**Evaluation the effectiveness of antagonistic endophytic fungi isolated from Taify rose on root-knot nematode, *Meloidogyne javanica***

**Alaa Baazeem1, Mohammed Al-Arabi2, Hadeer Darwesh2,3, Saqer S. Alotaibi2, Ahmed Nour El-Deen1,4**

1 Department of Biology, College of Science, Taif University, P.O.BOX 11099, Taif 21944, Saudi Arabia, [**aabaazeem@tu.edu.sa**](mailto:aabaazeem@tu.edu.sa)

**2** Department of Biotechnology, College of Science, Taif University, P.O.BOX 11099, Taif 21944, Saudi Arabia, [**saqer@tu.edu.sa**](mailto:saqer@tu.edu.sa)**,** [**maalorabi@hotmail.com**](mailto:maalorabi@hotmail.com)

3 Medicinal and Aromatic Plants Department, Horticulture Institute, Agriculture Research Center, Egypt, [**hadeer.darwesh@yahoo.com**](mailto:hadeer.darwesh@yahoo.com)

4 Agricultural Zoology Department, Faculty of Agriculture, Mansoura University, Egypt, [**ahnoureldeen2003@yahoo.com**](mailto:ahnoureldeen2003@yahoo.com)

**ABSTRACT**

The effectiveness of three Taify rose-endophytic fungal filtrates at different concentrations on juveniles mortality and egg hatching of *Meloidogyne javanica* after various exposure times was evaluated *in vitro*. Results revealed that exposition to culture filtrate of *Penicillium citrinum* isolate MN518391 at 8 % for 24 h significantly gave the highest reduction in the numbers of viable *M. javanica* J2s and hatched eggs. The characteristic appearances of the nematode J2s incubated for 24 h in the highest concentration of the fungal filtrates clear that most of the nematodes exposed to *P. citrinum* characterized with straight body shape, implying reduction in viability, whereas, it mostly exhibited the bent shape after exposing either to *Aspergillus niger* MK713445 or *A. niger* MN513383 filtrates. GC-MS analysis of the three fungal filtrates detected 22 chemical constituents those may be responsible for nematicidal properties. Of which, 6,8-dibromo-2-(3-pyridyl)-4-phenyl-quinazoline compound was found in major amount, whereas, squalane was less predominant compound. Our study proved that the three endophytic fungi isolated from Taify rose could be used for the biological control of root-knot nematodes.

**KEYWORDS**

Biological control, Endophytic fungi, Root-knot nematode, *Meloidogyne javanica*, Taify rose

**INTRODUCTION**

The root-knot nematodes (RKNs), *Meloidogyne* spp. are the economically important pests found all over the world and have been reported on different plant species causing quality and yield crop losses up to 80% in susceptible vegetable plants ([Kaskavalci, 2007](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6097824/" \l "b20-ppj-34-308)). In Saudi Arabia, the RKNs are widely distributed throughout the country different agricultural areas with M. incognita and M. javanica reported as the most common existed species parasitizing roses (Al-Hazmi et al., 1983; Nour El-Deen et al., 2015). When plants are infested by *Meloidogyne* spp., the normal root system is reduced to a limited number of severely galled roots with a completely disorganized vascular system. Rootlets are almost completely absent. The roots are seriously hampered in uptake and transport of water and nutrients (Netscher and Sikora, 1990). Most of effective nematicides used for controlling RKN are very expensive and have negative environmental impacts. In the last decades, bio-control agents received greater attention for nematode management. Antagonistic fungi have been successfully used for nematode control without causing environmental hazards (Holgado and Crump, 2003; Chen and Dickson, 2004; Lopez-Liorca and Jansson, 2006; Mukhtar, 2018). Devi and Bora (2018) recorded that culture filtrates of *Trichoderma viride*, *T. harzianum*, *Trichoderma* sp., *Fusarium* sp., *Penicillium* sp. and *Aspergillus* sp. significantly induced larval mortality and egg hatching inhibition of *Meloidogyne incognita* race 2. Several compounds with nematicidal activity have been reported from fungi (Li et al., 2007; Anke, 2010), but no major commercial product based on these natural fungal compounds has been developed yet for wide use. Using fungal endophytes against phytonematodes has previously been studied (Zabalgogeazcoa, 2008). There is evidence that endophytes may affect nematodes either directly, by synthesizing nematicidal compounds that kill or paralyse nematodes, or indirectly by eliciting plant defense responses toward nematode (Schouten, 2016). The endophytic fungus, *Acremonium implicatum* isolated from galled tomato roots has excellent potential for *M. incognita* control (Tian et al., 2014). Yan et al. (2011) found that cucumber seed treatment with the endophytic fungi, *Chaetomium* Ch1001 had the highest potential against *M. incognita* infection. *Fusarium oxysporium* strain Fo162 synthesized a number of compounds that have nematicidal effect on *M. incognita* (Hu et al., 2013). To date the research on using endophytic fungi for managing plant parasitic nematode is still rare. Therefore, the current study aimed to evaluate the nematicidal activity of three endophytic fungi isolated from Taify rose against root-knot nematode, *Meloidogyne javanica* in laboratory.

**MATERIALS AND METHODS**

This investigation was carried out in the Biology Department, Faculty of Science., Taif University, Kingdom of Saudi Arabia.

**Fungal filtrate preparation:**

Three endophytic fungal strains *Aspergillus niger* MK713445, *A. niger* MN513383 and *Penicillium citrinum* MN518391 isolated from leaves of Taify rose and previously identified molecularly were cultured on PDA medium. Agar discs from the 10 d-old PDA cultures of three fungus isolates were inoculated into 250 ml Erlenmeyer ﬂasks containing 150 ml potato dextrose broth and shaken at 100 r/min at 28 °C for 10 d. The cultures were then filtered through Whatman filter paper (No. 1) and 0.22 μm Micropore filter to separate the liquid from the mycelia. The filtrate was stored at 4°C until used.

**Nematode culture:**

Nematode inocula were obtained from a pure culture established from a single egg-mass of *M. javanica* isolated from Taify rose roots that previously identified according to the characteristics of its perineal pattern (Taylor and Sasser 1978), maintained and propagated on a highly susceptible tomato plants c.v. Strain B in a greenhouse. Nematode eggs were extracted from infected tomato roots using 0.5 % NaOCl solution and shaking for 2 min, while second stage juveniles (J2s) were obtained by hatching method for 7 days (Hussey and Barker, 1973). The inocula suspension was then adjusted to 100 eggs or 50 J2s per ml.

**Bioassay:**

To test the in vitro toxicity of the fungal filtrates to eggs and J2s of *M. javanica*, 1 ml culture filtrates of each fungus isolate at three concentrations i.e. 2, 4 and 8 %; over 100 eggs or 50 J2s suspension in 1 ml sterilized distilled water were poured into 24-well tissue culture plates. A 2.0 ml of potato dextrose broth and suspension of eggs or J2s served as control. Five replicates were used for both treatment and control and the experiment was repeated once. The numbers of dead larva were recorded after 6, 12 and 24 h. J2s death were confirmed if their body appeared no realistic movement when touched with a fine needle. Mortality rates (M) were corrected using Abbott’s formula (Abbott 1925): , where Mt means mortality percentage in treatment and Mc means mortality percentage in control. The rate of *M. javanica* egg hatching inhibition was assessed under a light microscope at 10 days of exposure. Hatch inhibition (HI) was calculated according to the formula: where C and T are the percentages of eggs hatched in the control and treatment, respectively. Characteristic shapes of *M. javanica* J2s were examined under the stereomicroscope after 24 h exposure to the highest concentration of the three fungal filtrates or control (Unexposed treatment).

**Analysis of fungal filtrates using gas chromatography mass spectrometry (GC-MS):**

Identification of the chemical constituents of filtrates of fungal isolates was analyzed by Gas Chromatography-Mass Spectrometer (GC-MS), Agilent Model 7890A-5975B [Column, DB 5 ms, Agilent form (30 m x 250 µm x 0.25 µm)] in the Unit of Analytical Chemistry, Department of Chemistry, Faculty of Science, Assiut University. The column was initially maintained for 2 min at 40o C, and then the temperature was increased to 50o C at a rate of 4o C/min and held for 3 min, then increased to 150o C at a rate of 10o C/min and held for 3 min, then increased to 220o C at a rate of 10o C/min and held for 6 min, ﬁnally increased to 280o C at a rate of 15o C/min and held for 10 min. Helium (purity 99.999%) was used as the carrier gas with a ﬂow rate of 0.5 ml/min for 10.9 min, then 1 ml/min per min to 1 ml/min for 30 min. Neither internal nor external chemical standards were used in this chromatographic analysis. The mass spectrum data of each peak of the chromatogram were interpreted using a computerized library-searching program (Willey 9 and NIST library) for the identiﬁcation of the chemical constituents of fungal filtrates.

**Statistical analysis:**

Data were subjected to one-way ANOVA and the means of the treatments were compared by Duncan’s multiple range test (p < 0.05) using the COSTATE software package.

**RESULTS**

1. **Larval mortality:**

The fungal filtrates of *Aspergillus niger* MK713445, *A. niger* MN513383 and *Penicillium citrinum* MN518391 at concentrations of 8, 4 and 2 % in comparison with potato dextrose broth media on mortality percentage of newly hatched juveniles of *M. javanica* after 6, 12 and 24 h are shown in figure (1). Data revealed that larval mortality percentages increased with increase in fungal filtrates concentrations and exposure periods tested. Exposition to culture filtrate of *P. citrinum* at 8 % for 24 h significantly gave the highest reduction in the number of viable *M. javanica* J2s to be 51.67, followed by *A. niger* MN513383 at the same concentration and duration (45 %), whereas, J2s mortality reached 34.17 % when exposed to culture filtrate of *A. niger* MK713445 (Fig. 1C). As shown in Fig. 1A&B, the number of viable J2s after a 6 or 12-h exposure to 8 % *P. citrinum* filtrate did not differ significantly from the number of viable J2s present following a 6 or 12-h exposure to 8 % *A. niger* MN513383 according to two way ANOVA, since it gave 35.42 and 32.5 %, respectively after 6 h exposure; and 41.25 and 40 %, respectively following 12 h exposure. Incubation of J2s for 12 or 24 h in the presence of *P. citrinum* resulted in remarkable larval mortality with mean reductions of 31.28 % each at 4 % and 15.79 or 22.27 % at 2 %, respectively. Exposition of J2s for 12 or 24 h to middle concentration of *A. niger* MN513383 also resulted in the same mortality percentage (27.57 %). It was noticed that low concentration of both *A. niger* isolates did not act as good nematostatic, since it reduced the number of viable J2s by 6.43, 8.91 and 15.79 % or 4.02, 5.67 and 7.7 % after 6, 12 and 24 h exposing to *A. niger* MN513383 or MK713445 isolates, respectively.

1. **Egg hatching:**

The susceptibility of *M. javanica* eggs in relation to hatching following exposure to fungal filtrates was demonstrated in figure (2). Likewise, using higher concentrations of the three tested fungi resulted in significantly higher egg hatching inhibition percentages. Moreover, similar trend concerning *P. citrinum* was recorded, since it significantly ranked first in inhibiting egg hatchability that was 89.27 and 86.49 % for concentrations of 8 and 4 %, respectively. However, exposing *M. javanica* eggs to *A. niger* MN513383 filtrate reduced hatching by 84.41 and 81.05 %, respectively at the same concentrations. No significant difference in the number of hatched eggs was recorded between *P. citrinum* and *A. niger* MN513383 at 2 %. The percentages of hatching inhibition of *M. javanica* eggs, which were exposed to *A. niger* MK713445 at 8, 4 and 2 % were considerable less than those of eggs under *P. citrinum* and *A. niger* MN513383 conditions (73.28, 69.35 and 53.8 %, respectively).

1. **Characterization of J2s:**

Visual examination of nematodes clear that most of the J2s in control treatment were mobile and retained the sigmoidal (∑-shape) characteristic typical for viable nematodes (Fig 3A). In contrast, a clear reduction in the number of viable nematodes could be observed after exposition to *P. citrinum* culture filtrate, as characterized in higher portion with straight body shapes (Fig 3B). Moreover, after exposing either to *A. niger* MK713445 or *A. niger* MN513383 (Fig 3C and 3D), the nematodes mostly exhibited the typical bent body (banana-shape), implying reduction in viability.

1. **GC-MS analysis of active fungal filtrates:**

In this work, filtrates of the three target fungal isolates were subjected to GC-MS analysis. A total of 22 chemical compounds were found from the filtrates of the fungal isolates. The chemical composition of the filtrate varied from fungi to other with the same number of compounds was found in all tested isolates (10 compounds), however a few of them were predominant (Table 1). The results showed that the most bioactive compounds produced in major amounts by *A. niger* MN513383 was 6,8-dibromo-2-(3-pyridyl)-4-phenyl-quinazoline, hexadecanoic acid (Palmitic acid) and octadecane representing 64.895%, 7.437% and 6.108%, respectively. The components of *P. citrinum* MN518391also consisted of 6,8-dibromo-2-(3-pyridyl)-4-phenyl-quinazoline (65.334%), palmitic acid (7.973%) and pentadecane (6.370%) as major components. Similarly, 6,8-dibromo-2-(3-pyridyl)-4-phenyl-quinazoline (58.889%), acetic acid, piperidide (15.037%) and triacontane (6.081%) were abundantly present in the filtrate of *A. niger* MK713445. In fungal isolates, other compounds such as octadecane (1.875%), docosane (1.593%), phytane (1.397%) and squalane (1.088%), have been reported to be a common minor component. A small amount of 10-Methoxy-nb-alpha-methylcorynantheol was only detected in *A. niger* MN513383 (1.548%), whereas, tetracosane (1.856%) was detected only in small amounts by *P. citrinum* MN518391. On the other hand, 1,1,2,2-Tetrachloroethane (1.654%) was estimated only in *A. niger* MK713445 (Table 1).

**DISCUSSION**

Apparently, screening entophytic fungi isolated from Taify rose leaves for the potential to be used as a bio-agent against *M*. *javanica* was examined in laboratory. Fungi have been appeared to be the most important control agents for regulating nematode numbers in soil (Chen and Dickson, 2004). In the present investigation, *M*. *javanica* J2s mortality percentages significantly increased as the concentrations of fungal filtrates and exposure times tested increased in comparison with control treatment. Among all tested fungi, *P. citrinum* significantly reduced the number of viable J2s and egg hatching, indicating that nematicidal compounds were produced in the potato dextrose broth. This result was in accordance with ([Gotlieb](https://www.researchgate.net/scientific-contributions/22899076_Dror_Gotlieb?_sg%5B0%5D=qBw1AKagJrX18XCrDnfr2LshGpnaE9bBVzAEZozz39S9untPDbejMZO1HkNOMKH0zcJUg2k.E-PGBPKAYKMiTTD2OWCO23jJ_lIvVsmRSZZCnNNVHF1KEHBemalULFweNwz4zxgSaQ0NKP41AtO_vWndSJiCZg&_sg%5B1%5D=PeYYHUMpBNybrpKjEMo00th_9nGVnwGbIaZOtfdzTP4gc38VAuzrwhTviwOoI1CCqTis1ZU.DTbYoNrdlpTPueNe6vKhkSUJxFjzdERykcaWN1KyU_2vQ0FPWB76IEpidHpJgOe1FoOdVRYIEBopYhvu43gBNg) et al., 2003) who recorded that incorporation of dry mycelium of *P. chrysogenum* into soil enhanced cucumber and tomato plant growth and reduced root galling caused by *M. javanica*. Several fungi are known to regulate the nematode densities in soil by exhibiting a range of antagonistic activity including production of nematoxic compounds (Lopez-Llorca and Jansson, 2006). Many endophytes have been found to secrete specialized metabolites and complex glycoproteins (Miller et al., 1998; Bashyal et al., 1999; Woropong et al., 2001; Castillo et al., 2002; Ezra et al., 2004), and some endophytic fungi emit volatile organic compounds (VOCs) (McAfee and Taylor, 1999; Morath et al., 2012; Stinson et al, 2003; Strobel et al., 2011; Mari et al., 2012) that may be biologically active, for example, as a biofumigant for controlling postharvest disease (Suwannarach et al., 2013). *Muscodor albus* is an endophytic fungus that exhibits nematistatic and nematicidal properties against plant-parasitic nematodes (Riga et al., 2008; Grimme et al., 2007). Nematicidal VOCs were observed in the model yeast *Saccharomyces cerevisiae*: exposure of second-stage juveniles of *M. javanica* to a synthetic mixture of the yeast VOCs resulted in nematode mortality (Fialho et al., 2012). Similarly, VOCs produced by *Fusarium oxysporum* isolated from rhizospheres of coffee plants were nematicidal against *M. incognita* nematodes (Freire et al., 2012). No significant difference was recorded between high concentration of *A. niger* MK713445 isolate and median concentration of *A. niger* MN513383 isolate in the reduction of J2s viability after 6 and 12 h of exposure. Previous records reported that nematicidal toxins produced by the species of *A. niger* were found to beeffective against *Meloidogyne* while *Penicillium* produced toxins active against *Aphelenchoides composticola* (Cayrol *et al*., 1989; Grewal *et al*., 1989). At all exposition durations, lower and median concentrations of *A. niger* MK713445 had no significant effect on J2s viability. These results are disagreed with those recorded by (Jang et al., 2016) who mentioned that culture filtrate of *A. niger* F22 was highly active against *M. incognita* with remarkable mortality of J2s and inhibition of egg hatching. The result of the compound assay from the filtrates of fungal isolates using GC-MS shows that there were several compounds contained in the filtrates. Based on the identification result using GC-MS, the characterized compounds were belonged to alkane hydrocarbon, fatty acid, alkaloid, aliphatic hydrocarbon, aromatic hydrocarbon, [heterocyclic](https://en.wikipedia.org/wiki/Heterocyclic_compound) [amine](https://en.wikipedia.org/wiki/Amine), carboxylic acid, chlorinated hydrocarbon and ketones groups. It is well known that a number of heterocyclic compounds containing nitrogen as quinazolinone derivatives exhibited a wide variety of biological activity. In the present study, high amounts of 6,8-dibromo-2-(3-pyridyl)-4-phenyl-quinazoline were detected in all tested fungal isolates. The highest percentage of this bioactive compound was recorded from *P. citrinum* MN518391 which had the best nematicidal properties in our study. These results were in agreement with Zheng et al. (2012) who mentioned that quinazoline alkaloid produced by endophytic fungus, *Penicillium vinaceum* which isolated from the corm of *Crocus sativus* exhibited potential anticariogenic and antifungal activities. El-Gazzar et al. (2009) recorded that the quinazolinone derivatives have emerged as antimicrobial agents of an immense interest because of their broad spectrum of in vitro and in vivo chemotherapeutic activities. Even though major components are usually responsible for the nematicidal activity of fungal filtrates, the effect of minor ones cannot be neglected. Results express the presence of minor bioactive compounds which had been detected in fungal filtrates during GC–MS analysis. Out of which, fatty acids *viz.* hexadecanoic (palmitic) acid was identiﬁed in *P. citrinum* MN518391 and *A. niger* MN513383, dodecanoic acid in *A. niger* MN513383 and palmitinic acid in *A. niger* MK713445. Bardhan et al. (2019) stated that the predominant fatty acids detected from *P. citrinum* isolate PKB20 were oleic acid (30.09%), palmitic acid (20.25%) and linoleic acid (33.14%). Likewise, hexadecenoic acid and hexadecanoic acid was the major compounds those detected from hexane and ethyl acetate fractions of the rhizobacteria, *Pseudomonas jessenii* strain R62 and *P. synxantha* strain R81 and may be responsible for the nematicidal activity (Sharma et al. 2018). Our results were also confirmed with the record of Zhang et al. (2012) who reported that butyric, caprylic, capric, lauric, myristic, palmitic, and oleic acids showed the nematicidal action differently, among which capric acid exhibited a strong nematicidal effect and might be a powerful active substance for integrated *M. incognita* management. Elsewhere, Oliveira et al. (2009) signaled that palmitic acid are known to be very toxic to different nematode species. Triacontane and dotriacontane are the long-chain alkanes were present as detectable content in the tested fungal isolates *A. niger* MK713445 and *P. citrinum* MN518391, respectively. Triacontane compound has recognized antibacterial and antifungal activities (Bordoloi et al. 2017). Small content of 1,1,2,2-Tetrachloroethane was present in the filtrate of *A. niger* MK713445*.* This compound is known as chlorinated hydrocarbon and exhibited a pronounced nematicidal activity on intestinal nematode parasites. A single dose of 0.12 ml per kg (maximum of 5.0 ml) cured 80% of *Necator americanus* and 25 % of *Ancylostoma duodenale* infections and may stimulate ascarids to migration (Marsden and Hoskins 1966). Another compound with minor content was 4-Oxopentanoic acid, p-tolylsulfonylhydrazone, ethyl ester that had been recovered from *A. niger* isolate MK713445 and classified as a keto acid. Shemshura et al. (2016) reported that antagonism of the fungus *A. candidus* against *M. incognita* is primarily due to the production of two major compounds that have been identified as citric acid (Compound 1) and 1,2- dimethyl citrate (3-hydroxy-5-methoxy-3-(methoxycarbonyl)-5-oxopentanoic acid) (Compound 2). When compound 1 and a citric acid standard tested at 50 mg mL−1 in water, hatchability of *M. incognita* eggs was decreased by more than 94%, and completely immobilized second-stage juveniles after 4–6 days exposure. Hawranik and Sorensen (2010) characterized 1,3-dimethyl citrate from *A. niger* and noted that it is also a constituent of numerous higher plants. The phenotypes of *M. javanica* J2s either exposed or unexposed to fungal filtrates was an interesting finding that might be useful for analyzing the major mode of toxic action of these bio-control agents. The present result indicated that the nematodes exposed to potato dextrose broth (Control) was viable and retained sigmoid (∑-shape); whereas, the nematodes treated with fungal filtrates mostly appeared paralyzed or dead and followed straight or bent shapes, similar to those killed by the pyrethroid that has an effect on the central nervous system of the nematodes. This finding is in accordance with those reported by Wiratno et al. (2009) and Nour El-deen and Issa (2016) who mentioned that the shapes of the dead nematodes differed in a characteristic way, and groups of pesticides or bio-agents could clearly be distinguished based on this phenomenon.

**CONCLUSION**

The results of the current study represented examples of endophytic fungi with nematicidal properties and their modes of action which introduced reliable base for promising nematode bio-control agent. Further studies are necessary for isolation the endophytic fungi from another plant hosts and detection the secondary products those have nematicidal activity under greenhouse and field conditions.

**ETHICS STATEMENTS**

**Not applicable**

**FIGURES AND TABLES**

***P. citrinum* MN518391 *A. niger* MK713445 *A. niger* MN513383**

**A**

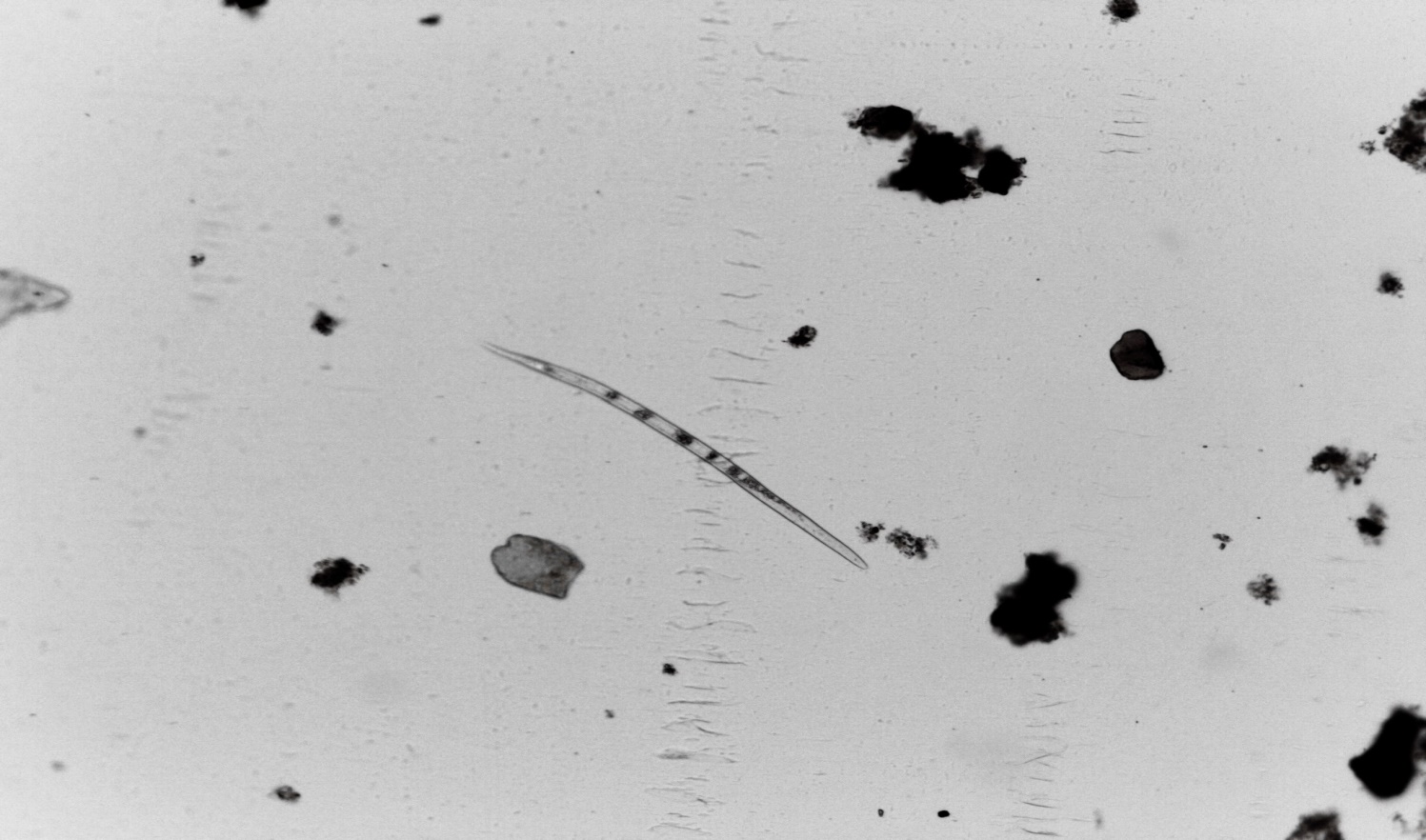
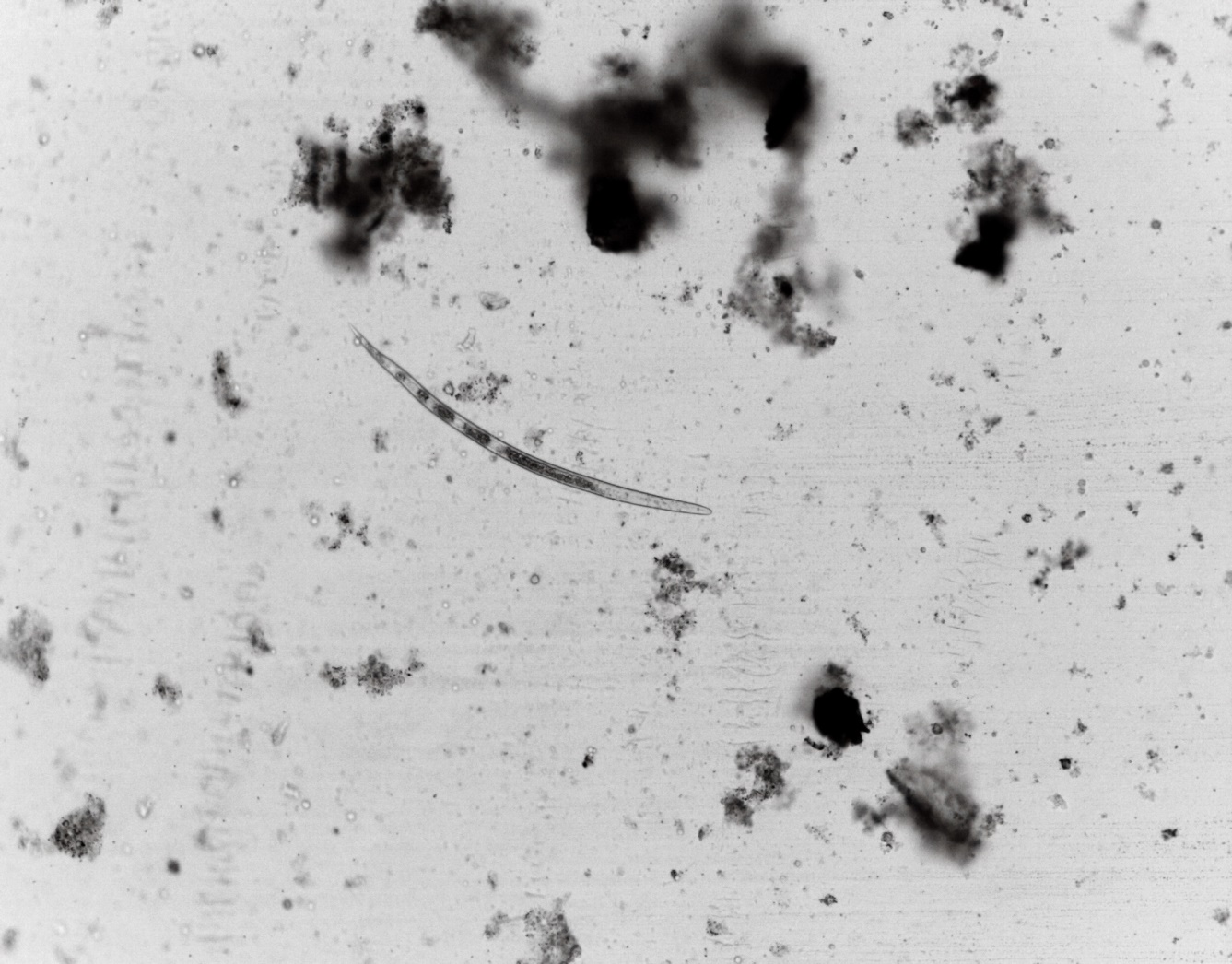
**% Larval mortality**

Sterile mycelium

**Fig. (1): Effect of endophytic fungi culture filtrates on *M*. *javanica* J2s mortality**

***P. citrinum* MN518391 *A. niger* MK713445 *A. niger* MN513383**

**Fig. (2): Effect of endophytic fungi culture filtrates on *M*. *javanica* egg hatching**



B

A

D

C

**Fig. (3): Characteristic shapes of *M*. *javanica* J2s following exposure to: A. Potato dextrose broth (Control)-sigmoid (∑-shape), B. *P. citrinum* MN518391-straight (I shape), C. and D. *A. niger* MK713445 and *A. niger* MN513383-bent (banana-shape).**

**Table 1. GC-MS analysis of the active components (in % Relative Content) in filtrates of fungal isolates**

|  |  |  |  |
| --- | --- | --- | --- |
| **Active compounds** | ***A. niger* MN513383** | ***P. citrinum* MN518391** | ***A. niger* MK713445** |
| Octadecane | 6.108 | 1.875 | -- |
| 11-Butyldocosane | 4.333 | -- | -- |
| Heptacosane | 3.933 | -- | -- |
| Squalane | 1.088 | 4.921 | -- |
| Dodecanoic acid | 4.704 | -- | -- |
| Hexadecanoic acid (Palmitic acid) | 7.437 | 7.973 | -- |
| 10-Methoxy-nb-alpha-methylcorynantheol | 1.548 | -- | -- |
| 3-Methyl-2-butenoic acid,  2,7-dimethyloct-7-en-5-yn-4-yl ester | 2.702 | -- | -- |
| Docosane | 3.251 | -- | 1.593 |
| 6,8-dibromo-2-(3-pyridyl)-4-phenyl-quinazoline | 64.895 | 65.334 | 58.889 |
| Boric acid, ethyl-, didecyl ester | -- | 2.494 | -- |
| Dotriacontane | -- | 2.715 | -- |
| Eicosane | -- | 5.065 | 3.442 |
| Pentadecane | -- | 6.370 | -- |
| Phytane | -- | 1.397 | 4.152 |
| Tetracosane | -- | 1.856 | -- |
| Triacontane | -- | -- | 6.081 |
| Acetic acid, piperidide | -- | -- | 15.037 |
| Palmitinic acid | -- | -- | 4.609 |
| 2-Methyl-,2-ethyl-2-[[(2-methyl-1-oxo-2-propenyl)oxy]methyl]-2-Propenoic acid | -- | -- | 2.462 |
| 1,1,2,2-Tetrachloroethane | -- | -- | 1.654 |
| 4-Oxopentanoic acid,  p-tolylsulfonylhydrazone, ethyl ester | -- | -- | 2.081 |

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**FUNDING**

The current work was funded by the deanship of the scientific research, Project number (1-439-6113), Taif university, Taif, Saudi Arabia.

**AUTHORS CONTRIBUTIONS**

Alaa Baazeem, Ahmed Nour El-Deen and Hadeer Darwesh carried out the research work. Saqer S. Alotaibi and Mohammed Al-Arabi carried out the modelling work. Alaa Baazeem and Ahmed Nour El-Deen drafted the manuscript. All authors contributed to the article and approved the submitted version.

**REFERENCES**

Abbott WS. (1925). A method of computing the effectiveness of and insecticide. J Am Mosq Control Assoc. 1987; 3: 302–303. PMID: 3333059

Al-Hazmi AS, Abl-Hayja ZM, Trabulsi IY. 1983. Plant parasitic nematodes in Al-Kharj area of Saudi Arabia. N ematol. Medit., 11: 207-212.

Anke, H. 2010. Insecticidal and nematicidal metabolites from fungi. In: *The Mycota: Industrial Applications*. 10: 2nd (Ed.): M. Hotrichter. Springer- Verlag, Berlin.

Bardhan P., Minakshi Gohain, Niran Daimary, Sumit Kishor, Pronobesh Chattopadhyay, Kuldeep Gupta, Chayanika Chaliha, Eeshan Kalita, Dhanapati Deka, Manabendra Mandal (2019). Microbial lipids from cellulolytic oleaginous fungus *Penicillium citrinum* PKB20 as a potential feedstock for biodiesel production. [Annals of Microbiology](https://link.springer.com/journal/13213) volume 69: 1135–1146.

Bashyal B, Li J, Strobel G, Hess W, Sidhu R. Seimatoantlerium nepalense, an endophytic taxol producing coelomycete from Himalayan yew (*Taxus wallachiana*). Mycotaxon. 1999; 72:33-42.

Bordoloi, M.; Sakia, S.; Bordoloi, P. K.; Kolita, B.; Dutta, P. P.; Bhuyan, P. D.; Dutta, S. C.; Rao, P. G. Isolation, characterization and antifungal activity of very long chain alkane derivatives from *Cinnamomum obtusifolium*, *Elaeocarpus lanceifolius* and *Baccaurea* *sapida*. *Journal of Molecular Structure*, **2017**, 1142.

Castillo UF, Strobel GA, Ford EJ, Hess WM, Porter H, Jensen JB, et al. Munumbicins, wide-spectrum antibiotics produced by Streptomyces NRRL 30562, endophytic on *Kennedia nigriscans*. Microbiology. 2002; 148(9):2675-85.

Cayrol, J.C., C. Djian and L. Pijarowski. 1989. Study of the nematicidal properties of the culture filtrate of the nematophagous fungus *Paecilomyces lilacinus*. *Revue-de-Nematologie*, 12(4): 331-336

Chen S.Y., D.W. Dickson. (2004). Biological control of nematodes by fungal antagonists. Z.X. Chen, S.Y. Chen, D.W. Dickson (Eds.), Nematology: advances and perspectives, vol II. Nematode management and utilization, Tsinghua University Press and CABI Publishing, Cambridge, MA (2004), pp. 343-403 979–1039

Devi G and L.C. Bora (2018). Effect of some biocontrol agents against root-knot nematode (*Meloidogyne incognita* race2). International Journal of Environment, Agriculture and Biotechnology, 3(5): 1748-1755.

El-Gazzar A.B.A., M.M. Youssef, AMS. Youssef, A.A. Abu-Hashem, F.A. Badria. Design and synthesis of azolopyrimidoquinolines, pyrimidoquinazolines as antioxidant, antiinflammatory and analgesic activities. Eur. J. Med. Chem., 44 (2009), pp. 609-624.

Ezra D, Castillo UF, Strobel GA, Hess WM, Porter H, Jensen JB, et al. Coronamycins, peptide antibiotics produced by a verticillate Streptomyces sp.(MSU-2110) endophytic on Monstera sp. Microbiology. 2004; 150(4):785-93.

Fialho MB, Bessi R, Inomoto MM, Pascholati SF. Nematicidal effect of volatile organic compounds (VOCs) on the plant-parasitic nematode *Meloidogyne javanica*. Summa Phytopathologica. 2012; 38 (2):152-154.

Freire E, Campos V, Pinho R, Oliveira D, Faria M, Pohlit A, et al. Volatile substances produced by *Fusarium oxysporum* from coffee rhizosphere and other microbes affect *Meloidogyne incognita* and *Arthrobotrys conoides*. Journal of nematology. 2012; 44(4):321. PMID: 23482720

[Gotlieb](https://www.researchgate.net/scientific-contributions/22899076_Dror_Gotlieb?_sg%5B0%5D=qBw1AKagJrX18XCrDnfr2LshGpnaE9bBVzAEZozz39S9untPDbejMZO1HkNOMKH0zcJUg2k.E-PGBPKAYKMiTTD2OWCO23jJ_lIvVsmRSZZCnNNVHF1KEHBemalULFweNwz4zxgSaQ0NKP41AtO_vWndSJiCZg&_sg%5B1%5D=PeYYHUMpBNybrpKjEMo00th_9nGVnwGbIaZOtfdzTP4gc38VAuzrwhTviwOoI1CCqTis1ZU.DTbYoNrdlpTPueNe6vKhkSUJxFjzdERykcaWN1KyU_2vQ0FPWB76IEpidHpJgOe1FoOdVRYIEBopYhvu43gBNg) D, [Oka](https://www.researchgate.net/scientific-contributions/13426640_Yuji_Oka?_sg%5B0%5D=qBw1AKagJrX18XCrDnfr2LshGpnaE9bBVzAEZozz39S9untPDbejMZO1HkNOMKH0zcJUg2k.E-PGBPKAYKMiTTD2OWCO23jJ_lIvVsmRSZZCnNNVHF1KEHBemalULFweNwz4zxgSaQ0NKP41AtO_vWndSJiCZg&_sg%5B1%5D=PeYYHUMpBNybrpKjEMo00th_9nGVnwGbIaZOtfdzTP4gc38VAuzrwhTviwOoI1CCqTis1ZU.DTbYoNrdlpTPueNe6vKhkSUJxFjzdERykcaWN1KyU_2vQ0FPWB76IEpidHpJgOe1FoOdVRYIEBopYhvu43gBNg) Y, [Ben-Daniel](https://www.researchgate.net/scientific-contributions/12668955_Bat-Hen_Ben-Daniel?_sg%5B0%5D=qBw1AKagJrX18XCrDnfr2LshGpnaE9bBVzAEZozz39S9untPDbejMZO1HkNOMKH0zcJUg2k.E-PGBPKAYKMiTTD2OWCO23jJ_lIvVsmRSZZCnNNVHF1KEHBemalULFweNwz4zxgSaQ0NKP41AtO_vWndSJiCZg&_sg%5B1%5D=PeYYHUMpBNybrpKjEMo00th_9nGVnwGbIaZOtfdzTP4gc38VAuzrwhTviwOoI1CCqTis1ZU.DTbYoNrdlpTPueNe6vKhkSUJxFjzdERykcaWN1KyU_2vQ0FPWB76IEpidHpJgOe1FoOdVRYIEBopYhvu43gBNg) B, [Cohen](https://www.researchgate.net/profile/Yigal_Cohen?_sg%5B0%5D=qBw1AKagJrX18XCrDnfr2LshGpnaE9bBVzAEZozz39S9untPDbejMZO1HkNOMKH0zcJUg2k.E-PGBPKAYKMiTTD2OWCO23jJ_lIvVsmRSZZCnNNVHF1KEHBemalULFweNwz4zxgSaQ0NKP41AtO_vWndSJiCZg&_sg%5B1%5D=PeYYHUMpBNybrpKjEMo00th_9nGVnwGbIaZOtfdzTP4gc38VAuzrwhTviwOoI1CCqTis1ZU.DTbYoNrdlpTPueNe6vKhkSUJxFjzdERykcaWN1KyU_2vQ0FPWB76IEpidHpJgOe1FoOdVRYIEBopYhvu43gBNg) Y (2003). Dry mycelium of *Penicillium chrysogenum* protects cucumber and tomato plants against the root-knot nematode *Meloidogyne javanica*. Phytoparasitica, 31(3): 217-225.

Grewal, P.S., H.S. Sohi, K. Grabbe and O. Hilber. 1989. Effect of various fungal metabolites on *Aphelenchoides composticola* Franklin and its multiplication on some fungi. pp. 813-820. *Mushroom Science*. *Proc*. *12th* *Int*. *Cong*. *Science & Cultivation of Edible Fungi*. Vol.2. Braunschweig, Germany.

Grimme E, Zidack N, Sikora R, Strobel G, Jacobsen B. Comparison of *Muscodor albus* volatiles with a biorational mixture for control of seedling diseases of sugar beet and root-knot nematode on tomato. Plant disease. 2007; 91(2):220-225.

Hawranik DJ, Sorensen JL. (2010). The isolation of citric acid derivatives from *Aspergillus niger*. FEMS Microbiol Lett, 306(2): 122-126.

Holgado, R. and D. H. Crump (2003). First record on the occurrence of nematophagous fungi parasitizing cyst nematodes in Norway. International Journal of Nematology 13: 65-71.

Hu, Y., Zhang, W., Zhang, P., Ruan, W., and Zhu, X. (2013) Nematicidal activity of chaetoglobosin A produced by *Chaetomium globosum* NK102 against *Meloidogyne incognita*. J Agric Food Chem 61: 41–46.

Hussey, R.S. and Barker, K.R. (1973). A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. Plant Disease Reporter, 57: 1925-1928.

Jang JY, Choi YH, Shin TS, Kim TH, Shin K-S, Park HW, et al. 2016. Biological control of *Meloidogyne incognita* by *Aspergillus niger* F22 producing oxalic acid. PLoS One 11: e0156230.

Kaskavalci G. Effect of soil solarization and organic amendments treatment for controlling *Meloidogyne incognita* in tomato cultivars in Western Anatolia. Turk J Agric For. 2007;31:159–167.

Li., G., K. Zhang, J. Xu, J. Dong and Y. Liu. 2007. Nematicidal substances from fungi. *Recent patents on Biotechnology,* 1: 1-22.

Lopez-Llorca L.V., Jansson H.B. (2006). Fungal parasites of invertebrates: multimodal biocontrol agents. pp. 310-335. In: Exploitation of f Fungi. (Eds.): G.D. Robson, P. vanWest and G.M. Gadd. Cambridge University Press, Cambridge.

Mari M, Martini C, Guidarelli M, Neri F. Postharvest biocontrol of *Monilinia laxa*, *Monilinia fructicola* and *Monilinia fructigena* on stone fruit by two *Aureobasidium pullulans* strains. Biological Control. 2012; 60 (2):132-140.

Marsden P.D. and Hoskins D.W. (1966). Progress in Gastroenterology: Intestinal Parasites. GASTROENTEROLOGY, 51(5): 701-720.

McAfee B, Taylor A. A review of the volatile metabolites of fungi found on wood substrates. Natural toxins. 1999; 7(6):283-303. PMID: 11122520

Miller C, Miller R, Garton-Kenny D, Redgrave B, Sears J, Condron M, et al. Ecomycins, unique antimycotics from *Pseudomonas viridiflava*. Journal of applied microbiology. 1998; 84(6):937-44. PMID: 9717277

Morath SU, Hung R, Bennett JW. Fungal volatile organic compounds: a review with emphasis on their biotechnological potential. Fungal Biology Reviews. 2012; 26(2):73-83.

Mukhtar T. (2018). Management of Root-Knot Nematode, *Meloidogyne incognita*, in Tomato with Two *Trichoderma* Species. Pakistan J. Zool., vol. 50(4), pp 1589-1592.

Netscher, C. and Sikora, R. A. (1990). Nematode parasites of vegetables. Pp 237-284 in M. Luc, R. A. Sikora and J. Bridge, eds. Plant parasitic nematodes in subtropical and tropical agriculture. CAB International.

Nour El-Deen, A.H. & Issa, A.A. (2016). Nematicidal properties of some algal aqueous extracts against root-knot nematode, *Meloidogyne incognita* in vitro. Egypt. J. Agronematol., 15(1), 67-78.

Nour El-Deen, A.H.; Darwesh Hadeer, Y.; El-Ghamdi A.A. and Samra, B.N. (2015). Evaluating the pathogenicity of nematodes infecting roses at Taif governorate, KSA. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 6(2): 1562-1567.

Oliveira D.F., H.W.P. Carvalho, A.S. Nunes, G.H. Silva, V.P. Campos, H.M.S. Júnior, A.J. Cavalheiro (2009). The activity of amino acids produced by *Paenibacillus macerans* and from commercial sources against the root-knot nematode *Meloidogyne exigua*. Eur J Plant Pathol, 124:57–63.

Riga E, Lacey LA, Guerra N. *Muscodor albus*, a potential biocontrol agent against plant-parasitic nematodes of economically important vegetable crops in Washington State, USA. Biological Control. 2008; 45(3):380-385.

Schouten, A. (2016) Mechanisms involved in nematode control by endophytic fungi. Annu Rev Phytopathol 54, 3.1– 3.22.

Sharma I.P., Sharma A.K., Prashad L., Rana V.S. (2018). Natural bacterial cell-free extracts with powerful nematicidal activity on root-knot nematode. Rhizosphere, 5: 67–70.

[Shemshura](javascript:;) O.N., [Bekmakhanova](javascript:;) N.E., [Mazunina](javascript:;) M.N., [Meyer](javascript:;) S.F., [Rice](javascript:;) C.P., [Masler](javascript:;) E.P. (2016). Isolation and identification of nematode-antagonistic compounds from the fungus *Aspergillus candidus. FEMS Microbiology Letters*, 363(5): 1-9.

Stinson M, Ezra D, Hess WM, Sears J, Strobel G. An endophytic Gliocladium sp. of *Eucryphia cordifolia* producing selective volatile antimicrobial compounds. Plant Science. 2003; 165(4):913-22.

Strobel G, Singh SK, Riyaz-Ul-Hassan S, Mitchell AM, Geary B, Sears J. An endophytic/pathogenic Phoma sp. from creosote bush producing biologically active volatile compounds having fuel potential. FEMS microbiology letters. 2011; 320(2):87-94. doi: 10.1111/j.1574-6968.2011.02297.x PMID:21535100

Suwannarach N, Kumla J, Bussaban B, Nuangmek W, Matsui K, Lumyong S. Biofumigation with the endophytic fungus *Nodulisporium* spp. CMU-UPE34 to control postharvest decay of citrus fruit. Crop protection. 2013; 45:63-70.

Taylor AL, Sasser JN. (1978). Biology, identification and control of root-knot nematodes (Meloidogyne species). Coop. Publ., Dep. Plant Pathol., North Carolina State Univ., and U.S. Agency Int. Dev., Raleigh, NC.: pp. 111.

Tian, [X.](https://www.tandfonline.com/author/Tian%2C+Xueliang), [Yao](https://www.tandfonline.com/author/Yao%2C+Yurong), Y., [Chen](https://www.tandfonline.com/author/Chen%2C+Guohua), G., [Mao](https://www.tandfonline.com/author/Mao%2C+Zhenchuan), Z., [Wang](https://www.tandfonline.com/author/Wang%2C+Xiaotian), X., [Xie](https://www.tandfonline.com/author/Xie%2C+Bingyan), B. (2014). Suppression of *Meloidogyne incognita* by the endophytic fungus *Acremonium implicatum* from tomato root galls. International Journal of Pest Management, 60(4): 239-245.

Wiratno, A.; D. Taniwiryonoc; H. Van den Bergb; J.A.G. Riksend; I.M.C.M. Rietjensb; S.R. Djiwantia; J.E. Kammengad and A.J. Murkb (2009). Nematicidal activity of plant extracts against the root-knot nematode, *Meloidogyne incognita*. The Open Natural Products Journal, 2: 77-85.

Woropong J, Strobel G, Ford E, Li J, Baird G, Hess W. *Muscodor albus* aman. nov. an endophyte from *Cinnamonum zeylanicum*. Mycotaxon. 2001; 79:67-69.

Yan Xiao-ning, Richard A. Sikora, Jing-wu Zheng (2011). Potential use of cucumber (*Cucumis sativus* L.) endophytic fungi as seed treatment agents against root-knot nematode *Meloidogyne incognita*. J Zhejiang Univ-Sci B (Biomed & Biotechnol), 12(3): 219-225.

Zabalgogeazcoa I. (2008). Fungal endophytes and their interaction with plant pathogens. Span. J. Agric. Res., 6:138-146.

[Zheng CJ](https://www.ncbi.nlm.nih.gov/pubmed/?term=Zheng%20CJ%5BAuthor%5D&cauthor=true&cauthor_uid=21517707), [Li L](https://www.ncbi.nlm.nih.gov/pubmed/?term=Li%20L%5BAuthor%5D&cauthor=true&cauthor_uid=21517707), [Zou JP](https://www.ncbi.nlm.nih.gov/pubmed/?term=Zou%20JP%5BAuthor%5D&cauthor=true&cauthor_uid=21517707), [Han T](https://www.ncbi.nlm.nih.gov/pubmed/?term=Han%20T%5BAuthor%5D&cauthor=true&cauthor_uid=21517707), [Qin LP](https://www.ncbi.nlm.nih.gov/pubmed/?term=Qin%20LP%5BAuthor%5D&cauthor=true&cauthor_uid=21517707). (2012). Identification of a quinazoline alkaloid produced by *Penicillium vinaceum*, an endophytic fungus from *Crocus sativus*. [Pharm Biol.](https://www.ncbi.nlm.nih.gov/pubmed/21517707), 50(2): 129-133. doi: 10.3109/13880209.