EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
MICROBIOLOGY

G

ISSN
2090-0872

WWW.EAJBS.EG.NET

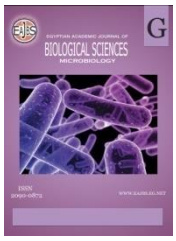
Vol. 14 No. 2 (2022)

Citation: *Egypt. Acad. J. Biolog. Sci. (G. Microbiolog) Vol.14 (2) pp.123- 133 (2022)*
DOI: 10.21608/EAJBSG.2022.262580

Egypt. Acad. J. Biolog. Sci., 14(2):123-133(2022)

Egyptian Academic Journal of Biological Sciences
G. Microbiology

ISSN: 2090-0872
https://eajbsg.journals.ekb.eg/



Endophytic *Streptomyces enissocaesilis* As a Nematicidal and Biostimulant Agent

Eman A. El-Akshar, Rasha M. El-Meihy*, Taha A. Tewfike and Hamed E. Abou-Aly
Department of Agricultural Microbiology, Faculty of Agriculture, Benha University, Moshtohor, Qaluybia, 13736, Egypt
*E. Mail: rashaelmehy@fagr.bu.edu.eg

ARTICLE INFO
Article History
Received:15/8/2022
Accepted:1/10/2022
Available:4/10/2022

***Keywords*:**
Endophytic *Streptomyces*, *Meloidogyne incognita*, Nematicide, Hydrolytic enzymes, Plant growth regulators.

ABSTRACT

The endophytic *Streptomyces enissocaesilis* OM182843 was investigated for its antagonistic activity against root-knot nematode (*Meloidogyne incognita*) as well as its ability to produce plant growth regulators. The nematicidal activity was performed using both culture and crude culture filtrate (CCF) of *Strep. enissocaesilis*. Both caused high mortality and inhibition of egg-hatching of *M. incognita* during all experimental intervals. Hydrolytic enzymes (chitinases, proteases and gelatinase), as well as plant growth regulators (indole acetic acid, gibberellic acid, salicylic acid, and proline), were produced by *Strep. enissocaesilis* in considerable quantities. Furthermore, volatile organic compounds (VOCs) released by *Strep. enissocaesilis* were analyzed by GC/MS and several of them were found to have nematicidal activity, the most abundant being 2,4-Decadienal, (E, Z) (CAS). This study opens new horizons for thinking about the use of microorganisms to combat pathogens such as root-knot nematodes, which avoid the harmful effects of synthetic nematicides, not only this but also can stimulate plant growth and thus provide a double benefit.

INTRODUCTION

Meloidogyne incognita, one of the most common species of root-knot root nematodes which capable of infecting approximately 3,000 plant species worldwide and resulting in approximately 5% of global crop loss (Lu *et al.*, 2022). The root-knot galls produced by *M. incognita* larvae cause the plant to lose nutrients through gall-like swellings known as root-knot galls. If the plants are young, the infection causes them to suffer and possibly die, but if they are mature, it causes a decrease in yield and increases their susceptibility to water and nutrient stress (El-Ashry *et al.*, 2022). Their widespread distribution, a vast variety of potential hosts, and connection to fungus, bacteria, and viruses provide a pressing problem (Wagh *et al.*, 2022). Because the disease's above-ground symptoms and indicators, such as incipient wilt, stunting, and low yields, are like those of nutrient insufficiency, many producers are really unaware of root-knot nematodes (Loan *et al.*, 2018).

Numerous nematicides and soil fumigants have been used extensively in the past several decades to manage the root-knot nematode disease, but these extremely toxic pesticides have had major negative effects on the environment, human health, and the development of nematode multi-drug resistance (Rajasekharan *et al.*, 2020).

To manage plant parasitic nematodes, biological control will become more and more crucial (Zhou *et al.,* 2021). The application of living organisms and their metabolites can potentially control root-knot nematodes and found to be a promising biocontrol manner (Loan *et al.,* 2018). Among these microorganisms, endophytes are microbes that colonize plant tissues and coexist with plant cells in a symbiotic relationship and cause immunological reactions in the plants that enable them to endure various biotic and abiotic challenges. In this relationship, plants produce many compounds such as proteins, secondary metabolites, and hormones in response to the presence of endophytes, which in turn participate in the process of resistance to infection with pathogens (Kumar *et al.,* 2021). Endophytic actinobacteria are effective biocontrol agents that widely spread in different types of soil, they can control *M. incognita* via many mechanisms such as producing various secondary metabolites and enzymes, as well as parasitism and other bio-controlling mechanisms (Kang *et al.,* 2022). The most prominent actinobacteria genus in the soil is *Streptomyces* sp. (Panneerselvam *et al.,* 2021), it produces a huge number of volatile organic compounds (VOCs) which protect the plant against various pathogens either directly or indirectly through fumigation. Furthermore, the production of volatile organic compounds plays an important role in both antagonism and parasitism between soil microbes (de Brito *et al.,* 2022).

Induced systemic resistance (ISR), systemic acquired resistance (SAR), and microbe-associated molecular pattern (MAMP)-triggered immunity are common examples of the priming process in plants. Defense responses against phytopathogenic are swiftly initiated by ISR, which is mediated by phytohormones like ethylene (ET) and jasmonic acid (JA). Unlike ISR, which uses salicylic acid (SA) as a signal substance of the plant resistance pathways, SAR-triggered immunity is produced as the first line of defense. The role of phytohormones in improving biotic stress tolerance and defense response in plants subjected to various stresses (Hanaka *et al.,* 2021).

This study aimed to evaluate a previously recognized endophytic actinobacterium, *Streptomyces enissocaesilis* OM182843, as an environmentally friendly biological control agent against *M. incognita*. The role of metabolites produced by this strain, particularly volatile organic compounds (VOCs) and extracellular hydrolysis enzymes as well as plant growth regulators has been evaluated.

MATERIALS AND METHODS

1.Sources of Actinobacteria:

In the current study, *Streptomyces enissocaesilis* OM182843, an endophytic actinobacterium (EA) previously isolated from healthy cucumber plants, was used (El-Akshar *et al.,* 2022). *Strep. enissocaesilis* was grown on the International *Streptomyces* project (ISP-4) medium (Shirling and Gottlieb, 1966) at 35±2°C for 7 days, then kept at 4°C for all subsequent experiments.

2.Sources of Nematode:

The larvae of the second stage juvenile (J2) of root-knot nematode (*Meloidogyne incognita*) utilized in this study were given by Egypt's Syngenta Company's (https://www.syngenta.com.eg/), Department of Nematology. Free eggs of root-knot nematode (*M. incognita*) were extracted from infected cucumber roots using the 0.5% NaOCl procedure (El-Ashry *et al*. 2019) and purified using two different size sieves (60 and 500 mesh). The J2s inoculum was prepared by incubating the collected egg masses in sterile distilled water at 25°C for 5 days. Then, the hatched juveniles were collected daily for a week, counted, and considered as the nematode inoculum for *in vitro* assays (Martinuz *et al.,* 2015). The hatched juveniles were continuously propagated on the susceptible cucumber cultivar Furore, grown in a large container with sandy loam soil in a greenhouse.

3.Mortality Percentage of *M. incognita* (J2s) and Egg-Hatching as Affected by *Strep. enissocaesilis* :

3.1. Effects of *Strep. enissocaesilis* Culture:

One ml of *Strep. enissocaesilis* liquid culture (7 days) and 1.0 ml of *M. incognita* suspension (the previously prepared J2s inoculum) were transferred to sterilized 5 ml-capacity cups (1000±50 juveniles/cup) (Kim and Riggs, 1991). Sterilized distilled water instead of *Strep. enissocaesilis* liquid cultures served as a control. Both treatment and control were replicated three. After that, all prepared cups were loosely covered (to allow slightly permitted aeration and reduced evaporation) and incubated at 30°C, then examined after different intervals (24, 48 and 72) hours.

Juveniles who exhibited no movement and attained the shape of a straight line were considered dead. Dead nematodes were counted and recorded using a 1.0 ml nematode counting slide (Chalex, USA) to calculate the mortality percentage and egg-hatching as described by (AbdelRazek *et al.*, 2020):

$$\text{Inhibition of egg} - \text{hatching}(\%) = \frac{\text{Control} \times \text{Treatment}}{\text{Control}} \times 100$$

$$\text{Mortality } (\%) = \frac{\text{Dead juveniles}}{\text{Total number of juveniles}} \times 100$$

$$\text{Juvenile mortality } (\%) = \frac{\text{T} - \text{C}}{100 - \text{C}} \times 100$$

Where, T: % of nematode mortality in the treatment, C: % of nematode mortality in the control.

3.2. Effects of Crude Culture Filtrate (CCF) of *Strep. Enissocaesilis*:

Both of the previously prepared J2s inoculum and CCF of *Strep. enissocaesilis* were used in this experiment. Like the effect of *Strep. enissocaesilis* culture, 1.0 ml of CCF in addition to 1.0 ml of nematode suspension (1000±50 juveniles) were transmitted to a sterilized cup, then incubated at 30°C and examined for dead nematodes after 24, 48 and 72 h. Each treatment was repeated three times. As previously mentioned, J2 mortality and egg hatching inhibition percentage were examined.

4.Production of Degrading Enzymes:

4.1. Chitinase Activity:

Strep. enissocaesilis was cultured on a colloidal chitin broth medium (Joe and Sarojini, 2017) and incubated for 7 days at 35±2°C under shaking (150 rpm). Then, the culture was filtered by Whatman No.1 filter paper and the filtrate was centrifuged at 10000 rpm for 20 min. To estimate chitinase, 1.0 ml of 1.0% colloidal chitin prepared in 0.05*M* citrate phosphate buffer (pH 6.6) was transferred to a clean test tube, and 1.0 ml of the EA supernatant was added, then the mixture was incubated under shaking (180 rpm) at 37°C. After 60 min, the tubes were immersed in boiling water for 5 min to stop the reaction, then allowing them to cool (about 25-30°C). The released reducing sugar N-acetylglucosamine (NAG) was spectrophotometrically estimated (540 nm) using 3,5-dinitrosalicylic acid (DNS) reagent. Chitinase activity (U), was calculated as the quantity of crude enzyme solution required to release 1.0 mol of NAG/h/37°C (Sadeghi *et al.*, 2012).

4.2. Protease Activity:

The proteolytic activity was assayed using casein (Hi Media, India) as a substrate according to Ao *et al.* (2018). Firstly, *Strep. enissocaesilis* was cultured in starch casein liquid medium containing (gL^{-1}): soluble starch (10.0), casein (0.3), potassium nitrate (2.0), magnesium sulfate heptahydrate (0.05), di-potassium hydrogen phosphate anhydrous (2.0), sodium chloride (2.0), calcium carbonate (0.02), ferrous sulfate heptahydrate (0.01); at 30°C in shaking incubator (150 rpm) for 7 days. Then, the culture was centrifuged at 10,000 rpm at 4°C for 20 min. 400µL casein solution {2% w/v in Tris-HCl buffer (50 m*M*; pH 7.2)} and 100µL of *Strep. enissocaesilis* supernatant was mixed in a glass tube and incubated at 30°C for 10 min in a water bath. Then, 2.0mL of trichloroacetic acid (24% w/v) was added and centrifuged at 14,000 rpm for 20 min. Finally,

3.0mL of NaOH (0.5*M*) and 0.5mL of Folin reagent were mixed with 0.5mL of the reaction mixture and left for 10 min., then read spectrophotometrically at 660 nm. One unit of protease activity (U) was defined as the amount of enzyme required to release trichloroacetic acid-soluble casein fragments, giving a blue color equivalent to 1.0 µmole of tyrosine per min under assay conditions.

4.3. Gelatinase Activity:

To reveal the ability of *Strep. enissocaesilis* to produce gelatinase, the gelatin agar medium containing (gL^{-1}): gelatin (40.0), meat extract (3.0), peptone (5.0), pH (7.0) was prepared in glass tubes at a rate of 4.0 mL/tube. *Strep. enissocaesilis* was inoculated into tubes and incubated at 30°C for 5 days, tubes without inoculation were used as control. Finally, gelatin hydrolysis was assessed by re-incubate all tubes in the fridge at 4ºC for 2 h. Then, make sure that the medium is still fluid, and that the gelatin didn't freeze under cooling conditions compared to control tubes (Minotto *et al.*, 2014).

5.Production of Secondary Volatile Metabolites:

GC-mass analysis of the produced secondary volatile metabolites by *Strep. enissocaesilis* was achieved at Central Laboratories Network, National Research Centre, Dokki, Cairo, Egypt (https://www.nrc.sci.eg/centrel-labs/) using a Thermo Scientific, Trace GC Ultra/ISQ Single Quadrupole MS, TG-5MS fused silica capillary column. For this experiment, *Strep. enissocaesilis* was cultured singly in 250 mL Erlenmeyer flasks containing 100mL of ISP-4 medium at 35℃ for 7 days under shaking (150 rpm). The secreted metabolites were extracted by ethyl acetate as described by Siddique et al. (2013). The culture was filtered through Whatman No. 41 filter paper and concentrated in a rotary evaporator at 45 °C, which was suspended in 200 mL of distilled water and successively partitioned with ethyl acetate (400 mL) in a separating funnel. Later on, the ethyl acetate fraction was evaporated. It was subjected to GC-MS analysis to identify volatile compounds. The distillate was diluted with ethyl acetate and the volume was reduced 100-fold prior to analysis. Tentative identification of the compounds was performed based on the comparison of their relative retention times and mass spectra with those of the NIST and WILLY library data of the GC/MS system.

6.Production of Plant Growth Regulators:

Erlenmeyer flasks (250-mL) containing 50 mL of ISP-4 broth medium amended with tryptophan (1.0 m*M*) (Rahal *et al.*, 2010) were inoculated with 1.0mL of spore suspensions of *Strep. enissocaesilis* and incubated for 7 days at 35°C±2 under shaking (150 rpm). Cell-free extract (CFE) was obtained by centrifugation at 10,000 rpm for 5 min under cooling, then kept at 4°C. Indole acetic acid (IAA) was estimated using Salkowski's reagent as described by (Gilickmann and Dessaux, 1995) and read at a spectrophotometer (535nm). Gibberellic acid (GA_3) was determined using a Folin-cicholate reagent according to (Patel *et al.*, 2015) and then read at a spectrophotometer (760nm). Proline (Pro) was estimated as described by (Theriappan *et al.*, 2011) and finally read at a spectrophotometer (520nm). Salicylic acid (SA) was assessed using the method described by (Lukkani and Reddy, 2014), then the absorbance was read spectrophotometrically (527nm).

RESULTS AND DISCUSSION

1.Mortality and Egg Hatching of *M. incognita* (J2s) as Affected by *Strep. Enissocaesilis*:

The suppression ability of *Strep. enissocaesilis* on the growth of second-stage juveniles J2 and hatching of *M. incognita* eggs was studied in vitro. Mortality (%) and inhibition of egg hatching after 24, 48 and 72 h were calculated. Results in (Fig. 1a) demonstrated that *Strep. enissocaesilis* culture and its CCF caused a great death rate compared to control, but the culture was superior to its filtrate over three intervals. The mortality increased gradually after 24h reaching the maximum rate after 72h in both culture and filtrate as well as in control. A severe lethal effect of *Strep. enissocaesilis* was observed after 24h, and more than 80%

of *M. incognita* juveniles were dead, this trend was true in juveniles treated with both culture and filtrate. Furthermore, the culture of *Strep. enissocaesilis* exhibited the highest mortality percentage of *M. incognita* J2 after 72 h at 97%. Based on these findings, it is possible to conclude that actinobacterial cultures outperformed cultural filtrates in terms of nematode *M. incognita* mortality. This may be due to the that the actinobacterial cells were able to produce nematocidal substances constantly in the media.

Both culture and CCF of *Strep. enissocaesilis* caused a significant decrease in the hatching rate of *M. incognita* eggs during all intervals (Fig. 1b). The inhibition of egg-hatching was gradually increased after 24h of exposure and reached its maximum ratio of 72h. The exposure of *M. incognita* egg masses to both culture and CCF of *Strep. enissocaesilis* inhibited the hatching by (85-95%) and (75-88%), respectively. Moreover, CFF was less effective in egg-hatching compared to culture. The obtained results are in harmony with those of Wang *et al.* (2021), who found that the bacterial culture has been shown to have little effect on nematode activity. *Streptomyces* sp. AE170020, an endophytic strain with significant nematocidal activity, successfully reduced the proliferation of nematodes (Kang *et al.* 2022), which confirmed the results of the current study. Moreover, the impact of VOCs generated by strain Pc-10 on different phases of the *M. incognita* life cycle (eggs, juveniles, and females) resulted in a reduction of up to 88 % in nematode egg hatching and juveniles (Pacheco *et al.*, 2022).

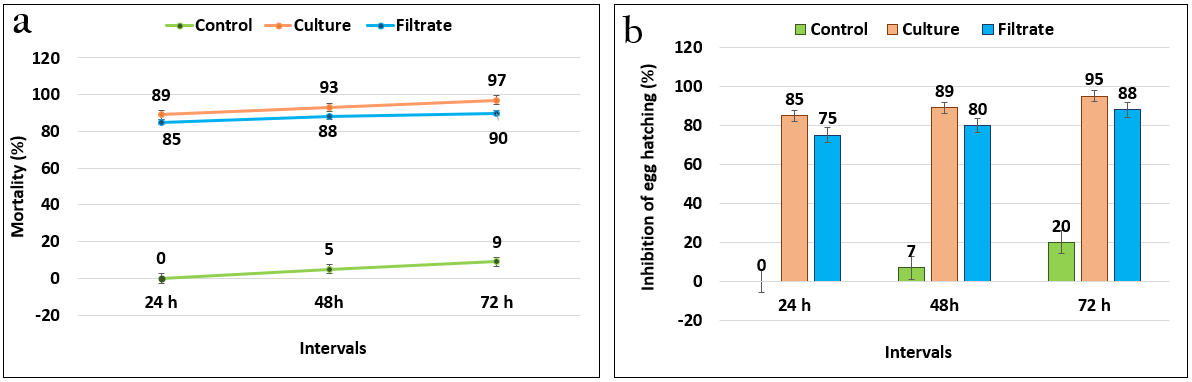


Fig. 1. Impacts of culture and crude culture filtrate of *Strep. enissocaesilis* on *M. incognita*; a) mortality of J2s (%), b) inhibition of egg hatching (%).

2.The Antinematicidal Activity of *Strep. Enissocaesilis*:

2.1. Degrading Enzymes:

Based on the results in (Fig. 2a and b), *Strep. enissocaesilis* was able to produce all estimated degrading enzymes (chitinase, protease and gelatinase) that are supposed to be responsible for the degradation of the nematode's wall and then subsequent death. *Strep. enissocaesilis* was highly producer of chitinase and proteases at a rate of 10.19 U/mL and 77.66 U/mL, respectively (Fig. 2a). Additionally, *Strep. enissocaesilis* was able to hydrolyze gelatin in the broth medium which indicates their ability to secrete gelatinase (Fig. 2b). As for the proteases and gelatinase enzymes, they are among the essential enzymes that degrade egg and nematode cell walls, thus enabling *Streptomyces* strains to inhibit their growth in addition to inhibiting egg hatching.

In a previous study done by Mitra *et al.* (2022), chitinase produced by actinobacteria has shown potential for the biocontrol activity of several phytopathogens, including nematodes. In addition, Alblooshi *et al.* (2022) found that *Streptomyces* sp. produced a set of chitinases and ß-1,3-glucanase to obtain nutrients through degradation of environmental chitin, including the cell wall of soil pathogen. Furthermore, many species of actinomycetes are able to produce a wide range of enzymes such as proteases as previously reported (Al-Agamy *et al.*, 2021; Janatinigrum and Lestari, 2022).

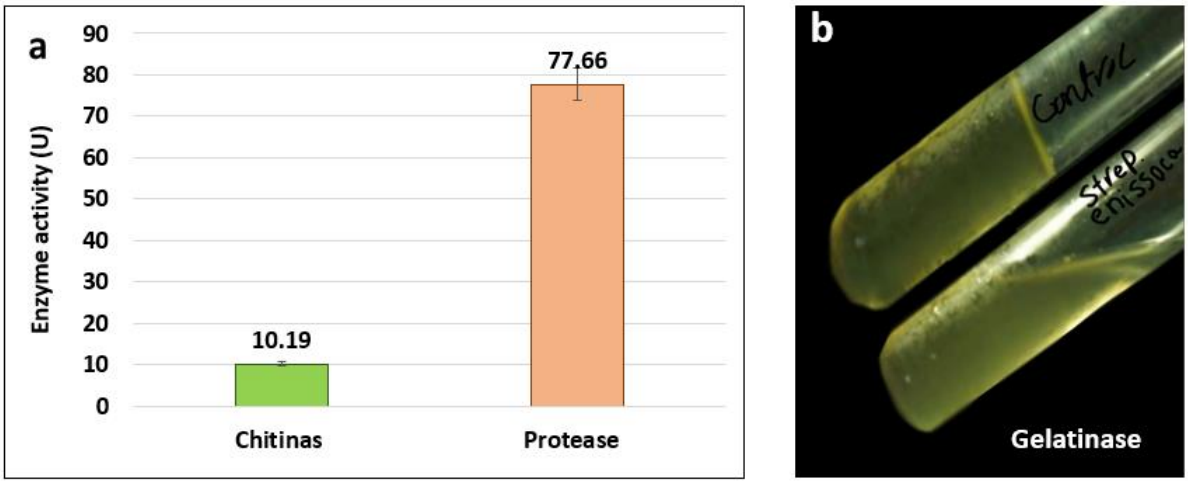


Fig. 2. Active compounds produced by *Strep. enissocaesilis;*
a) quantitative production of protease and chitinase,
b) b) qualitative assessment of gelatinase,

2.2. Volatile Organic Compounds (VOCs):

Analyses of the produced VOCs by *Strep. enissocaesilis* were done by GC-MS and their compositions are displayed in (Fig 3 and Table 1). produced many valuable metabolites that possess biological activities. GC-MS analysis revealed 10 volatile compounds in ethyl acetate extract, among them 2,4-Decadienal, (E, Z) (CAS) was the most abundant one, which was detected at a rate of 27.47% then 2-isopropyl-5-methyl-6-ox abicyclo[3.1].0] hexane-1-carboxaldehyde comes next with a 17.37% and has been recognized as antifungal and antioxidant compound as reported by (Ntalli *et al.*, 2021), and reported as antifungal and nematicidal by (Medaura *et al.*, 2021), while (Wu *et al.*, 2020) observed nematicidal activity of this compound. These results are in harmony with (Ayed *et al.*, 2021) who found that the analysis of produced VOC by *Streptomyces lydicus* revealed the presence of 14 compounds, including three major terpenoids: 3-carene-2,5-dione, geosmin, beta-cubebene, as well as one phenolic compound, Phenol, 2-(1,1-dimethylethyl)-6-methyl, accounted for 43, 20, 11, and 9.34% of total VOCs, respectively. Seven of the detected VOCs include di-methyl disulfide, 1-H-indene, 1-ethylideneociahydro-7a-methyl, 3-Cyclohexen-and hexane derivatives, 2-methyl-3-buten-2-ol, and 6-methyl-5-hepten-2-one were also detected by (Roy and Banerjee, 2019).

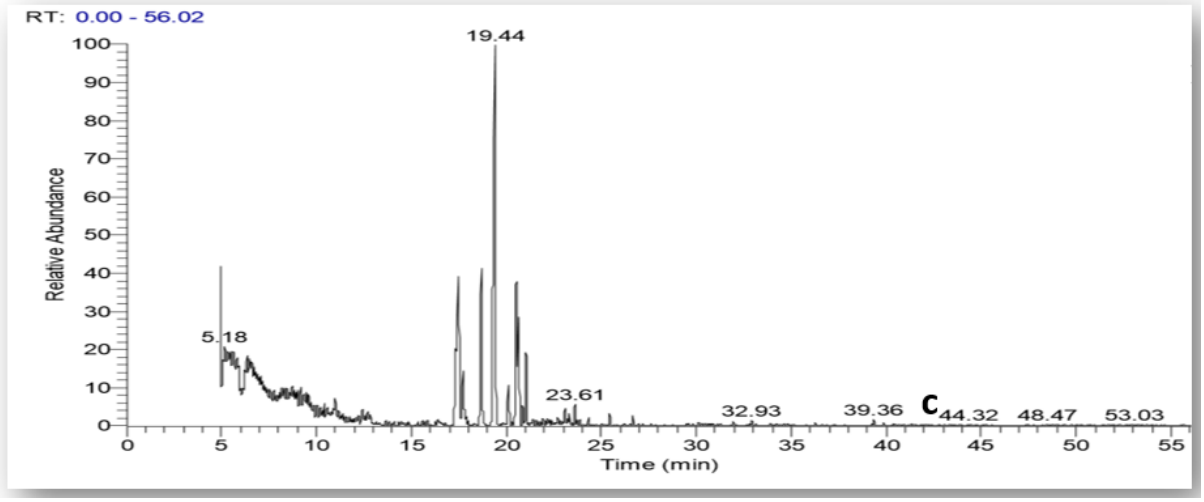


Fig. 3. GC-MS profile of volatile organic compounds.

Table 1. GC-mass analysis of secondary metabolites secreted by *Streptomyces enissocaesilis* and their activities.

Peak No.	R_t (min.)	Name	Formula	Area (%)	Activity	Reference
1	37. 88	3.85-2,2 Bis[4-[(4,6-dichloro1,3,5-triazin-2-yl) oxy] phenyl]-1,1,1,3,3,3-hexafluoropropane	C21H8Cl4F6N6O2	1.20	Antifungal-antibacterial	Wang *et al*., 2021
2	17. 50	2-Isopropyl-5-methyl-6-ox abicyclo[3.1.0]hexane-1-carboxaldehyde	C10H16O2	17.37	No activity found	-
3	17. 73	2-Decenal	C10H18O	2.77	Nematicidal	Deng *et al*., 2022
4	19. 43	2,4-Decadienal, (E,Z)(CAS)	C10H16O	27.47	Nematicidal- antioxidant	Ntalli *et al*., 2021
5	20. 12	1-Tridecanol (CAS)	C13H28O	2.41	Nematicidal	Díaz-Navarro *et al*., 2021
6	21. 04	Fumaric acid, decyl 3-methylbut-3-enyl ester	C19H32O4	3.52	Antifungal- nematicidal	Guo et al., 2021
7	38. 07	Dodecachloro-3,4-benzo phenanthrene	C18Cl12	7.03	Antifungal- nematicidal	Medaura *et al*., 2021
8	38 .15	14.11 Trans-9-octadece noic acid 1tms	C21H42O2SI	3.47	Antifungal-antibacterial	Espinosa-Salgado *et al*., 2022
9	26.38	Butylated Hydroxytoluene	C15H24O	5.78	Nematicidal	Wu *et al*., 2020
10	44. 99	Methyl 3,7 - dimethyl decanoate	C13H26O2	1.96	Nematicidal	Wagh *et al*., 2022

2.3. Plant Growth Regulation Ability by *Strep. Enissocaesilis:*

In this respect, data in (Fig. 4) indicated that *Strep. enissocaesilis* was able to produce all estimated compounds namely indole acetic acid (IAA), gibberellic acid (GA_3), salicylic acid (SA) and proline (Pro) at considerable amounts. These plant regulators frequently function as the primary signaling molecules that set off complex signaling cascades that stimulate physiological and morphological changes and ultimately result in tolerance or resistance to biotic and abiotic stresses (Nephali *et al.*, 2020). These results agree with those obtained by Ma *et al.* (2022) who have suggested that *Streptomyces* sp. TOR3209 exerts additional influence on tomato plants' ability to withstand biotic stress by controlling the auxins signaling system, stress-related metabolism, and photosynthesis. Additionally, (Verma *et al.*, 2021) found that the endophytic *Streptomyces geysiriensis* strain produces IAA and GA_3 which stimulate indigenous levels of plant hormones; thus, endophytes modulate developmental or signaling processes in plants. Moreover, numerous mechanisms of action for *Streptomyces* have been identified, including assisting plants in acquiring nutrients, creating growth regulators including (SA) and proline (Pro), and reducing levels of the stress hormone ethylene (Kour *et al.*, 2022). With the current advancement in microbial ecology, endophytic *Streptomyces* are gaining popularity. The plant's internal tissue serves as a unique habitat for a variety of endophytic microbes to thrive and provide essential nutrients (such as nitrogen, phosphorus, and potassium) and the synthesis of phytohormones like salicylic acid and gibberellins (Adeleke and Babalola, 2022).

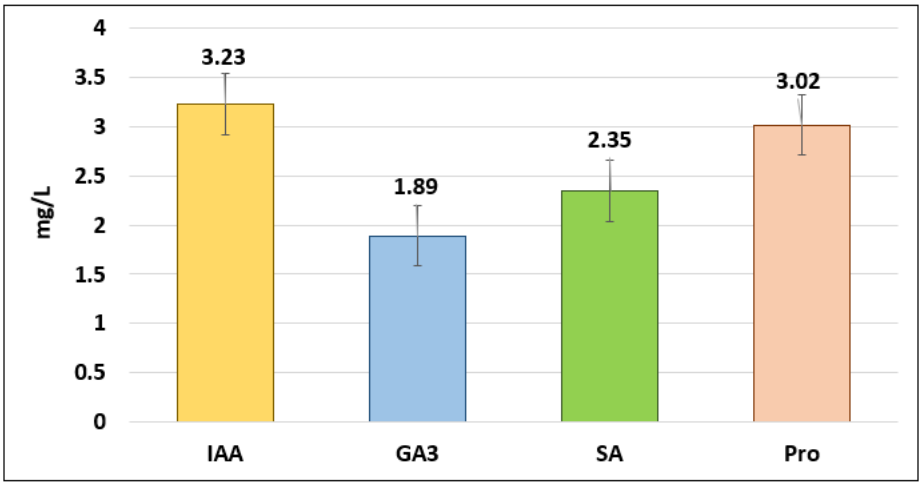


Fig. 4. Quantitative production of plant growth regulators by *Strep. enissocaesilis*

Conclusion

Streptomyces enissocaesilis OM182843, an endophytic actinobacterial strain, was able to create anti-nematode activities via the production of nematicidal compounds like cell wall-degrading enzymes as well as volatile organic compounds. Additionally, it was able to produce plant growth regulators namely indole acetic, gibberellic, and salicylic acids besides proline. In general, it can be concluded that *Strep. enissocaesilis* has the characteristics that qualify as a biological control agent for root-knot nematodes (*M. incognita*) and at the same time a plant growth stimulator. Therefore, we recommended the use of this endophytic strain to reduce nematode damage and increase plant resistance to stresses which ultimately leads to enhance plant health and increase productivity.

REFERENCES

AbdelRazek, G.M. and Yaseen, R. (2020). Effect of some rhizosphere bacteria on root-knot nematodes. *Egyptian Journal of Biological Pest control*, *30*(1), 1-11.

Adeleke, B.S. and Babalola, O.O. (2022). Meta-omics of endophytic microbes inagricultural biotechnology. *Biocatalysis and Agricultural Biotechnology*, 102332.

Al-Agamy, M.H.; Alhuzani, M.R.; Kelany, M.S. and Hamed, M.M. (2021). Production and partial characterization of α-amylase enzyme from marine actinomycetes. *BioMed Research International*, l, 1-15.

Alblooshi, A.A.; Purayil, G.P.; Saeed, E.E.; Ramadan, G.A.; Tariq, S.; Altaee, A.S. and AbuQamar, S.F. (2022). Biocontrol potential of endophytic actinobacteria against *Fusarium solani*, the causal agent of sudden decline syndrome on date palm in the UAE. *Journal of Fungi*, 8(1), 8.

Ao, X.L.; Yu, X.; Wu, D.T.; Li, C.; Zhang, T.; Liu, S.L. and Zou, L.K. (2018). Purification and characterization of neutral protease from *Aspergillus oryzae* Y1 isolated from naturally fermented broad beans. *AMB Express*, 8(1), 1-10.

Ayed, A.; Kalai-Grami, L.; Ben Slimene, I.; Chaouachi, M.; Mankai, H.; Karkouch, I. and Limam, F. (2021). Antifungal activity of volatile organic compounds from *Streptomyces* sp. strain S97 against *Botrytis cinerea*. *Biocontrol Science and Technology*, 31(12), 1330-1348.

de Brito, M.V.; Fonseca, W.L.; Mafezoli, J.; Barbosa, F. G.;Nunes, F.M.; de Mattos, M.C. and Oliveira, M.C. (2022). Biologically active volatile organic compounds (VOCs) produced by rhizospheric actinobacteria strains inhibit the growth of the phytopathogen *Colletotrichum musae*. *Journal of the Brazilian Chemical Society*, 1-18.

El-Akshar, E.A.; El-Meihy, R.M.; Tewfik, T.A.; Youssef, A.M. and Abou-Aly, H.E. (2022). Chitinolytic activity and antifungal potential of endophytic actinobacteria isolated from the cucumber plant. (In press).

El-Ashry, R.M.; El-Saadony, M.T.; El-Sobki, A E.; El-Tahan, A.M.; Al-Otaibi, S.; El-Shehawi, A.M and Elshaer, N. (2022). Biological silicon nanoparticles maximize the efficiency of nematicides against biotic stress induced by *Meloidogyne incognita* in eggplant. *Saudi Journal of Biological Sciences*, 29 (2), 920-932.

El-Ashry, R; Ali, A. and ElSobki, A. (2019). Nematicidal properties of three adjuvants for management of southern root-knot nematode, *Meloidogyne incognita* in vitro and under greenhouse conditions. *Journal of Plant Protection and Pathology*, 10(11), 511-519.

Glickmann, E. and Dessaux, Y. (1995). A critical examination of the

specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. *Applied and Environmental Microbiology*, 61(2), 793-796.

Hanaka, A.; Ozimek, E.; Reszczyńska, E.; Jaroszuk-Ściseł, J. and Stolarz, M. (2021). Plant tolerance to drought stress in the presence of supporting bacteria and fungi: An efficient strategy in horticulture. *Horticulturae*, 7(10), 390.

Joe, S. and Sarojini S.M. (2017). An efficient method of production of colloidal chitin for enumeration of chitinase producing bacteria. *Mapana Journal of Sciences*, 4(16), 37-45.

Kang, M. K.; Kim, J.H.; Liu, M.J.; Jin, C. Z.; Park, D.J.; Kim, J. and Son, K.H. (2022). New discovery on the nematode activity of *aureothin* and *alloaureothin* isolated from endophytic bacteria *Streptomyces* sp. AE170020. *Scientific Reports*, 12 (1), 1-16.

Kim, D.G. and Riggs, R.D. (1991). Characteristics and efficacy of a sterile hyphomycete (ARF 18), a new biocontrol agent for *Heterodera glycines* and other nematodes. *Journal of Nematology*, 23, 275-282.

Kour, D.; Khan, S.S.; Kaur, T.; Kour, H.; Singh, G.; Yadav, A. and Yadav, A.N. (2022). Drought adaptive microbes as bioinoculants for the horticultural crops. *Heliyon*,8(5), 2405-8440.

Kumar, M.; Kumar, P.; Das, P.; Solanki, R. and Kapur, M.K. (2021). Proactive role of *Streptomyces* spp. in plant growth stimulation and management of chemical pesticides and fertilizers. *International Journal of Environmental Science and Technology*, 1-20.

Loan, T.T.N.; Ja, Y.J.; Tae, Y.K.; Nan, H.Y.; Ae, R.P.; Seungki, L.; Chang-Hwan, B., Joo; H.Y.; Jae-Seoun, H.; Hae, W.P. and Jin-Cheo, K., (2018). Nematicidal activity of verrucarin A and roridin A isolated from *Myrothecium verrucaria* against *Meloidogyne incognita*. *Pesticide Biochemistry and Physiology*, 148, 133-143.

Lu, C., Meng, Y., Wang, Y., Zhang, T., Yang, G., Mo, M., Ji, K., Liang, L., Zou, C. and Zhang, K. (2022). Survival and infectivity of second-stage root-knot nematode *Meloidogyne incognita* juveniles depend on lysosome-mediated lipolysis. *Journal of Biological Chemistry*, 298(3), 101637.

Lukkani, N.J. and Reddy, E.S. (2014). Evaluation of plant growth promoting attributes and biocontrol potential of native fluorescent *Pseudomonas* spp. against *Aspergillus niger* causing collar rot of ground nut. *International Journal of Plant, Animal and Environmental Sciences*, 4(4), 256-262.

Ma, J.; Peng, J.; Tian, S.; He, Y.; Zhang, C.; Jia, N. and Hu, D. (2022). *Streptomyces* sp. TOR3209 alleviates cold stress in tomato plants. *New Zealand Journal of Crop and Horticultural Science*, pp, 1-21.

Martinuz, A.; Zewdu, G.; Ludwig, N.; Grundler, F.; Sikora, R.A. and Schouten, A. (2015). The application of *Arabidopsis thaliana* in studying tripartite interactions among plants, beneficial fungal endophytes and biotrophic plant-parasitic nematodes. *Planta*, 241(4), 10-25.

Medaura, M.C.; Guivernau, M.; Moreno-Ventas, X.; Prenafeta-Boldú, F.X. and Viñas, M. (2021). Bioaugmentation of native fungi, an efficient strategy for the bioremediation of an aged industrially polluted soil with heavy hydrocarbons. *Frontiers in Microbiology*, 12, 713.

Minotto, E.; Milagre, L.P.; Oliveira, M.T. and Van Der Sand, S.T. (2014). Enzyme characterization of

endophytic actinobacteria isolated from tomato plants. *Journal of Advanced Scientific Research*, 5(2), 16-23.

Mitra, D., Mondal, R.; Khoshru, B.; Senapati, A.; Radha, T.K., Mahakur, B. and Mohapatra, P.K.D. (2022). Actinobacteria-enhanced plant growth, nutrient acquisition, and crop protection: Advances in soil, plant, and microbial multifactorial interactions. *Pedosphere*, 32(1), 149-170.

Nephali, L.; Piater, L.A.; Dubery, I.A.; Patterson, V.; Huyser, J.; Burgess, K. and Tugizimana, F. (2020). Biostimulants for plant growth and mitigation of abiotic stresses: A metabolomics perspective. *Metabolites*, 10, 505.

Ntalli, N.; Skourti, A.; Nika, E.P.; Boukouvala, M.C. and Kavallieratos, N.G. (2021). Five natural compounds of botanical origin as wheat protectants against adults and larvae of *Tenebrio molitor* L. and *Trogoderma granarium* Everts. *Environmental Science and Pollution Research*, 28(31), 42763-42775.

Pacheco, P.V.M.; Campos, V.P.; Terra, W.C., Pedroso, M.P.; de Paula, L.L.; da Silva, M.S.G. and de Freitas, L.G. (2022). Attraction and toxicity: Ways volatile organic compounds released by *Pochonia chlamydosporia* affect *Meloidogyne incognita*. *Microbiological Researc h*, 255, 126925.

Panneerselvam, P.; Selvakumar, G.; Ganeshamurthy, A.N.; Mitra, D., and Senapati, A. (2021). Enhancing pomegranate (*Punica granatum* L.) plant health through the intervention of a *Streptomyces* consortium. *BiocontrolScience and Technology*, 31(4), 430-442.

Patel, P.; Cummings, R. and Roberts, B. (2015). Exploring the influence of the global fund and the gavi alliance on health systems in conflict affected countries. *Conflict Health*, 9, 7-11.

Rahal, A. Gh.; Zaghloul, R.A.; Neweigy, N.A.; Hanafy, E.A. and El-Meihy, R.M. (2010). Optimal environmental conditions for production of plant growth regulators by rhizobacteria. *Egyptian Journal of Microbiology (special issue)*, pp, 33-44.

Rajasekharan, S.K.; Kim, S.; Kim, J. and Lee, J., (2020). Nematicidal activity of 5-iodoindole against root-knot nematodes. *Pesticide Biochemistry and Physiology*, 163, 76-83.

Roy, S. and Banerjee, D. (2019). Volatile organic compounds from endophytic fungi. In: Yadav, (eds) Recent Advancement in White Biotechnology Through Fungi (pp, 149-175). Springer, Cham.

Sadeghi, A.; Karimi, E.; Dahaji, P.A.; Javid, M.G.; Dalvand, Y. and Askari, H. (2012). Plant growth promoting activity of an auxin and siderophore producing isolate of *Streptomyces* under saline soil conditions. *World Journal of Microbiology and Biotechnology*, 28(4), 1503-1509.

Shirling, E.B. and Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *International Journal of Systematic Bacteriology*, 16, 313-340.

Siddique, Y.H.; Mujtaba, S.F.; Jyoti, S. and Naz, F. (2013). GC–MS analysis of Eucalyptus citriodora leaf extract and its role on the dietary supplementation in transgenic Drosophila model of Parkinson's disease. *Food and Chemical Toxicology*, 55, 29-35.

Theriappan, P.; Gupta, A.K. and Dhasarrathan, P. (2011). Accumulation of proline under salinity and heavy metal stress in cauliflower seedlings. *Journal of Applied Sciences and Environmental Management*, 15(2), 251-255.

Verma, H.; Kumar, D.; Kumar, V.; Kumari, M.; Singh, S.K.; Sharma, V.K. and

Kumar, A. (2021). The potential application of endophytes in management of stress from drought and salinity in crop plants. *Microorganisms,* 9(8), 1729.

Wagh, P.R.; Sonkamble, V.V. and Wagh, N.S. (2022). Bioprospecting of endophytes: Recent advances in endophytic microbes for industrially important bioactive compounds. In: Verma, P. and Shah, M.P. (Eds.) Bioprospecting of Microbial Diversity (Chapter 7). Elsevier, ISBN: 978-0-323-90958-7, pp, 145-183.

Wang, J.Y.; Guo, C.;Zhao, P.; Yu, F.Y.; Su, Y.; Qu, J.P. and Zhou, B. (2021). Biocontrol potential of *Bacillus altitudinis* AMCC1040 against root-knot nematode disease of ginger and its impact on rhizosphere microbial community. *Biological Control*, 158, 104598.

Wu, F.; Tong, X.; Zhang, L.; Mei, L.; Guo, Y. and Wang, Y. (2020). Suppression of *Rhizopus* fruit rot by volatile organic compounds produced by *Paenibacillus polymyxa* CF05. *Biocontrol Science and Technology*, 30(12), 1351-1364.

Zhou, Y.; Chen, J., Zhu, X.; Wang, Y.; Liu, X.; Fan, H. and Chen, L. (2021). Efficacy of *Bacillus megaterium* strain Sneb207 against soybean cyst nematode (*Heterodera glycines*) in soybean. *Pest Management Science*, 77(1), 568-576.