

## Research Article

## Effect of *Acrostalagmus luteoalbus* ACRO1 on mortality of the second-stage juveniles of the citrus nematode *Tylenchulus semipenetrans* under laboratory conditions

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**ABSTRACT-** This study investigates the nematicidal activity of *Acrostalagmus luteoalbus* ACRO1 as a potential biocontrol agent against the citrus nematode *Tylenchulus semipenetrans* under laboratory conditions. The ACRO1 isolate was cultured in potato extract-dextrose broth, and its nematicidal metabolites were extracted using ethyl acetate. Its effectiveness was assessed in comparison to *Purpureocillium lilacinum*, a known biocontrol agent, and abamectin, a chemical nematicide. ACRO1 demonstrated remarkable nematicidal efficacy, achieving an average mortality rate of 81.5% within 72 hours, significantly surpassing *P. lilacinum* (46.3%) and the negative control (6.5%). In a secondary experiment, ACRO1 induced 58.7% mortality in 72 hours, again outperforming *P. lilacinum* (26.2%) and the control (6.25%). Its cell-free culture extract exhibited rapid activity, reaching 76.0% mortality within 24 hours, while its metabolites achieved complete nematicidal efficacy with 100% mortality. By comparison, abamectin induced mortality rates of 53.2%, 62.2%, and 100% at concentrations of 5 ppm, 10 ppm, and 3%, respectively. These findings underscore ACRO1's potential as an effective and environmentally friendly alternative to synthetic nematicides, offering a sustainable solution for managing the citrus nematode, a persistent threat to global citrus production.

## INTRODUCTION

Plant-parasitic nematodes (PPNs) are microscopic roundworms that pose a significant threat to agricultural production worldwide. Among these, the citrus nematode *Tylenchulus semipenetrans* is one of the most economically damaging species, primarily affecting citrus crops. This sedentary, semi-endoparasitic nematode establishes feeding sites within the root cortex, leading to adverse physiological effects, including stunted growth, leaf chlorosis, reduced fruit yield, and a gradual decline in tree vigor (Bozbuga et al., 2023; Labiadh et al., 2023). Extensive surveys in citrus-growing regions indicate that *T. semipenetrans* is highly prevalent, with infection densities ranging from 53 to 12,173 second-stage juveniles (J2s) per 100 g of soil. Its distribution is largely influenced by soil physico-chemical characteristics, such as texture, pH, and mineral composition (Bozbuga et al., 2023; Labiadh et al., 2023; Zoubi et al., 2022).

Given its widespread occurrence and severe impact on citrus production, *T. semipenetrans* is regarded as one of the most challenging nematode pests in the citrus industry. Chemical nematicides remain a widely used

method for controlling *T. semipenetrans*. However, concerns regarding their environmental persistence, toxicity to non-target organisms, and potential to induce nematode resistance have necessitated the exploration of sustainable alternatives. Consequently, biological control strategies employing nematophagous fungi have gained increasing attention. Certain fungal species, including *Trichoderma harzianum* and *Purpureocillium lilacinum*, have demonstrated efficacy in suppressing nematode populations, while simultaneously enhancing plant health by promoting root development and improving nutrient uptake (Saadoon et al., 2022). These fungi exert their biocontrol effects through multiple mechanisms, including direct parasitism, inhibition of egg hatching, reduction in juvenile survival, and suppression of feeding site formation (Saadoon et al., 2022). As a result, fungal-based biocontrol approaches not only mitigate nematode infestations but also serve as viable alternatives to chemical nematicides in integrated pest management (IPM) programs.

Beyond direct biocontrol activity, fungal metabolites have been identified as potent nematicidal agents. Metabolites derived from *T. harzianum* and *P. lilacinum* have been shown to significantly reduce *T.*

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*semipenetrans* populations in both in vitro and field studies (Bhagawati et al., 2021; El-Marzoky et al., 2023). These findings highlight the potential of fungal secondary metabolites as crucial components of nematode management strategies. Among promising fungal candidates, *Acrostalagmus luteoalbus*, a member of the Hypocreaceae family, has garnered attention due to its diverse metabolic capabilities. Commonly isolated from organic-rich environments, this fungus is known for its role in nutrient cycling and its ability to synthesize a wide array of bioactive compounds (Bondarenko et al., 2018; Rojas et al., 2008). Recent investigations suggest that *Acrostalagmus* species possess biocontrol properties, with crude extracts exhibiting antibacterial, antifungal, and nematicidal activities (Jensen, 1963; Buyan et al., 2023). Of particular interest, the ACRO1 isolate of *A. luteoalbus*, obtained from arugula vermicompost, has demonstrated nematicidal activity against root-knot nematodes (Gharin, 2023). This finding suggests its potential for broader applications in the management of *T. semipenetrans*, a target that remains relatively unexplored.

The present study aimed to assess the nematicidal efficacy of *A. luteoalbus* ACRO1 against *T. semipenetrans* under laboratory conditions. By comparing its effectiveness with established biocontrol agents and chemical nematicides, this research sought to determine its potential as a sustainable alternative for citrus nematode management.

## MATERIALS AND METHODS

### *Collection and preparation of the citrus nematode population*

Soil samples were collected from the rhizosphere of infected citrus trees in a citrus orchard located in Jahrom city, Fars Province. Sampling was conducted at a depth of 5–25 cm beneath the tree canopy. The collected samples were then transported to the Department of Plant Protection, School of Agriculture, Shiraz University, for further analysis. Active juveniles of the citrus nematode (*T. semipenetrans*) were extracted using the tray method described by Whitehead and Hemming (1965). The nematode population was quantified in triplicate by counting the number of nematodes present in 1 mL of the suspension under a stereomicroscope.

### *Preparation of fungal isolate*

The *A. luteoalbus* ACRO1 isolate was previously obtained from arugula vermicompost. Phylogenetic analysis, based on the internal transcribed spacer (ITS) region and beta-tubulin gene sequences, confirmed its taxonomic identity as *A. luteoalbus* (Gharin, 2023). The ACRO1 isolate was cultured on potato dextrose agar (PDA) medium, after which several fungal plugs were transferred to flasks containing potato dextrose broth (PDB) and incubated under continuous shaking for three days. A spore suspension was subsequently prepared at a concentration of  $1 \times 10^7$  spores/mL using a hemocytometer (Banihashemi, 2010). As a positive biological control, *P. lilacinum* was included in the study at a concentration of  $1.2 \times 10^8$  spores/mL, following the

manufacturer's recommendations. In a subsequent experiment, the spore concentrations of both fungal treatments were standardized to  $1 \times 10^7$  spores/mL. Additionally, the chemical nematicide Nematex® (abamectin 2% Suspension concentrate) was prepared at concentrations of 5 ppm and 3% for cell-free experiments and at 10 ppm and 3% for metabolite-based experiments.

### *Preparation of Acrostalagmus luteoalbus ACRO1 isolate cell-free culture extract*

Mycelial plugs of *A. luteoalbus* ACRO1 were excised from the PDA medium and transferred into 100 mL of sterilized PDB. The inoculated flasks were then incubated at  $28 \pm 2$  °C for 15 days. To obtain the cell-free culture extract, the fungal biomass was first filtered using Whatman® No. 4 filter paper and subsequently centrifuged at 10,000 rpm for 20 minutes. The resulting supernatant was collected and stored at -20 °C until further analysis (Santra & Banerjee, 2023).

### *Extraction of Acrostalagmus luteoalbus ACRO1 Metabolites*

Metabolites were extracted from the ACRO1 cell-free culture extract using the organic solvent ethyl acetate (EA). To obtain the cell-free culture extract, the fungal biomass was first filtered through a muslin cloth and then centrifuged at 10,000 rpm for 20 minutes. Ethyl acetate was added to the filtrate at a ratio of 2:1 (200 mL EA per 100 mL filtrate) and stirred for 30 minutes using a magnetic stirrer. The organic phase was subsequently separated using a separatory funnel and concentrated under reduced pressure (105 mbar) at  $45 \pm 1$  °C using a vacuum rotary evaporator to remove excess solvent. The resulting 250 mL of concentrated extract was lyophilized, and the dried residue was weighed and stored for further analysis. To evaluate nematicidal activity, the dried extract was reconstituted in distilled water (Santra & Banerjee, 2023).

### *In vitro experiment*

#### *Nematophagous effect of ACRO1 isolate*

A spore suspension of *A. luteoalbus* ACRO1 ( $1 \times 10^7$  spores/mL) was tested against 200 juveniles of *T. semipenetrans* in 6 cm Petri dishes. Distilled water was used as a negative control and *P. lilacinum* served as a positive control. Each treatment had four replicates. Petri dishes were incubated at  $28 \pm 2$  °C and observations were recorded at 24, 48, and 72 hours. The mortality rates were calculated using the following equation (He et al., 2020):

$$\text{Mortality rate (\%)} = (\text{number of dead nematodes per mL} / \text{total number of nematodes per mL}) \times 100.$$

Eq. (1)

Only nematodes that were mechanically parasitized by the fungal organs were considered dead. This experiment was conducted in duplicate.

#### *Nematicidal effect of ACRO1 isolate*

The nematicidal activity of *A. luteoalbus* ACRO1 was evaluated by exposing *T. semipenetrans* juveniles to its cell-free culture extract and metabolic compounds. Two

concentrations of the cell-free culture extract (1 and 5 mL) and two concentrations of nematicidal metabolites (50 and 100  $\mu\text{L/mL}$ ) were tested against 200 *T. semipenetrans* juveniles in 6 cm Petri dishes. Distilled water was used as a negative control, while abamectin served as the chemical control. Each treatment was performed in four replicates. Petri dishes were incubated at  $28 \pm 2^\circ\text{C}$ , and nematode mortality, determined by immobility, was assessed after 24 hours. Immobile nematodes were subsequently rinsed with distilled water and examined under a light microscope to confirm mortality based on alterations in internal organ structure. The experiment was conducted in duplicate to ensure reproducibility.

#### Data analysis

Data were statistically analyzed using SAS software (version 9.4; SAS Institute Inc., Cary, NC). Analysis of variance (ANOVA) was performed using the General Linear Model (GLM) procedure to identify significant differences among the treatments. Tukey's post-hoc test was applied to compare group mean values ( $P < 0.01$ ).

## RESULTS AND DISCUSSION

#### Nematophagous effect of ACRO1 isolate

The analysis of variance indicated that *A. luteoalbus* ACRO1 and *P. lilacinum* had a highly significant effect ( $P < 0.01$ ) on nematode mortality (Table 1).

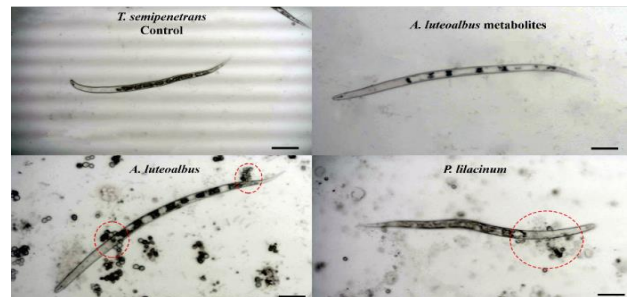
**Table 1.** Source of variation as mean square for the first and second experiments in the nematophagous test

Source of variation	DF	24 hours	48 hours	72 hours
<b>First experiment</b>				
Fungi	2	3580**	3782**	5630**
Error	9	1.75	1.83	1.11
CV (%)	-	4.14	3.74	2.35
<b>Second experiment</b>				
Fungi	2	1270**	1589**	2808**
Error	9	5.77	9.75	6.69
CV (%)	-	13.1	13.72	8.50

\*\*: significant at 1% probability level.

Treatment with *A. luteoalbus* ACRO1 led to a significant reduction in the citrus nematode population at all times, outperforming both the control and *P. lilacinum* treatments. The mortality rates of nematodes for each treatment are summarized in Table 2, which highlights the superior efficacy of *A. luteoalbus* ACRO1. In the first experiment, the mortality rate for *A. luteoalbus* ACRO1 increased progressively, reaching 63.5% at 24 hours, 67% at 48 hours, and 81.5% at 72 hours (Fig. 1; Table 2). In contrast, *P. lilacinum* treatment achieved mortality rates of 28.3%, 36.5%, and 46% at the same time intervals. The second experiment showed lower overall mortality rates but still demonstrated the superior efficacy of *A. luteoalbus*. At 24, 48, and 72 hours, the mortality rates for the *A. luteoalbus* ACRO1 isolate were 37%, 43.5%, and 58.7%, respectively, compared to 16.5%, 21%, and 26.2% for the *P. lilacinum*

treatment. These findings underscore two key advantages of *A. luteoalbus* ACRO1: its rapid initial action and its sustained efficacy over time. With a mortality rate exceeding 60% within 24 hours in the first experiment, *A. luteoalbus* ACRO1 shows strong potential as a biocontrol agent, providing a natural and effective alternative to chemical pesticides.



**Fig. 1.** Parasitic behavior towards *Tylenchulus semipenetrans* by fungi *Acrostalagmus luteoalbus*, *Purpureocillium lilacinum*, and a metabolite derived from *Acrostalagmus luteoalbus* after a three-day period. Bars = 20 $\mu$ .

Certain species of filamentous fungi have demonstrated good potential as effective biological control agents against plant-parasitic nematodes. However, only a limited number of fungal genera, including *Trichoderma*, mycorrhizal fungi, and endophytic fungi, possess the necessary mechanisms to effectively mitigate nematode-induced damage.

**Table 2.** Comparison of the effect of *Acrostalagmus luteoalbus* ACRO1 and *Purpureocillium lilacinum* on the mortality rate of second-stage juveniles of the citrus nematode *Tylenchulus semipenetrans* in vitro at different time points

Treatment	Mortality of second stage juveniles (J2s) (%)			
	24 hours	48 hours	72 hours	Mean
<b>First experiment</b>				
Control	4.00 <sup>c</sup> $\pm$ 0.40	5.3 <sup>c</sup> $\pm$ 0.25	6.50 <sup>c</sup> $\pm$ 0.50	5.3 <sup>c</sup> $\pm$ 1.96
ACRO1	63.5 <sup>a</sup> $\pm$ 9.40	67.0 <sup>a</sup> $\pm$ 1.47	81.5 <sup>a</sup> $\pm$ 1.93	70.7 <sup>a</sup> $\pm$ 1.96
<i>P. lilacinum</i>	28.3 <sup>b</sup> $\pm$ 2.05	36.5 <sup>b</sup> $\pm$ 1.32	46.0 <sup>b</sup> $\pm$ 1.88	37.0 <sup>b</sup> $\pm$ 1.96
<b>Second experiment</b>				
Control	1.50 <sup>c</sup> $\pm$ 0.64	3.75 <sup>c</sup> $\pm$ 0.62	6.25 <sup>c</sup> $\pm$ 0.48	3.83 <sup>c</sup> $\pm$ 0.78
ACRO1	37.0 <sup>a</sup> $\pm$ 1.58	43.5 <sup>a</sup> $\pm$ 2.46	58.7 <sup>a</sup> $\pm$ 2.01	46.41 <sup>a</sup> $\pm$ 0.78
<i>P. lilacinum</i>	16.5 <sup>b</sup> $\pm$ 1.19	21.0 <sup>b</sup> $\pm$ 0.91	26.2 <sup>b</sup> $\pm$ 0.85	21.25 <sup>b</sup> $\pm$ 0.78

The data represent the mean of four replicates. Data in each column with the same letter are not significantly different according to Tukey's test ( $P < 0.01$ ).

Filamentous fungi employ three primary strategies to combat nematodes: parasitism, antibiosis, and the production of lytic enzymes. For instance, *P. lilacinum* directly invades and parasitizes nematodes, effectively neutralizing them. In contrast, species of *Trichoderma* release bioactive compounds that inhibit nematode development and reproduction through antibiosis. These



fungi can also enhance plant tolerance by triggering hormone-mediated defense responses, such as the production of salicylic and jasmonic acid, which help plants repel nematode attacks (Lawal et al., 2022; Poveda et al., 2020). Additionally, filamentous fungi improve plant health by enhancing nutrient and water uptake, as well as modifying root morphology. These changes not only increase plant resilience but also reduce competition with nematodes for resources, thereby promoting healthier plant growth (Lawal et al., 2022; Poveda et al., 2020). By inducing plant resistance, these fungi serve as a sustainable alternative to chemical nematicides. Several studies have demonstrated that certain filamentous fungi can significantly reduce nematode populations in agricultural environments. In vitro tests have shown that fungi such as *Cladosporium* sp., *Trichoderma* sp., and *Fusarium equiseti* significantly reduce the number of infective nematode juveniles (Zarrin et al., 2015). Notably, both *T. harzianum* and *T. album* enhance plant resistance to nematodes while simultaneously promoting plant growth. These fungi exhibit strong nematostatic properties in vitro; at optimal concentrations, *T. album* achieved over 90% mortality in *T. semipenetrans* juveniles (Kerry, 1990; Verdejo-Lucas & Kaplan, 2002). Certain fungal species, including *P. lilacinum* and *Arthrobotrys conoides*, also exhibit nematicidal properties against *T. semipenetrans*. The *P. lilacinum* isolate cAUMC 10620 has shown promising results in both in vitro and in vivo laboratory studies, as well as under field conditions. In in vitro tests, this fungus induced high immobility in *T. semipenetrans* juveniles and inhibited egg hatching. At a concentration of  $5 \times 10^7$  spores/mL, it achieved up to 89.01% immobility of the juveniles after 48 hours (El-Marzoky et al., 2023). *A. conoides*, another nematophagous fungus, has been widely studied for its ability to trap and kill nematodes. This fungus can be used in combination with other biocontrol agents, such as the mycorrhizal fungus *Glomus mosseae*, which improves soil health and enhances plant resilience, thereby supporting the effectiveness of biocontrol strategies against citrus nematodes (Sweelam et al., 2019). Additionally, fungal metabolites, such as those from *Gliocladium roseum*, have been shown to inhibit egg hatching and cause juvenile mortality in nematodes, indicating their significant potential as biocontrol agents (Wang et al., 2011).

#### Nematicidal effect of ACRO1 isolate

The analysis of variance showed that the main factors ACRO1 and *P. platinum* had a significant effect ( $P < 0.01$ ) on the nematicidal activity test (Table 3).

**Table 3.** Source of variation as mean square for the first and second experiments in the nematicidal effect

Source of variation	DF	Cell-free	Metabolite
Nematicidal	4	6365**	7097**
Error	15	2.83	0.76
CV (%)	-	2.56	1.19

\*\* : significant at 1% probability level.

Cell-free culture extracts of *A. luteoalbus* demonstrated a mortality rate of 76.0% in response to a 1 mL dose. When the dose was increased to 5 mL, the mortality rate increased to 96.3%, indicating a dose-

dependent response. In comparison, the two abamectin doses (3% and 5 ppm) caused mortality rates in the cell-free culture extract experiments to be 100% and 53.2%, respectively (Table 4).

**Table 4.** Effect of various concentrations of cell-free culture extract from ACRO1 isolate on citrus nematode juveniles after 24 hours

Treatment	Cell-free
Control	2.50 <sup>e</sup> ± 0.29
Abamectin 3%	100 <sup>a</sup> ± 0.00
Abamectin 5 ppm	53.2 <sup>d</sup> ± 0.48
ACRO1 1 mL	76.0 <sup>c</sup> ± 1.29
ACRO1 5 mL	96.3 <sup>b</sup> ± 1.25

The doses for extracellular substances (cell-free) were 1 and 5 mL. The data represent the average of four replicates. The mean values with the same letter are not significantly different at  $P < 0.01$ , according to the Tukey's test.

The metabolites of the ACRO1 isolate resulted in a 100% mortality rate after 24 hours at both doses. In comparison, abamectin at doses of 10 ppm and 3% caused mortality rates of 62.2% and 100%, respectively (Table 5). These results indicated that the metabolites of *A. luteoalbus* exhibited highly potent nematicidal activity against citrus nematodes. Moreover, the metabolites showed more significant bioactive properties than the cell-free culture extract. The high mortality rates observed in this study suggest that secondary metabolites may play a crucial role in the nematicidal effect.

**Table 5.** Effect of various concentrations of metabolites from ACRO1 isolate on citrus nematode juveniles after 24 hours

Treatment	Metabolite
Control	3.75 <sup>c</sup> ± 0.48
Abamectin 3%	100 <sup>a</sup> ± 0.00
Abamectin 10 ppm	62.2 <sup>b</sup> ± 0.85
ACRO1 100 µL/mL	100 <sup>a</sup> ± 0.00
ACRO1 50 µL/mL	100 <sup>a</sup> ± 0.00

The doses of the metabolites were 100 and 50 µL/mL. The data represent the average of four replicates. The mean values with the same letter are not significantly different at  $P < 0.01$ , according to Tukey's test.

Several factors likely contribute to the nematicidal mechanisms of *A. luteoalbus* and its metabolites. Previous research has demonstrated that fungal secondary metabolites can target the nervous systems of nematodes, disrupt energy metabolism, and even damage cellular membranes. Between 1969 and 2022, over 50 natural compounds were successfully isolated from the *Acrostalagmus* genus, with alkaloids (56%) and terpenoids showing particularly potent nematicidal effects. Specific alkaloids, such as loline and pyrrolizidine, have been shown to inhibit the migration of root-knot nematodes (*Meloidogyne* spp.) and prevent their establishment in plant roots (Wang et al., 2012; Wen et al., 2013). On the other hand, terpenoids and benzene derivatives have also exhibited strong nematicidal properties against these nematodes (Khoshkhoo et al., 1994; Echeverrigaray et al., 2010; Xu et al., 2015). Notably, 72% of the natural products extracted from

*Acrostalagmus* exhibit significant biological activity, with 50% of these compounds showing efficacy comparable to or exceeding that of standard positive controls (Cao et al., 2021).

Among the most highly bioactive compounds, 67% belonged to the epipolythiodioxopiperazine family, suggesting that this structural class could serve as a promising foundation for the development of new pharmaceutical agents (Yu et al., 2018). Our findings align with previous studies that have emphasized the potential of fungal biocontrol agents in managing plant-parasitic nematodes. Similar nematicidal effects have been observed in other fungal species, as reported by Meyer et al. (2004) and Cayrol et al. (1989). In this study, we evaluated the efficacy of *A. luteoalbus* as an innovative biocontrol agent against citrus nematode infestation. While *A. luteoalbus* shows considerable potential, further research is needed to optimize formulations, determine application methods, and assess field efficacy.

This is the first study to report the remarkable nematicidal activity of *A. luteoalbus* ACRO1 and its metabolites against *T. semipenetrans*. The high efficacy demonstrated across all bioassays suggests that these metabolites could serve as viable alternatives to chemical nematicides in sustainable agricultural practices. Future studies are essential to elucidate the precise mechanisms of action underlying this effect and to develop practical biocontrol formulations for agricultural applications.

## CONCLUSION

This study demonstrated the strong nematicidal potential of the *A. luteoalbus* ACRO1 isolate and its secondary metabolites that stand against the *T. semipenetrans*. The 100% mortality rate observed within 24 hours showed the effectiveness of these metabolites as viable, sustainable alternatives to chemical nematicides. Future research should focus on isolating the active compounds, refining their mechanisms of action, and developing practical formulations for use in integrated pest management systems. To the best of our knowledge, this is the first study to report the nematicidal activity of *A. luteoalbus* ACRO1 and its metabolites against *T. semipenetrans*.

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## CRedit AUTHORSHIP CONTRIBUTION STATEMENT

Conceptualization: Reza Gharin and Akbar Karegar; Methodology: Reza Gharin, Akbar Karegar and Maryam Mirtalebi; Software: Reza Gharin; Validation: Reza Gharin, Akbar Karegar and Maryam Mirtalebi; Formal analysis: Reza Gharin; Investigation: Reza Gharin; Resources: Reza Gharin; Data curation: Reza Gharin; Writing—original draft preparation: Reza Gharin; Writing—review and editing: Reza Gharin, Akbar Karegar and Maryam Mirtalebi; Visualization: Reza Gharin; Supervision: Akbar Karegar and Maryam Mirtalebi; Funding acquisition: Akbar Karegar.

## DECLARATION OF COMPETING INTEREST

The authors declare no conflicts of interest.

## ETHICAL STATEMENT

All authors are aware of the content of the manuscript and have consented to its submission to the *Iran Agricultural Research Journal*. This manuscript has not been submitted to any other journal. No experiments involving humans or animals were conducted in this study.

## DATA AVAILABILITY

Raw data supporting this research, including mortality rate records, statistical analyses, and experimental protocols, are available upon request of the reviewers and editors.

## REFERENCES

- Banihashemi, Z. (2010). Reaction of Cucumis melo cultivars to races of *Fusarium oxysporum* f. sp. *melonis* the cause of melon vascular wilt. *Iranian Journal of Plant Pathology*, 46, 5–7. (In Persian)
- Bhagawati, B., Choudhary, B. N., & Kurulkar, U. (2021). Bio management of citrus nematode, *Tylenchulus semipenetrans* infecting citrus in Assam. *Indian Journal of Nematology*, 51(2), 123-128. <https://doi.org/10.5958/0974-4444.2021.00019.6>
- Bondarenko, S. A., Ianutsevich, E. A., Sinitsyna, N. A., Georgieva, M. L., Bilanenko, E. N., & Tereshina, B. M. (2018). Dynamics of the cytosol soluble carbohydrates and membrane lipids in response to ambient pH in alkaliphilic and alkalitolerant fungi. *Microbiology*, 87(1), 21-32. <https://doi.org/10.1134/S0026261718010034>
- Bozbuga, R., Yildiz, S., Yuksel, E., Özer, G., Dababat, A. A., & İmren, M. (2023). Nematode–citrus plant interactions: Host preference, damage rate and molecular characterization of citrus root nematode *Tylenchulus semipenetrans*. *Plant Biology*, 25(6), 871-879. <https://doi.org/10.1111/plb.13566>
- Buyan, Z., Zhifeng, L., Qinqin, L., Ling, Z., Changgeng, Y., Liyun, W., & Yulin, S. (2023). Molecular identification, secondary metabolites and biological activities of a deep-sea-derived fungus 101#. *Journal of Tropical Oceanography*, 42(5), 104-114. <https://doi.org/10.11978/2022242>
- Cao, J., Li, X. M., Li, X., Li, H. L., Konuklugil, B., & Wang, B. G. (2021). Uncommon N-Methoxyindoleketopiperazines from *Acrostalagmus luteoalbus*, a marine algal isolate of endophytic fungus. *Chinese Journal of Chemistry*, 39(10), 2808-2814. <https://doi.org/10.1002/cjoc.202100368>
- Cayrol, J. C., Djian, C., & Pijarowski, L. (1989). Study of the nematicidal properties of the culture filtrate of the nematophagous fungus *Paecilomyces lilacinus*. *Revue de Nematologie*, 12(4), 331-336.
- Echeverrigaray, S., Zacaria, J., & Beltrão, R. (2010). Nematicidal activity of monoterpenoids against the root-knot nematode *Meloidogyne incognita*. *Phytopathology*, 100(2), 199-203. <https://doi.org/10.1094/PHYTO-100-2-0199>

- El-Marzoky, A. M., Elnahal, A. S., Jghef, M. M., Abourehab, M. A., El-Tarabily, K. A., & Ali, M. A. (2023). *Purpureocillium lilacinum* strain AUMC 10620 as a biocontrol agent against the citrus nematode *Tylenchulus semipenetrans* under laboratory and field conditions. *European Journal of Plant Pathology*, 167(1), 59-76. <https://doi.org/10.1007/s10658-023-02684-1>
- Gharin, R. (2023). *Study of the inhibitory effect of the fungi isolated from vermicompost of arugula (Eruca sativa Mill.) on the root-knot nematode Meloidogyne javanica in tomato*. (Master's thesis, Shiraz University, Shiraz)
- He, Q., Wang, D., Li, B., Maqsood, A., & Wu, H. (2020). Nematicidal evaluation and active compounds isolation of *Aspergillus japonicus* ZW1 against root-knot nematodes *Meloidogyne incognita*. *Agronomy*, 10(9), 1222. <https://doi.org/10.3390/agronomy10091222>
- Jensen, H. L. (1963). Carbon nutrition of some microorganisms decomposing halogen-substituted aliphatic acids. *Acta Agriculturae Scandinavica*, 13(4), 404-412. <https://doi.org/10.1080/00015126309435665>
- Kerry, B. R. (1990). An assessment of progress toward microbial control of plant-parasitic nematodes. *Journal of Nematology*, 22(4S), 621.
- Khoshkhoo, N., Hedin, P. A., & McCarty Jr, J. C. (1994). Terpenoid aldehydes in root-knot nematode susceptible and resistant cotton plants. *Journal of Agricultural and Food Chemistry*, 42(1), 204-208. <https://doi.org/10.1021/jf00037a037>
- Labiadh, M., Mhamdi, B., Loulou, A., & Sadreddine, K. (2023). Impact of *rhizobacteria* community of citrus root on *Tylenchulus semipenetrans* and on citrus plant growth. *Biocontrol Science and Technology*, 33(3), 241-257. <https://doi.org/10.1080/09583157.2023.2175785>
- Lawal, I., Fardami, A. Y., Ahmad, F. I. I., Yahaya, S., Abubakar, A. S., Sa'id, M. A., Musa, M., & Maiyadi, K. A. (2022). A review on nematophagous fungi: A potential nematicide for the biocontrol of nematodes. *Journal of Environmental Bioremediation and Toxicology*, 5(1), 26-31. <https://doi.org/10.54987/jebat.v5i1.677>
- Meyer, S., Huettel, R., Liu, X. Z., Humber, R., Juba, J., & Nitao, J. (2004). Activity of fungal culture filtrates against soybean cyst nematode and root-knot nematode egg hatch and juvenile motility. *Nematology*, 6(1), 23-32.
- Poveda, J., Abril-Urias, P., & Escobar, C. (2020). Biological control of plant-parasitic nematodes by filamentous fungi inducers of resistance: *Trichoderma*, *mycorrhizal* and endophytic fungi. *Frontiers in Microbiology*, 11, 992-1006. <https://doi.org/10.3389/fmicb.2020.00992>
- Rojas, N. L., Cavalitto, S. F., Cabello, M., Hours, R. A., & Voget, C. E. (2008). Alkaline polysaccharidases produced in solid state cultures by alkalophilic fungi isolated from Argentina. *Journal of Pure and Applied Microbiology*, 2(1), 1-10.
- Saadoon, S. M., Sergany, M. I., Mona, H. E., Reham, A. M., & Gad, S. B. (2022). The efficiency of using some natural compounds for management of citrus nematode *Tylenchulus semipenetrans*. *Arab Journal of Plant Protection*, 40(4), 346. <https://doi.org/10.22268/AJPP-40.4.346350>
- Santra, H. K., & Banerjee, D. (2023). Antifungal activity of volatile and non-volatile metabolites of endophytes of *Chloranthus elatior* Sw. *Frontiers in Plant Science*, 14, 1156323. <https://doi.org/10.3389/fpls.2023.1156323>
- Sweelam, M. E., Abokorah, M. S., Taka, S. M. A., & Ellatief, S. M. A. (2019). Biological control of the citrus nematode, *Tylenchulus semipenetrans* attacking citrus sinensisnaval orange trees. *Menoufia Journal of Plant Protection*, 4(5), 179-188. <https://dx.doi.org/10.21608/mjapam.2019.123021>
- Verdejo-Lucas, S., & Kaplan, D. T. (2002). The citrus nematode: *Tylenchulus semipenetrans*. In *Plant resistance to parasitic nematodes*. (pp. 207-219). Wallingford UK: CABI Publishing.
- Wang, F.-Z., Huang, Z., Shi, X.-F., Chen, Y.-C., Zhang, W.-M., Tian, X.-P., Li, J., Zhang, S. (2012). Cytotoxic indole diketopiperazines from the deep sea-derived fungus *Acrostalagmus luteoalbus* SCSIO F457. *Bioorganic & Medicinal Chemistry Letters*, 22(23), 7265-7267. <https://doi.org/10.1016/j.bmcl.2012.08.115>
- Wang, L., Xu, Y., Li, S., Li, C., & Xue, A. G. (2011). Effects of metabolites of *Gliocladium roseum* on egg hatch and juvenile mortality of *Meloidogyne incognita*. *Soybean Science*, 30, 818-822.
- Wen, Y., Meyer, S. L., Masler, E. P., Zhang, F., Liao, J., Wei, X., & Chitwood, D. J. (2013). Nematotoxicity of drupacine and a cephalotaxus alkaloid preparation against the plant-parasitic nematodes *Meloidogyne incognita* and *Bursaphelenchus xylophilus*. *Pest Management Science*, 69(9), 1026-1033. <https://doi.org/10.1002/ps.3548>
- Whitehead, A. G., & Hemming, J. R. (1965). A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology*, 55(1), 25-38. <https://doi.org/10.1111/j.1744-7348.1965.tb07864.x>
- Xu, Y. Y., Lu, H., Wang, X., Zhang, K. Q., & Li, G. H. (2015). Effect of volatile organic compounds from bacteria on nematodes. *Chemistry & Biodiversity*, 12(9), 1415-1421. <https://doi.org/10.1002/cbdv.201400342>
- Yu, G., Wang, Y., Yu, R., Feng, Y., Wang, L., Che, Q., Gu, Q., Li, D., Li, J., Zhu, T. (2018). Chetracins E and F, cytotoxic epipolythiodioxopiperazines from the marine-derived fungus *Acrostalagmus luteoalbus* HDN13-530. *RSC Advances*, 8(1), 53-58. <https://doi.org/10.1039/C7RA12063J>
- Zarrin, M., Rahdar, M., & Gholamian, A. (2015). Biological control of the nematode infective juveniles of *Trichostrongylidae* family with filamentous fungi. *Jundishapur Journal of Microbiology*, 8(3), 1-20. <https://doi.org/10.5812/jjm.17614>
- Zoubi, B., Mokrini, F., Dababat, A. A., Amer, M., Ghoulam, C., Lahlali, R., Laasli, S. E., Khfif, Kh., Imren, M., Akachoud, O., Benkebboura, A., Housseini, A. I., & Qaddoury, A. (2022). Occurrence and geographic distribution of plant-parasitic nematodes associated with citrus in Morocco and their interaction with soil patterns. *Life*, 12(5), 637. <https://doi.org/10.3390/life12050637>