill nematodes by different mechanisms, including parasitism, antibiosis production of toxins, indirectly by interfering with the recognition of host plants inducing systemic resistance and by improving plant health [47]. They can synthesize various molecules that are toxic to nematodes [11, 36]. For example, *B.*

thuringiensis shows nematicidal activity towards *M. incognita* and *Heterodera glycines* by producing crystal inclusions, a family of toxic proteins to a wide range of insect species including nematodes [17]. A number of studies showed that the secondary metabolites of certain *Bacillus* spp. that are responsible for their nematicidal activity [45, 46, 50, 51]. It is however unclear what the exact metabolites are responsible for nematicidal activity. These unknown variables lead to questionable efficacy and reproducibility of bionematicide products, compounded by the lack of standards for such products. *Bacillus amyloliquefaciens* (Ba-14.5) was an isolate from the rhizosphere area of yams in the fields of CTCRI. The preliminary results have shown that the crude extract of the culture filtrate of *B. amyloliquefaciens* had showed strong nematicidal activity against root knot nematode. In this present investigation, the potential nematicidal compounds were investigated from the fore-said bacterial secondary metabolites.

2. Materials and Methods

2.1. Culturing of *B. amyloliquefaciens*

Fresh culture of *B. amyloliquefaciens* was prepared in slants by inoculating culture from mother culture and incubated at room temperature for 24 h. The fresh cultures from slants were inoculated to one litre nutrient broth (5 g of Peptone, 5 g of Sodium Chloride, 1.5 g of meat extract and 1.5 g of yeast extract) and were incubated in gyro-rotatory shaker at 30 °C for three days. The cultures were grown as batch culture in 500 ml conical flasks containing 100 ml of the nutrient broth.

2.2. Extraction of bacterial metabolites from *B. amyloliquefaciens*

The culture media were then centrifuged (10000 g, 20 min, 4 ºC) followed by filtration through a 0.45 µm Whatman filter paper to obtain cell-free culture filtrate. 1000ml of that filtrate was concentrated to 5 ml at 30 °C using a rotary flash evaporator. The extract was further eluted using silica gel column chromatography with hexane and ethyl acetate solvents. No fractions obtained with both hexane and ethyl acetate solvents. After elution with non-polar solvents the column was subjected to polar solvents such as methanol which yielded 16 fractions. Thin layer chromatography was demonstrated to understand the number of compounds present in each fraction. Based on TLC profile, methanol fractions 1–3, 4–6, 7–10 and 11–16, were then pooled, respectively, into four fractions (F_M1–F_M4).

2.3. Nematicidal activity assay

The sample of 2ml each methanol fractions (F_M1–F_M4) were added to petri dish containing ~20 second juveniles of root knot nematodes. Each treatment was replicated thrice with sterile water as control at 25 °C. The number of live and dead where calculated after incubation of 8, 12 and 24 hours. The nematodes (J_2s) were considered as dead when they are straight and are not responding to physical stimuli (touch with needle) while observing under a light microscope [9, 32]. Mortality values were calculated according to Abbott's formula [2].

(%) = [(mortality percentage in treatment – mortality percentage in control)/ (100 – mortality percentage in control)] × 100.

2.4. GC - MS analysis

Based on nematicidal bioassay study, potential methanol fractions were selected and extracted by using rotary evaporator to obtained viscous semi solid material. The semi dry methanol crude extract was suspended in water and sent outsourcing for GC-MS analysis. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology having more than 62,000 patterns. The spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

3. Results and Discussion:

3.1. Effect of methanol fractions of *B. amyloliquefaciens*

Four fractions i.e. F_M1, F_M2, F_M3, F_M4 were tested against root knot nematode under lab conditions. Solvent methanol is highly toxic to nematodes. So, all four methanol fractions were lyophilised, so that methanol solvent got evaporated leaving behind the compounds in powder form. This powdered form compounds were dissolved in water and tested it against nematodes and water as control.

Table 1: Effects of the cell-free culture filtrate of *B. amyloliquefaciens* on mortality of *M. incognita in vitro*

Mortality %	8h	12h	24h
	Mean ± S.E (Mortality %)		
F_M1	12.00 ± 0.57 (60%)	17.00 ± 0.88 (85.00%)	20 ± 0 (100%)
F_M2	1.33 ± 0.33 (6.65%)	4.66 ± 0.33 (23.33%)	6.33 ± 0.88 (31.65%)
F_M3	7.66 ± 0.33 (38.30%)	11.66 ± 1.2 (58.3%)	17.66 ± 0.88 (88.33%)
F_M4	12.66 ± 0.33 (63.33%)	17.33 ± 0.88 (86.65%)	20 ± 0 (100%)
control	0	0	0
C.D	1.18	2.24	2.06

Results revealed that 100% mortality of second stage juveniles (J2) was observed with two methanol fractions (F_M1 and F_M4) within 24 h incubation time (Table 1). Whereas, 88.3 % and 31.6% mortality observed with methanol fractions F_M3 and F_M2 respectively. The effect of nematicidal activity of this fractions was in this order F_M1=F_M4 >F_M3>F_M2. F_M1 and F_M4 showed high nematicidal activity followed by F_M3. But fraction F_M2 showed very less nematicidal activity.

3.2. GC-MS analysis of the secondary metabolites of *B. amyloliquefaciens*

Two methanol fractions F_M1 and F_M4 were selected to identify compounds/metabolites present in culture filtrate of *B. amyloliquefaciens*. Chromatogram GC-MS analysis of the two methanol fractions of *B. amyloliquefaciens* showed the presence of thirteen major peaks at respective temperature (Figure 1 & 2). The peak areas (or percentage compositions of the metabolites shown in the brackets) are relative to other constituents within the crude extracts and whose match factors were greater than 700.

Compounds that are identified through GC-MS analysis were tabulated in tables 1 and 2. The compound prediction is based on National Institute Standard and Technology Database. The results revealed the presence of stigmasta-3,5-dien-7-one (9.82%), Pregna-5, 16-dien-20-one, 3-(acetyloxy)-, (3.beta)- (5.12%), Benzeneacetaldehyde (3.96%), N-acetyl-3-methyl-

1,4-diazabicyclo[4.3.0]nonan-2,5-dione (3.65%), Pyrrolo(1,2-1)pyrazine-1,4-dione,hexahydro-(39.57%), 2,4,6,8-Tetramethyldecan-1-ol (0.54%), Pyrrolo(1,2-1)pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)- (4.96%), Hexadecanoic acid, methyl ester (0.35%), 5, 10-Diethoxy-2,3,7,8-tetrahydro-1H, 6H-dipyrrolo(1,2-a (1.35%), n-Octadecanoic acid methyl ester (0.39%), 3,6-Disobutyl-2,5-Piperazinedione (4.21%), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (9.79%) and Octadecanoic acid, 2,3-dihydroxypropyl ester (21.48%).

In the present study, the GC-MS analysis of the methanolic fractions of *B. amyloliquefaciens* showed the presence of thirteen compounds. Nematicidal activity are shown by Benzeneacetaldehyde, 2-hydroxy-1-(hydroxymethyl) ethyl ester and Octadecanoic acid, 2,3-

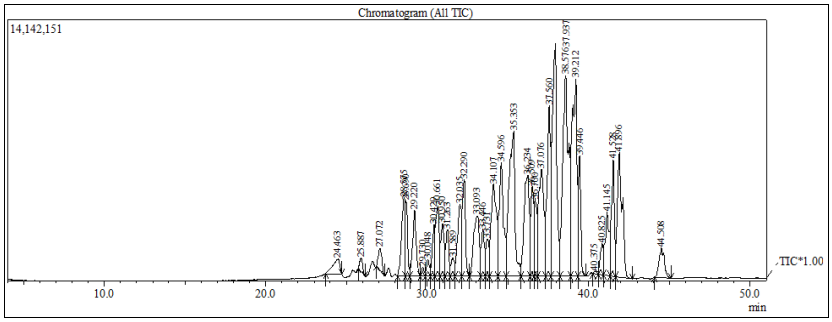

Fig 1: Chromatogram of GC-MS analysis of methanol fraction (F_M1) of secondary metabolites produced by *B. amyloliquefaciens*

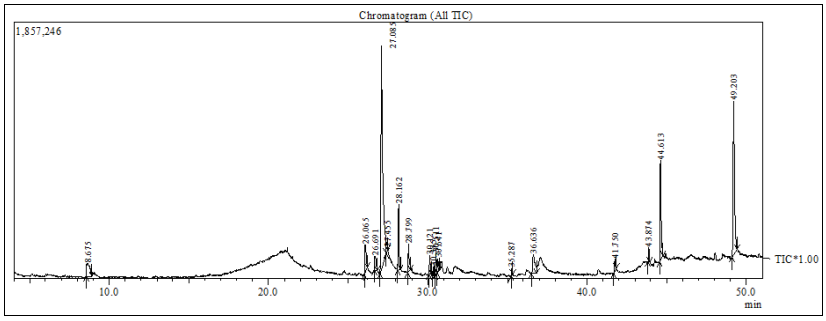

Fig 2: Chromatogram of GC-MS analysis of methanol fraction (F_M-4) of secondary metabolites produced by *B. amyloliquefaciens*

Table 2: Compounds identified after GC-MS analysis of methanol fraction (F_M-1) extracted from secondary metabolites produced by *Bacillus amyloliquefaciens*

S. No	Retention time	Area %	Compound	Molecular formula	Molecular weight	Activity	References
1.	24.46, 25.88, 27.07, 29.22, 29.73, 30.04, 31.58, 32.03, 34.10, 33.09, 33.70, 38.57	9.82	stigmasta-3,5-dien-7-one	$C_{29}H_{46}O$	410	free radical scavenging, anti-diabetic,anticancer, free radical scavenging, anti-inflammatory	[6, 14, 36]
2.	41.896	5.12	Pregna-5, 16-dien-20-one, 3- (acetyloxy)-, (3.beta.)-	$C_{23}H_{32}O_3$	356.49	anticancer agents	-

Table 3: Compounds identified after GC-MS analysis of methanol fraction (F_M-4) extracted from secondary metabolites produced by *Bacillus amyloliquefaciens*

S. No	Retention time	Area %	Compound	Molecular formula	Molecular weight	Activity	Reference
1.	8.675	3.96	Benzene acetaldehyde	C_8H_8O	120.1485	Nematicidal activity	[21, 23]
2.	26.065	3.65	N-acetyl-3-methyl-1,4-diazabicyclo[4.3.0]nonan-2,5-dione	$C_{10}H_{14}N_2O_3$	210.22	No activity reported	[41]
3.	27.085	39.57	Pyrrolo(1,2-1)pyrazine-1,4-dione, hexahydro-	$C_7H_{10}N_2O_2$	154.1665	antioxidant activity, antibiotic activity, antifungal compound	[20, 28, 39]
4.	27.455	0.54	2,4,6,8-Tetramethyldecan-1-ol	$C_{14}H_{22}O$	206.328	Volatile compound in sex pheromone of *Margarodes prieskaensis*	[7]
5.	28.162	4.96	Pyrrolo (1,2-1) pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	$C_{11}H_{18}N_2O_2$	210	Antifungal activity	[12]
6.	30.335	0.35	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O$	270	Antibacterial, antifungal, Antioxidant, nematicide, insecticide, lubricant, antiandrogenic, haemolytic	[4]
7	30.641	1.35	5, 10-Diethoxy-2,3,7,8-tetrahydro-1H, 6H-dipyrrolo (1,2-a	$C_{14}H_{22}N_2O_2$	250	Antimicrobial	[27]
8.	35.287	0.39	n-Octadecanoic acid methyl ester	$C_{18}H_{36}O_2$	284	Antimicrobial activity	[37]
9	36.636	4.21	3,6- Disobutyl-2,5-Piperazinedione	$C_9H_{16}N_2O_2$	184.24	Antifungal activity	[3]
10.	44.613	9.79	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330	Haemolytic, pesticide, flavour, antioxidant, nematicide, Insectifuge	[4]
11.	49.203	21.48	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	358.5558	Antimicrobial, Anticancer, nematicide, hepatoprotective, Anti-arthritic, anti-asthma, diuretic	[37]

dihydroxypropyl ester possess nematicidal activity [23],[4],[37]. Tyagi and Agarwal, (2017) [48] isolated compounds like N-Hexadecanoic acid, Ethyl ester from Pistia and Eichornia which showed nematicidal and antimicrobial properties. Similarly, nematicidal compounds like Octadecadienoic acid was identified in bacterium-based resources by Kanagarajan *et al*., 2016 [25]. These compounds were fatty acid in nature and has been suggested that they derive their nematicidal effects by adversely interfering with the nematode cuticle or hypodermis via a detergent (solubilization) effect, or through direct interaction of the fatty acids and the lipophilic regions of target plasma membranes [13]. Several Volatile organic compounds like aldehydes, ketones, alkyls, alcohols, alkenes, esters, alkynes, acids, ethers, and heterocyclic, and phenolic compound emitted by soil bacterial showed fumigant, repellent and attractive properties towards nematodes. benzeneacetaldehyde (Volatile organic compound) produced by soil bacteria showed nematicidal activity [21]. From this information, the above mentioned three compounds might be responsible for nematicidal activity of present studied bacteria, *B. amyloliquefaciens* (Ba-14.5). Remaining compounds identified from methanol solvents of present studied bacterial strain possess antifungal, antimicrobial, antioxidant, antihelminthic, and insecticidal properties.

The observed nematicidal activity may be attributed to one or synergetic effect of two or more compounds. Therefore, further work is required to know the nematicidal activity of individual compounds and also their synergistic effect against root-knot nematode.

4. Conclusion

The GC-MS analysis showed the presence of thirteen compounds from the methanol extracts from *B. amyloliquefaciens* culture filtrates. Among thirteen compounds, three compounds like octadecanoic acid, n-hexadecanoic acid, Hexadecanoic acid- ethyl ester possess nematicidal properties based on previous studies. This finding should be considered when formulating biocontrol compounds or establishing a biocontrol strategy. This suggested that some of the secondary metabolites of bacteria could be used as main skeletons for developing novel nematicidal agents by further chemical modifications.

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6. References

1. Abad P, Favery B, Rosso MN, Sereno CP, Root-knot nematode parasitism and host response: Molecular basis of a sophisticated interaction. Molecular Plant Pathology. 2003; 4:217-224.
2. Abbott WS. A method of computing the effectiveness of an insecticide J Am. Mosq. Control Assoc. 1987; 3:302-303.
3. Amita S, Mahendra GK, Pradeek KS, Pankaj S. Extracellular Release of Non-Peptide Group Compounds by Antifungal *Bacillus* and *Brevibacillus* Strains. Current Bioactive Compounds. 2017; 13(9):259-267.
4. Arora S, Ganesh K, Sonam M. Gas chromatography-mass spectroscopy analysis of root of an economically important plant, *Cenchrus ciliaris* L. from thara-desert, Rajasthan (India). Asian journal of pharmaceutical and clinical research. 2017; 10(9):64-69.
5. Atkinson HJ, Lilley CJ, Urwin PE. Strategies for transgenic nematode control in developed and developing world crops. Food Biotechnology and Plant Biotechnology. 2012; 23(2):251-256.
6. Balogun OS, Oladosu IA, Akiinnusi A, Zhiqiang L, Fatty acid composition α-glucosidase inhibitory potential and cytotoxicity activity of Oncoba spinosa Forssk. Elixir Applied Chemistry. 2013; 59:15637-15641.
7. Burger BV, Klerk CA, Morr M. Burger WJG. Identification, Synthesis, and Field Tests of the Sex Pheromone of *Margarodes prieskaensis* (Jakubski). Journal of chemical ecology. 2017; 43:94-105.
8. Butt TM, Jackson CW, Magan N. Fungi as Biocontrol Agents: Progress, Problems and Potential, Wallingford, Oxon, UK: CAB International, 2001.
9. Cayrol JC, Djian C, Pijarowski L. Study of the nematicidal properties of the culture filtrate of the nematophagous fungus *Paecilomyces lilacinus*. Review of Nematology. 1989; 12:331-336.
10. Chandrasekaran M, Senthilkumar A, Venkatesalu V. Antibacterial and antifungal efficacy of fatty acidmethyl esters from leaves of Sesuvium portulacastrum L. European Review for Medical and Pharmacological Sciences. 2011; 15:775-780.
11. Cui HY, Jin Liu Q, Yan ZQ. Nematicidal metabolites from roots of *Stellera chamaejasme* against *Bursaphelenchus xylophilus* and *Bursaphelenchus mucronatus*. Pest Management Science. 2014; 70:827-835.
12. Dash S, Jin C, Lee OO, Xu Y, Qian PY. Antibacterial and antilarval-settlement potential and metabolite profiles of novel sponge-associated marine bacteria. Journal of Industrial. Microbiology Biotechnology Journal of Industrial Microbiology & Biotechnology. 2009; 36:1047-1056.
13. Davis EL, Meyers DM, Dullum CJ. Feitelson JS. Nematicidal activity of fatty acid esters on soybean cyst and root-knot nematodes. Supplementary Journal of Nematology.1997; 29:677-840.
14. Delazar A, Nazifi E, Movafeghi A, Nazemiyey H, Hemmati S, Nahar L. Analyses of phytosterols and free radical scavengers in the bulbs of Ornithogalum cuspidatum Bertol. Bol. Latinoam. Caribe Plant. Med. Aromat. 2010: 9:87-92.
15. Djian CC. Root-knot nematodes (*Meloidogyne* spp.), a growing problem in French vegetable crops. OEPP/EPPO Bulletin. 2012; 42:1-12.
16. Dong JY, Li XP, Li L, Li GH, Liu YJ, Zhang KQ. Preliminary results on nematicidal activity from culture filtrates of basidiomycetes against the pine wood nematode, *Bursaphelenchus xylophilus* (Aphelenchoididae). Annals of Microbiology. 2006; 56(2):163-166.
17. Dong. Nematicidal effect of freshwater fungal cultures against the pine-wood nematode, *Bursaphelenchus xylophilus*. Fungal Diversity. 2004; 15:125-135.
18. Duncan DB. Multiple range and multiple F tests. Biometrics. 1955; 11:1-42.
19. Gao H, Qi G, Yin R, Zhang H, Li C, Zhao X. *Bacillus cereus* strain S2 shows high nematicidal activity against *Meloidogyne incognita* by producing sphingosine. Scientific Reports. 2016; 6:605.
20. Gopi M, Dhayanithi NB, Devi KN, Kuma R TTA. Marine natural product, Pyrrolo [1,2-a] pyrazine-1,4-

dione, hexahydro- (C7H10N2O2) of antioxidant properties from Bacillus species at Lakshadweep archipelago. Journal of coastal life medicine. 2014; 2(8):632-637.
21. Gu QY, Ming HM, Jun PZ, Chang SZ, Ke QZ. Evaluation and identification of potential organic nematicidal volatiles from soil bacteria. Soil Biology and Biochemistry. 2007; 39(10):2567-2575.
22. Hallmann J, Davies KG, Sikora R. Biological control using microbial pathogens, endophytes and antagonists, *In* Perry RN, Moens M, Starr JL. (ed), Root knot nematodes. CAB International, Wallingford, United Kingdom, 2009, 380-411.
23. Huang Y, Xu C, Ma L, Zhang K, Duan CQ, Mo MH. Characterisation of volatiles produced from *Bacillus megaterium* YFM3.25 and their nematicidal activity against *Meloidogyne incognita*. European journal of plant pathology. 2010; 126:417-422.
24. Jones JT, Haegemen A, Danchin EGJ, Gaur HS, Helder J, Jones MGK. Perry RN (Top 10 plant-parasitic nematodes in molecular plant pathology. Molecular Plant Pathology. 2013; 14:946-961.
25. Kanagarajan M, Devimarudachalam DPT, Ponnuraj S, Jagathan D. Synergistic effect of ethno medicinal plants against biofilm forming *Streptococcus pyogenes* isolated from upper respiratory tract infection. International journal of phytomedicine. 2016; 8:208-216.
26. Kerry BR. Rhizosphere interactions and the exploitation of microbial agents for the biological control of plant-parasitic nematodes. Annual Review of Phytopathology. 2000; 38:423-441.
27. Khattab A, Babiker EH, Saeed HA. International Current Pharm J. 2016; 5:27.
28. Kiran GS, Priyadharshini S, Sajayan A, Ravindran A, Selvin J. An antibiotic agent pyrrolo [1, 2-*a*] pyrazine-1, 4-dione, hexahydro isolated from a marine bacteria *Bacillus tequilensis* MSI45 effectively controls multi-drug resistant *Staphylococcus aureus*. RSC Advances. 2018; 8(32):17837-17846.
29. Koenning SR, Overstreet C, Noling JW, Donald PA, Becker JO, Fortnum BA. Survey of crop losses in response to phytoparasitic nematodes in the United States for 1994. Journal of Nematology. 1999; 31:587-618.
30. Kumazawa S, Kanda M, Utagawa M. Mk7924, a novel metabolite with nematicidal activity from *Coronophora gregaria*. Journal of antibiotics. 2003; 56:652-654.
31. Leyns F, Lambert B, Joos H, Swings J. Antifungal bacteria from different crops. In: Biological Control of Soil-borne Plant Pathogens (Hornby D., ed.). C.A.B International, Wallingford, U.K, 1990, 331-335.
32. Lucas VS, Cortada L, Sorribas FJ, Ornat C. Selection of virulent populations of *Meloidogyne javanica* by repeated cultivation of Mi resistance gene tomato rootstocks under field conditions. Plant Pathology. 2009; 58:990-998.
33. Meyer SLF. United States Department of Agriculture-Agricultural Research Service research programs on microbes for management of plant-parasitic nematodes. Pest Management Science. 2003; 59:665-670.
34. Mhatre PH, Karthik C, Kadirvelu K, Divya KL, Venkatasalam EP. Srinivasan S. Ramkumar G et al. Plant growth promoting rhizobacteria (PGPR): A potential alternative tool for nematodes bio-control. Biocatalysis and Agricultural Biotechnology. 2019; 17:119-128.
35. Mongkolthanaruk W. Classification of *Bacillus* beneficial substances related to plants, humans and animals. Journal of Microbiology and Biotechnology. 2012; 22:1597-1604.
36. Park SJ, Kim YW, Park MK, Byun SH, Kim SC, Lee JR. Anti-inflammatory steroid from *Phragmitis rhizoma* modulates LPS-mediated signalling through inhibition of NF-κB pathway. Inflammation. 2016; 39(2):727-34.
37. Rahuman AA, Gopalakrishnan G, Ghouse BS, Arumugam S, Himalayan B. Effect of Feronia limonia on mosquito larvae. Fitoterapia. 2000; 71:553-555.
38. Rastogi G, Tech JJ, Coaker GL, Leveau JH. A PCR-based toolbox for the culture-independent quantification of total bacterial abundances in plant environments. Journal of Microbiology Methods. 2010; 83:127-132.
39. Sanjenbam P, Krishnan K, Bioactivity of Pyrrolo [1,2-A]pyrazine-1,4-dione,hexahydro-3-(phenylmethyl)-Extracted from Streptomyces sp. VITPK9 Isolated from the Salt Spring Habitat of Manipur, India. Asian Journal of Pharmaceutics. 2016; 10(4):265-270.
40. Schneider SM, Rosskopf EN, Leesch JG, Chellemi DO, Bull CT, Mazzola M. Research on alternatives to methyl bromide: pre-plant and post-harvest. Pest Management Science. 2003; 59:814-826.
41. Sharma P, Thakur D. Antimicrobial biosynthetic potential and diversity of culturable soil actinobacteria from forest ecosystems of Northeast India. Scientific reports. 2010; 10:4104.
42. Siddiqui IA. Suppression of *Meloidogyne javanica* by *Pseudomonas aeruginosa* and *Bacillus subtilis* in tomato. Nematologica Mediterranea. 2002; 30:125-130.
43. Siddiqui ZA, Mahmood I. Role of bacteria in the management of plant parasitic nematodes: A review. Bioresource technology. 1999; 69:167-179.
44. Stirling GR. Biological Control of Plant Parasitic Nematodes. Progress, Problems and Prospects. Wallingford: CAB International, 1991.
45. Tadigiri S, Kumar HK, Veena SS. *In-vitro* evaluation of potential bio agents on hatching and mortality of root knot nematode, *Meloidogyne incognita*. 2020; 8(3):767-770.
46. Terefe M, Tefera T, Sakhuja PK. Effect of a formulation of *Bacillus firmus* on root-knot nematode *Meloidogyne incognita* infestation and the growth of tomato plants in the greenhouse and nursery. Journal of Invertebrate Pathology. 2009; 100:94-99.
47. Tian BY, Yang JK, Lian LH, Wang CY, Zhang KQ. Role of neutral protease from *Brevibacillus laterosporus* in pathogenesis of nematode. Applied Microbiology and Biotechnology. 2007; 74:372-380.
48. Tyagi T, Agarwal M. Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) solms. Journal of Pharmacognosy and Phytochemistry. 2017; 6(1):195-206.
49. Weller DM. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annual Review of Phytopathology. 1988; 26:379-407.
50. Xiong J, Zhou Q, Luo H, Xia L, Li L, Sun M, Yu Z. Systemic nematicidal activity and biocontrol efficacy of *Bacillus firmus* against the root-knot nematode *Meloidogyne incognita*. World Journal of Microbiology & Biotechnology; 2015; 31(4):661-667.
51. Zhang J, Li Y, Yuan H, Sun B, Li H. Biological control of the cereal cyst nematode (*Heterodera filipjevi*) by *Achromobacter xylosoxidans* isolate 09X01 and *Bacillus cereus* isolate 09B18. Biological Control. 2016; 92:1-6.