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# Attraction of Root Knot Nematodes, *Meloidogyne incognita*, to Living Mycelium of Nematophagous Fungi

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**MH designed the study, collected the data and wrote the manuscript. MZ and PR supervised the study and proofread the manuscript.**

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**Nematophagous fungi, Root-knot nematodes, Lecanicillium muscarium, Arthrobotrys arthrobotryoides, Attraction assay.**

## ABSTRACT

This study was designed to detect the intensity of the attraction and repulsion of nematodes to several fungi. Of the 14 fungi tested, 11 attracted root-knot nematodes, one repelled nematodes, and two were neutral. The attraction intensity was directly proportional to time and based on the nutrients provided by the nematodes. In this experiment, the nematophagous fungi *Arthrobotrys oligospora, A. superba, A. musiformis, Dactylella oviparasitica, Clonostachys rosea, Stropharia rugosoannulata, Lecanicillium muscarium*, *Trichoderma harzianum, T. viride, Pleurotus ostreatus*, and *Monacrosporium ellipsosporum* demonstrated a prominent attraction intensity. The attraction intensity of all these fungi increased with time, while that of two fungi, *Dactylaria gracilis* and *A. dactyloides*, remained neutral throughout the experiment. Only the fungus species *A. arthrobotryoides* repelled the nematodes.

## INTRODUCTION

Parasitism or predation, mechanisms by which individuals from one species utilize or kill the individuals of another species as for sources of food (Abrams, 2000), exert substantial pressure on the prey of parasites or predators. To avoid being eaten, prey must evolve specific behaviors or strategies, such as camouflage, avoidance, trickery and threat displays, against their enemies (parasites or predators) in order to enhance their survival chances. Similarly, predators and parasites have also developed aggrandized predatory or parasitic competencies to secure the food requirements for their survival and reproduction. Therefore, the evolutionary race between prey and their predators or parasites is continuous and occurs on both sides to sustain the balance of ecosystems (Dawkins and Krebs, 1979; Anwar *et al*., 2007).

The mechanism used by plant parasitic nematodes to be attracted towards their hosts is still controversial among nematologists (Jones, 1960; Klinger, 1965), although several factors, such as temperature, electrical potential (Caveness and Panzer, 1960), carbon dioxide, various organic and inorganic substances (Croll, 1970; Jansson and Nordbring-Hertz, 1979) and root exudates (Bird, 1959; Van Gundy and Rackham, 1961), are thought to be involved. It is believed that nematode amphids have chemoreceptive organs that could be involved in locating hosts (Steiner, 1925). Some believe that nematodes locate hosts by moving at random in the rhizosphere and becoming trapped around root zones that are free of water and lacking the possibility of escape (Sandstedt and Schuster, 1962). Townshend (1964) reported that *Aphelenchus avenae* and *Bursaphelenchus fungivorus* were attracted to 57 of 59 plant pathogenic and saprophyte fungi. Nematodes directly oscillated to fungi colonies without any random movements. The carbon dioxide in the rhizosphere helps nematodes locate hosts, but nematode nervous and sensory systems are sufficiently developed to detect hosts from a certain distance (Chitwood and Chitwood, 1937). Nematophagous fungi are an important and prevalent group of soil microorganisms that facilitate the suppression of nematode populations (Nordbring-Hertz *et al*., 2011). These fungi are widely distributed in both terrestrial and aquatic ecosystems from the tropics to the Polar Regions (Pramer, 1964; Nordbring-Hertz *et al*., 2011). These fungi are categorized into four broad groups according to their modes of action. They are (i) nematode-trapping fungi using adhesive or mechanical hyphal networks, (ii) endoparasitic fungi using spores, (iii) egg-parasitic fungi invading nematode eggs with their sharp hyphal tips, and (iv) toxin-producing fungi paralyzing nematodes before penetration (Swe *et al*., 2011; Moosavi and Zare, 2012). All these fungi attract the nematodes towards them by diverting them from their path to a

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plant's roots. Some fungi impede nematode movement by producing convoluted networks and prey devices, while others use toxic metabolites.

The objectives of this study were (i) to investigate the attraction of the root-knot nematode *Meloidogyne incognita* to the living mycelium of different types of nematophagous fungi and (ii) to determine the extent of nematode attraction or repulsion.

## MATERIALS AND METHODS

*Nematode culture*

The *Meloidogyne incognita* population was prepared by inoculating a single egg mass onto the susceptible tomato variety Beril. Infected roots were brought into the lab, washed with running tap water, surface sterilized in 0.5% sodium hypochlorite for 2 min, washed three additional times with sterile water, and then placed in 10 ml tubes supplemented with the antibiotic streptomycin at a concentration of 1 g/l before incubation at 25°C. After 10 days, the contents of each tube were poured onto tissue in Baermann funnels, and after 24 h, the J2 that hatched were collected, counted and used for tests (Hussain *et al*., 2016a, b, 2017a, 2018).

*Fungi culture*

Fungi already maintained on potato dextrose agar (PDA) at 20°C were cultured on corn meal agar (Difco), diluted 10-fold (CMA 1:10) and subcultured once a month. During the attraction/repulsion tests, fungi were allowed to grow almost to the edges of the plates, which took 1 to 3 weeks, depending on the species. The growth of some fungi was relatively slower than the growth of others, and some fungi were observed to produce trap devices after 3 weeks (Jansson and Nordbring-Hertz, 1979).

*Attraction assay*

The attraction and repulsion intensities of the fungi were determined based on the methods described by Jansson and Nordbring-Hertz (1979). Discs of fungal colonies (1 cm diameter) were cut with a cork borer from the edge of a colony growing on CMA 1:10 (0.2% agar, 2 mm thickness) and placed in two quadrants (I, III) on fresh CMA 1:10 plates 1 cm from the edge of the plate. In the other two quadrants (II, IV), control discs of media CMA 1:10 without fungi were placed as described in Figure 1. The plates were incubated for 24 h to allow the successful development of the diffusion of concentrated substance gradients.

A distilled water drop containing the nematode suspension (with approximately 100 juveniles) was placed in the middle of the plate. The nematodes present in, on and under the discs were counted using a stereomicroscope after 24 h. The average value was taken from quadrants I and III and similarly from quadrants II and IV. To avoid nematodes amassing in the middle of the plates, they were dried for at least 2 or 3 days before being observed under the microscope. Amassing could happen if a large quantity of nematodes were used in the plate. The number of traps (constricting rings) induced in response to the nematodes was recorded after 24 h with a microscope at 100× magnification. The trap devices were counted around the fungus base and the middle and sides of the petri plates with 9 inches diameter.

Dilute corn meal agar facilitated microscopic observations of both the mycelium and nematodes by furnishing a thin mycelial mat. The Petri plates were incubated at 28±1°C with five replications.

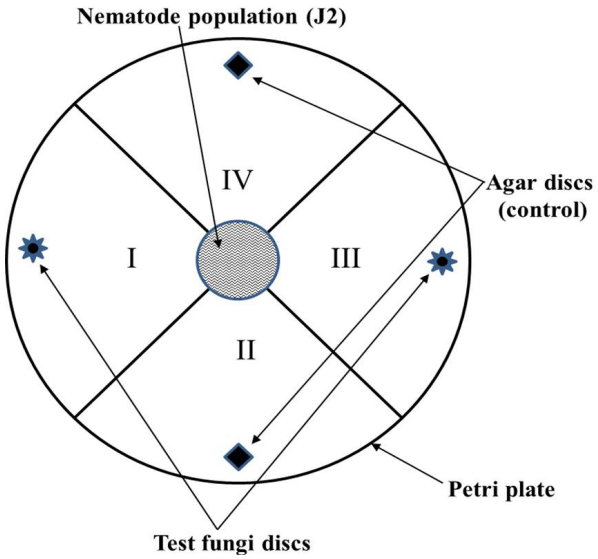


Fig. 1. Placement of test fungi and nematodes in Petri plates to determine the attraction assay.

*Attraction intensity assay*

The same procedure was adopted as described above, except that the number of nematodes aggregated under each disc was counted hourly. To ensure that the nematodes were not captured by trap-bearing nematophagous fungi, the assay was restricted to 7 h (Jansson and Nordbring-Hertz, 1979).

*Ultraviolet (UV) irradiation*

The plates were irradiated with UV light (254 nm, 2 W, 25 cm, 20 min) to neutralize the effects of nematophagous fungi. Immediately after the UV treatment, nematodes were introduced to the plates to complete the assay (Jansson and Nordbring-Hertz, 1979).

*Data analysis*

The lab experiment was conducted two times with five replications. Data were pooled and subjected to ANOVA tests; means were partitioned by the least significant difference (LSD) test by using the software Statistics 8.1.

**Table I.- Attraction of the root-knot nematodes, *Meloidogyne incognita* to several fungi on CMA 1:10 after 24 h.**

| Nematophagous fungi | Formation of trap devices | Attraction/ repulsion* | Attraction/ repulsion after UV treatment λ |
|---|---|---|---|
| *A. oligospora* | + | + | 0 |
| *A. superba* | − | + | 0 |
| *A. musiformis* | − | + | 0 |
| *A. dactyloides* | + | 0 | 0 |
| *A. arthrobotryoides* | + | − | − |
| *C. rosea* | − | + | + |
| *S. rugosoannulata* | + | + | ND |
| *L. muscarium* | − | + | 0 |
| *T. harzianum* | − | + | 0 |
| *T. viride* | − | + | 0 |
| *P. ostreatus* | − | + | ND |
| *M. ellipsosporum* | + | + | + |
| *D. oviparasitica* | + | + | 0 |
| *D. gracilis* | + | 0 | 0 |

ND, not determined; * +, attraction; −, repulsion; 0, neither attraction nor repulsion. All values significant at $P < 0.001$ at ten replications. λ, attraction/repulsion of fungi killed by UV irradiation prior to addition of nematodes.

## RESULTS

*Attraction assay*

Based on results of the attraction assay, the fungi can be divided into three groups. The first group of fungi attracted nematodes, the second did not show any activity during the experiment, and the third proved to be repellant to nematodes. Moreover, the highest intensity of the attraction of nematodes to the fungi was found in two fungi species, *L. muscarium* and *S. rugusoannulata*, which attracted over 80% of the nematodes in both quadrants (I, III) after 24 h. Out of the fourteen fungi species, eleven attracted nematodes, and two had an undetermined effect because they did not demonstrate any level of attraction. The fungus species *A. arthrobotryoides* was repellant to nematodes. Based on the trap devices observed after 24 h, the fungi with the highest attraction capability were *S. rugusoannulata*, *A. oligospora*, *A. dactyloides*, and *M. ellipsosporum*. The second highest capability was observed for *Arthrobotrys oligospora* and *Trichoderma harzianum*, which attracted more than 70% of the nematodes from both quadrants (Table I). The lowest attraction capability, irrespective of trap devices, was recorded for *Monacrosporium ellipsosporum*, as shown in Table II. Furthermore, *Clonostachys rosea, A. superba,* and *Monacrosporium ellipsosporum* were observed to be slow-growing species on corn meal agar.

**Table II.- Attraction of root knot nematodes, *Meloidogyne incognita* to different fungi after 24 h.**

| Nematophagous fungi | Fungal treated Quadrant I, III (Mean) | Control Quadrant II, IV (Mean) |
|---|---|---|
| *L. muscarium* | 41a | 3gh |
| *S. rugosoannulata* | 40a | 1hi |
| *A. oligospora* | 37b | 3gh |
| *T. harzianum* | 36b | 4fg |
| *P. ostreatus* | 28c | 4fg |
| *T. viride* | 28c | 7f |
| *C. rosea* | 27c | 1hi |
| *A. musiformis* | 26c | 4fg |
| *A. superba* | 22d | 6f |
| *D. oviparasitica* | 22d | 7f |
| *M. ellipsosporum* | 12e | 1hi |
| *A. arthrobotryoides* | 0i | 6f |
| *D. gracilis* | 0i | 7f |
| *A. dactyloides* | 0i | 6i |

All values significant at $P < 0.001$ at ten replications.

*Attraction intensity assay*

The attraction intensity assay was performed for all fungi. The results showed that with increasing time, the attraction intensity of all fungi also increased, except for three species (*A. arthrobotryoides, D. gracilis, A. dactyloides)*, which were declared as neutral during the experiment. The maximum increase in attraction over time was observed for the species *L. muscarium, S. rugusoannulata* and *Pleurotus ostreatus*, while the minimum value was found for *M. ellipsosporum, D. oviparasitica* and *A. superba*, as shown in Figure 2. The rest of the fungi showed moderate attraction intensity over time (Fig. 2).

*Ultraviolet (UV) irradiation*

To investigate the attraction/repulsion impression of nematophagous fungi, a UV irradiation experiment was employed. The results showed that after fungi were irradiated with UV light, they no longer attracted nematodes, except for the species *M. ellipsosporum*, which maintained its attraction capability. The activity of the neutral fungi remained constant.

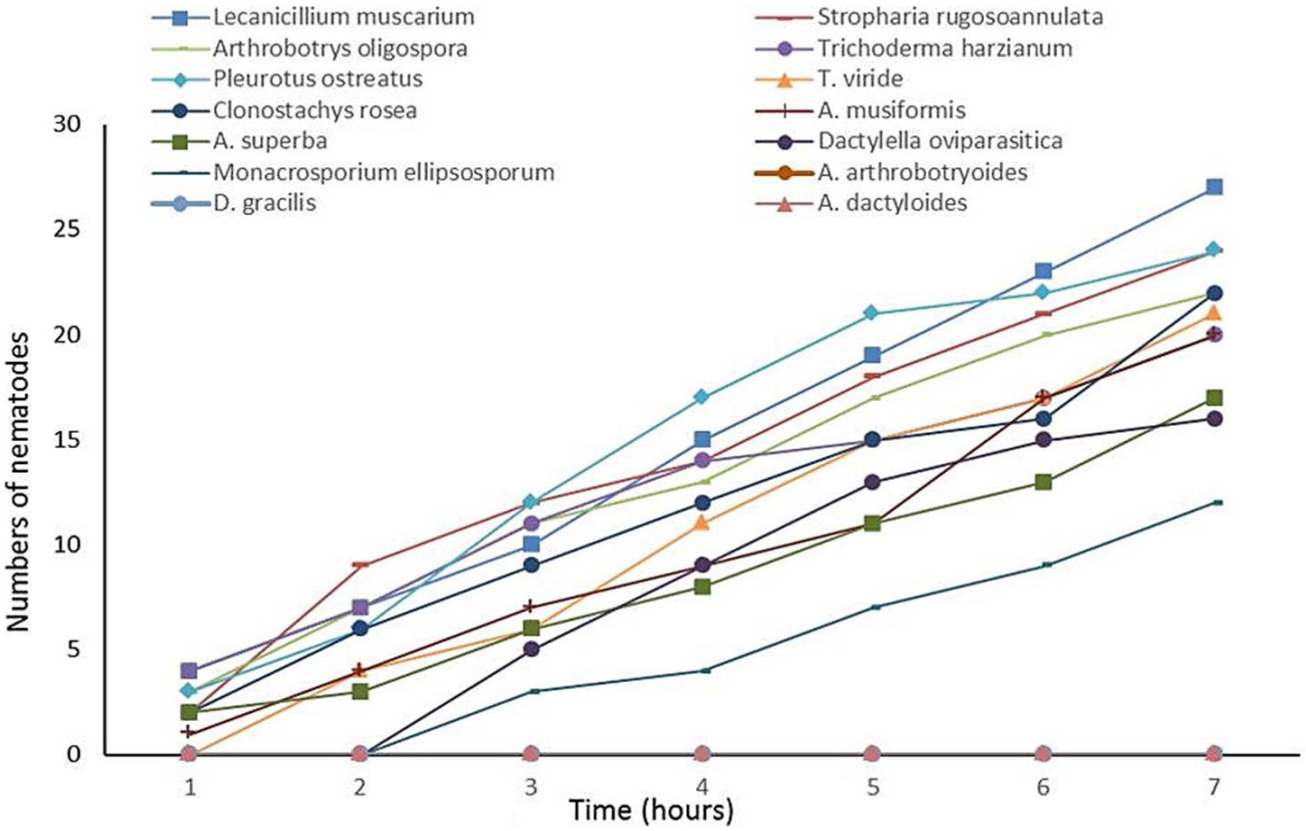


Fig. 2. Attraction intensities of nematodes to nematophagous fungi over time. Each point is the mean from five replicate plates.

## DISCUSSION

The study not only determined the attraction and repulsion of nematodes towards nematophagous fungi but also facilitated a comparison of the effects among fungi species. We observed that most of the fungi were nematophagous, except for a few that were neutral during the experiments. The attraction/repulsion intensity of the neutral fungi showed that they were non-nematophagous. We observed that fungi were capable of attracting nematodes irrespective of trap formation. According to our study, trap devices are not important to attract the nematode (Field and Webster, 1977; Jansson and Nordbring-Hertz, 1979) but are key weapons to capture and infect nematodes when fungi switch from the saprophytic to predacious phase (Yang *et al*., 2011). Moreover, it has been revealed that *A. arthrobotryoides* produces trap devices but repels the nematodes (Jansson and Nordbring-Hertz, 1979). In this study, *A. dactyloides* and *D. gracilis* produced trap devices but did not attract the nematodes. We assume that for these kinds of fungi, nematodes must be near the traps to be parasitized, and the fungi do not produce any attractant or stimulant enabling them to attract the nematodes. On the other hand, there is a small polypeptide or amino acid called "nemin" present in most nematodes (Pramer and Kuyama, 1963) that may also be responsible for the induction of trap formation. Our results contradict those of the studies conducted by Singh *et al*. (2007) reporting that *A. dactyloides* was the most efficient nematophagous fungi during *in vitro* studies by the induction of constricting rings against *Meloidogyne graminicola*. Our investigation is more closely related to attraction phenomena than parasitism. Certainly, when the nematodes get closer to the constricting rings, they could efficiently be captured and consumed by *A. dactyloides*.

The fungi *L. muscarium*, *S. rugosoannulata* and *A. oligospora* showed the maximum intensity of attraction to nematodes, which seems to suggest that they are better parasites of nematodes (Hussain *et al*., 2017b, c, d, e, f). These fungi may contain signaling substances that attract the nematodes towards them. This study also observed that almost all fungi lost their attraction capabilities after they were killed with UV light, except for *C. rosea* and *M. ellipsosporum*. This result suggests that there are volatile substances present that have the ability to attract nematodes (Jansson and Nordbring-Hertz, 1979). The measurement

of attraction intensity over time also proved that with the increase in time, attraction increases. According to Hsueh *et al*. (2017), if not lured by volatile compounds, nematodes could escape from *A. oligospora* before trap devices are fully functional after 12 h.

## CONCLUSION

Our data shows that the nematodes are attracted to nematophagous fungi during the period of trap formation and later captured by the functional traps.

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*Statement of conflict of interest*

All authors declare that there is no conflict of interest in this study.

## REFERENCES

Abrams, P.A., 2000. The evolution of predator-prey interactions: theory and evidence. *Annu. Rev. Ecol. Syst*., **31**: 79-105. https://doi.org/10.1146/annurev.ecolsys.31.1.79

Anwar, S.A., Zia, A., Hussain, M. and Kamran, M., 2007. Host suitability of selected plants to Meloidogyne incognita in the Punjab, Pakistan. *Int. J. Nematol.*, **17**: 144-150.

Bird, A.F., 1959. The attractiveness of roots to the plant parasitic nematodes *Meloidogyne javanica* and *M. hapla*. *Nematologica,* **4**: 322-335. https://doi.org/10.1163/187529259X00534

Caveness, F.E. and Panzer, J.D., 1960. Nemic galvanotaxis. *Proc. helminthol. Soc. Wash*., **27**: 73-74.

Chitwood, B.G. and Chitwood, M.B., 1937. *An introduction to nematology*, Chapter 2. Monumental Printing Co., Baltimore, Md.

Croll, N.A., 1970. *The behaviour of nematodes*. Edward Arnold, London.

Dawkins, R. and Krebs, J.R., 1979. Arms races between and within species. *Proc. R. Soc. B: Biol. Sci.*, **205**: 489-511. https://doi.org/10.1098/rspb.1979.0081

Field, J.I. and Webster, J., 1977. Traps of predacious fungi attract nematodes. *Trans. Br. Mycol. Soc.,* **68**: 467-469. https://doi.org/10.1016/S0007-1536(77)80210-6

Hsueh, Y.P., Gronquist, M.R., Schwarz, E.M., Nath, R.D., Lee, C.H., Gharib, S., Schroeder, F.C. and Sternberg, P.W., 2017. Nematophagous fungus *Arthrobotrys oligospora* mimics olfactory cues of sex and food to lure its nematode prey. *eLife*, **6**: 1-23. https://doi.org/10.7554/eLife.20023

Hussain, M., Zouhar, M., Ryšánek, P. and Anwar, S.A., 2016b. Relationship between Meloidogyne incognita density and plant growth of okra. *J. Anim. Plant Sci.,* **26**: 739-744.

Hussain, M., Kamran, M., Singh, K., Zouhar, M., Ryšanek, P. and Anwar, S.A., 2016a. Response of selected okra cultivars to Meloidogyne incognita. *Crop Prot.,* **82**: 1-6.

Hussain, M., Zouhar, M. and Ryšánek, P., 2017a. Potential of some nematophagous fungi against *Meloidogyne hapla* infection in Czech Republic. *Pakistan J. Zool*., **49**: 35-43. https://doi.org/10.17582/journal.pjz/2017.49.1.35.43

Hussain, M., Zouhar, M. and Ryšánek, P., 2017b. Effect of some nematophagous fungi on reproduction of a nematode pest, *Heterodera schachtii*, and growth of sugar beet. *Pakistan J. Zool.,* **49**: 189-196. https://doi.org/10.17582/journal.pjz/2017.49.1.189.196

Hussain, M., Zouhar, M. and Ryšánek, P., 2017c. Comparison between biological and chemical management of sugar beet nematode, *Heterodera schachtii. Pakistan J. Zool.,* **49**: 45-50. https://doi.org/10.17582/journal.pjz/2017.49.1.45.50

Hussain, M., Zouhar, M. and Ryšánek, P., 2017d. Comparison between biological and chemical management of root knot nematode, *Meloidogyne hapla. Pakistan J. Zool.,* **49**: 205-210. https://doi.org/10.17582/journal.pjz/2017.49.1.205.210

Hussain, M., Zouhar, M. and Ryšánek, P., 2017e. Population dynamics of a nematophagous fungus, *Lecanicillium muscarium,* and root knot nematode, *Meloidogyne incognita* to assess the disease pressure and its management. *Pakistan J. Zool.,* **49**: 197-204. https://doi.org/10.17582/journal.pjz/2017.49.1.197.204

Hussain, M., Zouhar, M. and Ryšánek, P., 2017f. Effects of nematophagous fungi on viability of eggs and juveniles of *Meloidogyne incognita. J. Anim. Pl. Sci*., **27**: 252-258.

Hussain, M., Zouhar, M. and Ryšánek, P., 2018. Suppression of *Meloidogyne incognita* by the entomopathogenic fungus *Lecanicillium muscarium*. *Pl. Dis.,* **102**: 977-982. https://doi.org/10.1094/PDIS-09-17-1392-RE

Jansson, H-B. and Nordbring-Hertz, B., 1979. Attraction of nematodes to living mycelium of nematophagous

fungi. *J. gen. Microbiol*., **112**: 89-93. https://doi.org/10.1099/00221287-112-1-89

Jones, F.G.W., 1960. Some observations and reflections on host finding by plant nematodes. *Meded. Landbouwhogesch. Opzoekingssta. Staat Gent*, **25**: 1009-1024.

Klingler, J., 1965. On the orientation of plant nematodes and of some other soil animals. *Nematologica*, **11**: 4-18. https://doi.org/10.1163/187529265X00438

Moosavi, M.R. and Zare, R., 2012. Fungi as biological control agents of plant-parasitic nematodes. In: *Plant defence: Biological control, progress in biological control* (eds. J.M. Merillon and K.G. Ramawat). Springer Science+Business Media, Dordrecht. pp. 67-107. https://doi.org/10.1007/978-94-007-1933-0_4

Nordbring-Hertz, B., Jansson, H.B. and Tunlid, A., 2011. *Nematophagous Fungi-eLS*. John Wiley & Sons, Ltd., Chichester.

Pramer, D. and Kuyama, S., 1963. Symposium on biochemical bases of morphogenesis in fungi. II. Nemin and the nematode-trapping fungi. *Bact. Rev.,* **27**: 282-292.

Pramer, D., 1964. Nematode-trapping fungi. *Science*, **144**: 382-388. https://doi.org/10.1126/science.144.3617.382

Sandstedt, R. and Schuster, M.L., 1962. Liquid trapping of *Meloidogyne incognita* about roots in agar medium. *Phytopathology*, **52**: 174-175.

Singh, K.P, Jaiswal, R.K, Kumar, N. and Kumar, D., 2007. Nematophagous fungi associated with root galls of rice caused by *Meloidogyne graminicola* and its control by *Arthrobotrys dactyloides* and *Dactylaria brochopaga*. *J. Phytopathol*., **155**: 193-197. https://doi.org/10.1111/j.1439-0434.2007.01208.x

Steiner, G., 1925. The problem of host selection and host specialization of certain plant-infesting nemas, and its application in the study of nemic pests. *Phytopathology*, **15**: 499-534.

Swe, A., Li, J., Zhang, K.Q., Pointing, S.B., Jeewon, R. and Hyde, K.D., 2011. Nematode-trapping fungi. *Curr. Res. environ. appl. Mycol*., **1**: 1-26

Townshend, J.L., 1964. Fungus hosts of *Aphelenchus avenae* Bastian, 1865 and *Bursaphelenchus* fungivorus Franklin & Hooper, 1962 and their attractiveness to these nematode species. *Can. J. Microbiol*., **10**: 727-737. https://doi.org/10.1139/m64-092

Van Gundy, S.D. and Rackham, R.L., 1961. Studies on the biology and pathogenicity of *Hemicycliophora arenaria*. *Phytopathology*, **51**: 393-397.

Yang, J.K., Wang, L., Ji, X.L., Feng, Y., Li, X.M., Zou, C.G., Xu, J., Ren, Y., Mi, Q., Wu, J., Liu, S., Liu, Y., Huang, X., Wang, H., Niu, X., Li, J., Liang, L., Luo, Y., Ji, K., Zhou, W., Yu, Z., Li, G., Liu, Y., Li, L., Qiao, M., Feng, L. and Zhang, K.Q., 2011. Genomic and proteomic analyses of the fungus *Arthrobotrys oligospora* provide insights into nematode-trap formation. *PLoS Pathogens*, **7**: e1002179. https://doi.org/10.1371/journal.ppat.1002179