ISSN 2449-8955 European Journal of Biological Research Review Article

DOI: http://dx.doi.org/10.5281/zenodo.10015641

Use of *Arthrobotrys* spp. in biocontrol of the root-knot nematode *Meloidogyne incognita*

Cafer Eken [1], Gülsüm Uysal [2], Dudu Demir [3], Selda Çalişkan [2], Emre Sevindik [1]*, Kardelen Çağlayan [3]

[1] Department of Agricultural Biotechnology, Faculty of Agriculture, Aydın Adnan Menderes University, Aydın, Türkiye

[2] Batı Akdeniz Agricultural Research Institute, Antalya, Türkiye

[3] Department of Agricultural Biotechnology, Faculty of Agriculture, Isparta University of Applied Sciences, Isparta, Türkiye

* Corresponding author e-mail: ph.d-emre@hotmail.com

Received: 22 May 2023; **Revised submission:** 03 September 2023; **Accepted:** 30 September 2023

https://jbrodka.com/index.php/ejbr

ABSTRACT: Plant parasitic nematodes are well-known and devastating pathogens of many agricultural crops around the world. Among the plant phytoparasitic, root-knot nematodes (*Meloidogyne* spp.) are the economically important limiting factors in agricultural productivity and the quality of crops. One of the most destructive species of root-knot nematodes is *Meloidogyne incognita* among the most important plant pests which cause severe problems in economically important crops such as vegetables, fruits, and ornamental plants. Root-knot nematodes can be managed by resistant cultivars, crop rotation, cultural practices, chemical nematicides and biocontrol agents. However, the use of nematicides can cause significant problems, including environmental pollution and long-term residue issues. Therefore, biological control with fungus is agriculturally useful an exciting and rapidly developing research area and especially there is growing attention to the exploitation of fungi for the control of nematodes. Nematophagous fungi are an important group of soil microorganisms that can suppress the populations of plant parasitic nematodes. These fungi can be divided into four main categories: endoparasitic fungi, nematode-trapping fungi, fungi that parasitic egg and female, and toxin-producing fungi. Among the nematophagous fungi, nematode-trapping fungi which are natural enemies of nematodes are the most studied. The nematode-trapping fungi develop hyphal structures, such as hyphal, knobs, hyphal rings or branches, adhesion or mechanical capture. *Arthrobotrys* spp. are a well-known nematode-trapping fungus with biocontrol potential against root-knot nematodes, including *M. incognita*. The objective of this paper is to summarize the data on the potential for use of *Arthrobotrys* spp. in biocontrol of the root-knot nematode *M. incognita*.

Keywords: *Arthrobotrys*; Biocontrol; *Meloidogyne incognita*; Root-knot nematode.

1. INTRODUCTION

Nematodes are non-segmented roundworms that live in various habitats and are important for ecology and agricultural economy [1]. They interact with many different organisms in many food webs in a few ways, influence physical processes such as decomposition, carbon and nutrient cycling, and are used for biomonitoring or indicating broader ecosystem properties such as resilience, evolutionary hotspots, energy or nutrient transfers

[2]. According to studies, approximately 26,000 nematode species have been reported so far, with 4100 of them being plant parasites [3].

Nematode damage on plants has a high economic value. However, plant-parasitic nematodes (PPNs) are a serious threat to crops, causing an estimated annual yield loss of over 215 billion $US worldwide [4]. Root-knot nematodes (RKNs; *Meloidogyne* spp.) (Heteroderidae, Tylenchida) are the most yield-limiting group of PPNs. RKNs are obligate sedentary endoparasites which can easily reproduce in roots of over 5.500 plant species [5]. On the other hand, RKNs are the most extensively distributed in tropical and subtropical countries, and their population in the soil increase easily under appropriate temperature, host and soil conditions. RKNs prefers to sandy loam soils and are more threatening in tropical climates than in temperate climates [6]. The infective second-stage juveniles (J2) enter to plant roots and feed on the roots, they trigger a proliferation process of the attacked cells, and cause swellings (root knots) that can be easily seen. Furthermore, wilting, stunting, and chlorotic symptoms were easily visible in the affected plants' foliar parts [7]. Besides, the injury caused by RKNs during the root penetration process facilitates the invasion of other harmful microorganisms such as bacteria and fungi [8].

Arthrobotrys spp. are a well-known nematode-trapping fungus with biocontrol potential against root-knot nematodes, especially *M. incognita* [9]. The objective of this paper is to summarize the data on the potential for use of *Arthrobotrys* spp. in biocontrol of the root-knot nematode *M. incognita*.

2. *MELOIDOGYNE INCOGNITA* NEMATODE AND IMPORTANCE OF BIOCONTROL

The genus *Meloidogyne* have 105 described species in the world, the most common of which are *Meloidogyne incognita*, *M. javanica*, *M. arenaria*, *M. chitwoodi*, *M. fallax* and *M. hapla* [10,11]. *Meloidogyne incognita* belongs to the Nematoda: Heteroderidae (Table 1) and is responsible for 8 to 25% yield losses in agricultural crops worldwide [8].

Table 1. Taxonomic classification of *Meloidogyne incognita* [37].

Taxonomic ranks	
Domain	Eukaryota
Kingdom	Metazoa
Phylum	Nematoda
Class	Secernentea
Order	Thylenchida
Sub-order	Tylenchina
Superfamily	Tylenchoidea
Family	Heteroderidae
Subfamily	Meloidogyninae
Genus	*Meloidogyne*
Species	*Meloidogyne incognita* (Kofoid & White, 1919) Chitwood 1949

Meloidogyne incognita is a serious threat to the agricultural production and economy in tropical and subtropical areas of the world, particularly to vegetable crops, including tomato [12]. For instance, a study on tomato plants infested with *M. incognita* caused a reduction in yield and productivity of about 27% [13]. *M. incognita* has a very broad host range, and once introduced, it is difficult to control. Several strategies have been employed to control *M. incognita* induced crop damage, including biological control, crop rotation, chemical nematicides, the use of resistant cultivars and integrated pest management. For many years, the use of chemical nematicides has been the first choice for controlling this nematode [14]. However, chemical nematicides may cause human health problems, the destruction of natural pest enemies, the transformation of previously harmless species into pests, harm to non-target species, persistence in the food chain, and environmental pollution [14, 15-17]. The majority of conventional chemical nematicides have been banned or restricted in use recently. As a result, an immediate and effective method of controlling *M. incognita* is required [18]. Some of the environmentally friendly treatments that have been studied for their efficacy against RKN include the use of live microbes (bacteria, fungi, etc.) and/or their secondary metabolites, essential oils, plant extracts, individual and mixed acids such as organic and amino acids, natural bioactive substances, green manure, and industrial wastes. Biocontrol agents such as fungi and bacteria are considered as environmental alternative methods for the management of RKNs [19]. Biological control agents for RKN management have gained popularity in recent years, as has the biopesticide market and interest in microbial control research [20]. Among biological control agents, fungi have different suitable strategies for controlling RKN. There are many kinds of nematophagous (nematode-feeding) fungi which capture and kill nematodes in soil [9].

3. NEMATOPHAGOUS FUNGI AND USING OF *ARTHROBOTRYS* SPP. IN BIOCONTROL OF *MELOIDOGYNE INCOGNITA*

Nematophagous fungi have been studied as a source of biological nematodes in order to reduce the significant economic damage caused by plant-parasitic nematodes due to their association in the rhizosphere. More than 700 nematophagous fungi species have been described [21]. There are four main groups of nematophagous fungi categorized based on their mechanisms of attacking nematodes; (1) the nematode-trapping fungi (about 380 species) that use specialized trapping devices to capture free-living nematodes (*Arthrobotrys* sp., *Drechslerella* sp., *Nematoctonus* sp.), (2) the endo-parasitic fungi (about 120 species) that use adhesive spores to infect nematodes (*Drechmeria* sp.), (3) the egg- and cyst-parasitic fungi that infect these stages with their hyphal tips (*Pochonia* sp., *Lecanicillium* sp.), and 4) the toxin-producing fungi (about 270 species) that secrete a toxin that immobilizes nematodes prior to hyphae penetration through the nematode cuticle (*Pleurotus* sp.) [9,22].

Many soil-dwelling fungus, particularly *Purpureocillium lilacinum*, *Trichoderma harzianum*, *Fusarium* spp., *Pochonia* spp., *Chlamydosporium*, *Aspergillius* spp., and *Penicillium* spp., have been proven to be effective biological control agents [23]. Nematode-trapping and endoparasitic fungi occupied more than 73-76% of total research efforts focusing on PPNs natural enemies and antagonists [24]. Nematode-trapping fungi are found in all regions of the world, from the tropics to Antarctica [25]. They are commonly found in soils and decaying leaf litter, and wood, dung, compost and mosses [21].

The majority of nematode-trapping fungi are asexual taxa, mostly known as hyphomycetes; they are found in Zygomycota, Ascomycota and Basidiomycota, including species in the genera *Arthrobotrys, Cystopage*, *Dactylellina*, *Dactylella*, *Drechslerella*, *Hohenbuehelia*, *Hyphoderma*, *Monacrosporium*, *Nematoctonus*, *Orbilia*, *Stylopage*, *Tridentaria*, T*riposporina*, and *Zoophagus* [9,21,25]. Members of the Orbiliaceae (Ascomycota) represent the largest group of nematode-trapping fungi, which include about 96 species and are currently assigned to the asexual genera *Arthrobotrys* (53 species), *Dactylellina* (28 species), and *Drechslerella* (14 species) [9,25].

Hyphae of nematode-trapping fungi form trapping structures with an adhesive to catch the nematodes. The most common structures are three-dimensional adhesive nets of *Arthrobotrys* spp. The fungal hyphae form rings that constrict upon nematode passage, and the hyphae then penetrate the cuticle and feed on the nematode [26].

Arthrobotrys oligospora is a well-known and extensively studied nematophagous fungus that is regarded as the model of the nematode-trapping fungus. Because of the presence of a variety of trapping structures with functional nematode-capturing devices, it is also well-studied in laboratory conditions as a biocontrol agent [27]. In the studies of the fungus against root-knot nematodes under glasshouse conditions indicated that *A. oligospora* offers a good opportunity to be used as a potential biocontrol agent against *M. incognita* in tomato [7, 28-31]. Furthermore, adding *A. dactyloides* to the soil at an early stage of tomato plant development protects against *M. incognita* penetration for 10 weeks [32], which is long enough to prevent major plant damage. There has been great promise and much research in the use of nematode-trapping fungi *Arthrobotrys* species for the biocontrol of *M. incognita* (Table 2).

Table 2. Examples of *Arthrobotrys* species used for biocontrol of the root-knot nematode *Meloidogyne incognita* in a variety of crops.

***Arthrobotrys* species**	**Crops**	**References**
A. conoides	Tomato	[38]
A. dactyloides	Faba bean	[39]
A. dactyloides	Peanut	[40]
A. dactyloides	Tomato	[32]
A. dactyloides	Soil	[41]
A. irreggularis	Tomato	[34,35]
A. musiformis	Tomato	[38]
A. oligospora	Cucumber	[42]
A. oligospora	Okra	[43]
A. oligospora	Tomato	[7, 27-30]
A. oligospora	*Withania somnifera*	[33]
A. robusta	Tomato	[38]
Arthrobotrys spp.	Tomato	[44,45]

Furthermore, the application of *A. oligospora* against the root-knot nematode *M. incognita* has a significant impact on plant growth parameters [28-31, 33]. The increase in plant growth parameters may be referred to as affecting the root system by the fungus application as the root system became more effective in water and nutrient uptake and transition. There is an immediate need to develop some efficient mass production system of the fungi at a commercial scale for field application if *A. oligospora* is to be widely used as biological control agent against RKNs. Although the potential for use of the nematode-trapping fungi *Arthrobotrys* is high there have been few successes resulting in commercial products (Table 3).

Table 3. Commercial biological nematicides based on *Arthrobotrys* spp.

Products	**Country of origin**	***Arthrobotrys* spp.**	**References**
Royal 350	France	*A. irregularis*	[46]
Royal 300	France	*A. robusta*	[46]
Adx-1004	Egypt	*A. dactyloides*	[40]
REM G®	Italy	*Arthrobotrys* spp.	[47]

Arthrobotrys irregularis has been tested for management of *M. incognita* on tomato. A commercial product based on *A. irregularis*, Royal 350, was used in pot and field experiments [34,35], as well as in the laboratory [34]. Sterilized soil was used in the pot experiment, and *Arthrobotrys* was applied 40 days before planting and inoculation with *M. incognita* J2s. In field tests, the fungus was applied to soil infested with *M. incognita*. Both researchers used the same dose (140 g/m^2) and achieved significant control. It is necessary to improve some efficient mass production system of the fungi at a commercial scale for field application.

4. CONCLUSION

Controlling RKNs is usually difficult due to being polyphagous and their high reproduction rate and short generation time [36]. However, biocontrol agents such as *Arthrobotrys* spp. fungi provide long-term and effective solutions against RKNs. Furthermore, the fungi promote plant growth in the plants that they colonize, resulting in a beneficial double effect. Plants will be better protected against RKNs in the long run if biological control agents are used in conjunction with other management strategies as part of an Integrated Pest Management (IPM) program. To protect the environment and human health from chemical pesticides, commercial bio-nematicides can be developed for global use. Isolating indigenous strains is the best way to ensure the success of these biological control agents because they are then adapted to the pest and the environment in which they will be used. The formulation of these strains should be developed to ensure both good microorganism conservation and high effectiveness against pests. On the other hand, the most significant impediment we face is the bureaucracy of product registration.

Author Contributions: Conceptualization: CE. Data Curation: GU, SÇ. Formal Analysis: ES. Funding Acquisition: CE. Investigation: DD, ES. Methodology: GU, SÇ. Project Administration: CE. Resources: DD, KÇ. Supervision: CE. Visualization: DD. Writing - original draft: CE, KÇ. Writing - review and editing: CE, GU, DD, SÇ, ES, KÇ. All authors read and approved the final version of the manuscript.

Conflict of interest: The author declares no potential conflict of interest.

Acknowledgments: We thank the Scientific and Technological Research Council of Türkiye (TUBITAK) for the support of this work (TOVAG 221O399).

Source of Funding: This study was supported by a grant from the Scientific and Technological Research Council of Türkiye (TUBITAK-TOVAG 221O399).

REFERENCES

1. Hodda M. Phylum Nematoda: A classification, catalogue and index of valid genera, with a census of valid species. Zootaxa. 2022; 5114(1): 1-289.
2. Hodda M. Phylum Nematoda: Feeding habits for all valid genera using a new, universal scheme encompassing the entire phylum, with descriptions of morphological characteristics of the stoma, a key, and discussion of the evidence for trophic relationships. Zootaxa. 2022; 5114(1): 318-451.
3. Bhat KA, Mir RA, Farooq A, Manzoor M, Hami A, Allie KA, et al. Advances in nematode identification: A journey from fundamentals to evolutionary aspects. Diversity. 2022; 14(7): 536.
4. Ferreira JM, Carreira DN, Braga FR, Soares FEF. First report of the nematicidal activity of *Flammulina velutipes*, its spent mushroom compost and metabolites. 3 Biotech. 2019; 9: 410.
5. AbdelRazek GM, Yaseen R. Effect of some rhizosphere bacteria on root-knot nematodes. Egypt J Biolog Pest Control. 2020; 30(1): 140.
6. De Waele D, Elsen A. Challenges in tropical plant nematology. Annu Rev Phytopathol. 2007; 45(1): 457-485.

7. Soliman MS, El-Deriny MM, Ibrahim DSS, Zakaria H, Ahmed Y. Suppression of root-knot nematode *Meloidogyne incognita* on tomato plants using the nematode trapping fungus *Arthrobotrys oligospora* Fresenius. J Appl Microbiol. 2021; 131(5): 2402-2415.
8. Rocha TL, Soll CB, Boughton BA, Silva TS, Oldach K, Firmino AAP, et al. Prospection and identification of nematotoxic compounds from *Canavalia ensiformis* seeds effective in the control of the root knot nematode *Meloidogyne incognita*. Biotechnol Res Innov. 2017; 1(1): 87-100.
9. Yang J, Zhang K. Biological control of plant-parasitic nematodes by nematophagous fungi. In: Nematode-trapping fungi. Springer, 2014: 231-262.
10. Ghaderi R, Karssen G. An updated checklist of *Meloidogyne* Göldi, 1887 species, with a diagnostic compendium for second-stage juveniles and males. J Crop Prot. 2020; 9(2): 183-193.
11. Maleita C, Cardoso J, Rusinque L, Esteves I, Abrantes I. Species-specific molecular detection of the root knot nematode *Meloidogyne luci*. Biology. 2021; 10(8): 775.
12. Tariq-Khan M, Munir A, Mukhtar T, Hallmann J, Heuer H. Distribution of root-knot nematode species and their virulence on vegetables in northern temperate agro-ecosystems of the Pakistani-administered territories of Azad Jammu and Kashmir. J Plant Dis Prot. 2016; 124(3): 201-212.
13. Kaur D, Sharma SK, Sultan MS. Effect of different chemicals on root knot nematode in seed beds of tomato. Plant Dis Res. 2011; 26: 170-170.
14. Ntalli NG, Caboni P. Botanical nematicides: A review. J Agric Food Chem 2012; 60(40): 9929-9940.
15. Berny P. Pesticides and the intoxication of wild animals. J Vet Pharmacol Ther. 2007; 30(2): 93-100.
16. Elbadri GA, Lee DW, Park JC, Yu HB, Choo HY. Evaluation of various plant extracts for their nematicidal efficacies against juveniles of *Meloidogyne incognita*. J Asia Pac Entomol. 2008; 11(2): 99-102.
17. Xiang N, Lawrence KS Donald PA. Biological control potential of plant growth-promoting rhizobacteria suppression of *Meloidogyne incognita* on cotton and *Heterodera glycines* on soybean: A review. J Phytopathol. 2018; 166(7-8): 449-458.
18. Zukerman BM, Esnard J. Biological control of plant nematodes-current status and hypotheses. Nematol Res. 1994; 24: 1-13.
19. Collange B, Navarrete M, Peyre G, Mateille T, Tchamitchian M. Rootknot nematode (Meloidogyne) management in vegetable crop production: the challenge of an agronomic system analysis. Crop Prot. 2011; 30: 1251-1262
20. Radwan MA, Farrag SAA, Abu-Elamayem MM, Ahmed NS. Biological control of the root-knot nematode, Meloidogyne incognita on tomato using bioproducts of microbial origin. Appl Soil Ecol. 2012; 56: 58-62.
21. Zhang Y, Li GH, Zhang KQ. A review on the research of nematophagous fungal species. Mycosystema. 2011; 30(6): 836-845.
22. Lopez-Llorca LV, Maciá-Vicente JG, Jansson HB. Mode of Action and Interactions of Nematophagous Fungi. In: Ciancio A, Mukerji KG (Eds.), Integrated Management and Biocontrol of Vegetable and Grain Crops Nematodes. Springer 2008: 51-76.
23. Khan RAA, Najeeb S, Mao Z, Ling J, Yang Y, Li Y, Xie B. Bioactive secondary metabolites from *Trichoderma* spp. against phytopathogenic bacteria and root-knot nematode. Microorganisms. 2020; 8: 401.
24. Bilgrami AL. Biological control potentials of predatory nematodes. In: Ciancio A, Mukergi KG (Eds.). Integrated Management and Biocontrol of Vegetable and Grain Crops Nematodes, Springer, 2008: 3-28.
25. Jiang X, Xiang M, Liu X. Nematode-trapping fungi. Microbial Spectrum. 2017; 5(1): 12.
26. Lamovšek J, Urek G, Trdan S. Biological control of root-knot nematodes (*Meloidogyne* spp.): Microbes against the pests. Acta Agric Slov. 2013; 101(2): 263-275.

27. Khan T, Shadab S, Afroz R, Aziz MA, Farooqui M. Study of suppressive effect of biological agent fungus, natural organic compound and carbofuran on root knot nematode of tomato (*Lycopersicon esculentum*). J Microbiol Biotechnol Res. 2011; 1(1): 7-11.
28. Singh UB, Sahu A, Sahu N, Singh RK, Renu S, et al. *Arthrobotrys oligospora*-mediated biological control of diseases of tomato (*Lycopersicon esculentum* Mill.) caused by *Meloidogyne incognita* and *Rhi*zoctonia *solani.* J Appl Microbiol. 2012; 114(1): 196-208.
29. Singh UB, Sahu A, Sahu N, Singh BP, Singh RK, Renu Singh DP, et al. Can endophytic *Arthrobotrys oligospora* modulate accumulation of defence related biomolecules and induced systemic resistance in tomato (*Lycopersicon esculentum* Mill.) against root knot disease caused by *Meloidogyne incognita*. Appl Soil Ecol. 2013; 63: 45-56.
30. Bakr R, Mahdy M, Mousa ES. Biological control of Root-knot nematode *Meloidogyne incognita* by *Arthrobotrys oligospora*. EJCP. 2014; 9(1): 1-11.
31. Quevedo A, Vera-Morales M, Espinoza-Lozano F, Castañeda-Ruiz RF, Sosa del Castillo D, Magdama F. Assessing the predatory activity of *Arthrobotrys oligosporus* strain C-2197 as biocontrol of the root-knot nematode *Meloidogyne* spp. Bionatura. 2021; 6(1): 1586-1592.
32. Kumar D, Singh KP. Assessment of predacity and efficacy of *Arthrobotrys dactyloides* for biological control of root knot disease of tomato. J Phytopathol. 2006; 154(1): 1-5.
33. Sharma P, Pandey R. Biological control of root-knot nematode; *Meloidogyne incognita* in the medicinal plant; *Withania somnifera* and the effect of biocontrol agents on plant growth. Afr J Agric Res. 2009; 4(6): 564-567.
34. Cayrol J. Lutte biologique contre les *Meloidogyne* au moyen d'*Arthrobotrys irregularis*. Revue Nématol. 1983; 6: 265-273.
35. Pelagatti O, Nencetti V, Caroppo S. UtiIizzazione del formulato R350 a based i *Arthrobotrys irregularis* nel controlto di *Meloidogyne incognita*. Redia. 1986; 89: 276-283.
36. Jang JY, Choi YH, Shin TS, Kim TH, Shin K-S, Park HW, et al. Biological control of *Meloidogyne incognita* by *Aspergillus niger* F22 producing oxalic acid. PLoS ONE. 2016; 11(6): e0156230.
37. CABI. Invasive Species Compendium. 2019; CABI, Wallingford, UK.
38. Santos M, Ferraz S Muchovej JJ. Evaluation of 20 species of fungi from Brazil for biocontrol of *Meloidogyne incognita* race 3. Nematropica. 1992; 22: 183-192.
39. EL-Shanshoury A, El-Sayed SA, Mahmoud YAG, Khalefa DM. Evaluation of *Pochonia chlamydosporia*, *Paecilomyces lilacinus* and *Arthrobotrys dactyloide* as biocontrol agents for *Meloidogyne incognita* under greenhouse condition. Pak J Biol Sci. 2005; 8(11): 1511-1516.
40. Noweer E, Al-Shalaby EM. Evaluation of nematophagous fungi *Dactylaria brochopaga* and *Arthrobotrys dactyloides* against *Meloidogyne incognita* infesting peanut plants under field conditions. ABJNA. 2014; 5(5): 193-197.
41. Kumar D, Maurya N, Kumar P, Singh H, Addy SK. Assessment of germination and carnivorous activities of a nematode-trapping fungus *Arthrobotrys dactyloides* in fungistatic and fungicidal soil environment. Biol Control. 2015; 82: 76-85.
42. Soleiman AS, Moursy FI, Khalil AEM, Taher AA. Biological control of root-knot nematode, *Meloidogyne incognita* on cucumber. Annals RSCB. 2021; 25(4): 11459-11470.
43. Dhawan SC, Satyandra S. Biological control activity of *Arthrobotrys oligospora* against *Meloidogyne incognita* infesting okra. Indian J Nematol. 2011; 41(1): 70-78.
44. Eken C, Söğüt MA, Demir D, Alkan N, Göze Özdemir F. *Arthrobotrys* spp.'nin kök ur nematodlarına karşı biyonematisit olarak kullanılma olanaklarının araştırılması. 2016; TAGEM.
45. Mosa I, Ehwaeti M, Akkuzu E. Isolation of fungi associated with *Meloidogyne incognita* and their effect on its eggs hatching in the Green Mountain region of Libya. Kastamonu Univ J Forest Faculty. 2017; 17(2): 362-367.

46. Whitehead A. Plant Nematode Control. CAB International. 1998.

47. Tranier M-S, Pognant-Gros J, Quiroz RD la C, González CNA, Mateille T, Roussos S. Commercial biological control agents targeted against plant-parasitic root-knot nematodes. Braz Arch Biol Technol. 2014; 57(6): 831-841.