In vitro screening of macro basidiomycetous fungi against root knot nematode, *Meloidogyne incognita*

M Themuhi, A Shanthi, AS Krishnamoorthy, N Swarnakumari and D Amirtham

**Abstract**

Biological control would be a prospective alternative to the existing chemical nematicides to keep the root knot nematode, *Meloidogyne incognita* regimented. Macrofungi are fungal species that produce fruiting bodies visible to the naked eye. Many of the macro basidiomycetous fungi produce several compounds which recorded with nematicidal and insecticidal properties. In this study, eleven macro basidiomycetes were screened in vitro against *M. incognita*. The crude culture filtrates were extracted and evaluated for their antagonistic effect on egg hatching and juvenile mortality of *M. incognita*. Crude culture filtrates of *Ganoderma lucidum* and *Lentinus edodes* were found to be toxic to egg masses and exhibit the highest mortality rate. The results revealed that the crude culture filtrates of *G. lucidum* reduced the *M. incognita* egg hatching 89.8% and increased the juvenile mortality by 93.5% which was followed by *L. edodes* which inhibited the egg hatching by 85% and caused juvenile mortality by 90.83%.

**Keywords**: Macro basidiomycetous fungi, *Meloidogyne incognita*, egg hatching, juvenile mortality

1. **Introduction**

Southern root knot nematode, *Meloidogyne incognita* is a sedentary endoparasite which is considered as a major nematode problem in India. *M. incognita* is probably the most widely distributed and economically important species of plant parasitic nematode in tropical and subtropical regions. It impedes the translocation and absorption of nutrients. Thereby it alters the physiology of the host plant and predisposing the host to diseases and environmental stresses [3]. Management of *M. incognita* is difficult as it has polyphagous nature. Current nematode management strategies are largely dependent upon highly toxic nematicides, which are harmful to the physical environment and reduce soil biodiversity by eliminating a variety of non-target species. On the other hand current effective nematicides, such as carbofuran and others will be prohibited in the future because of their negative environmental impact [7]. To overcome this negative impact of using nematicides, alternative biological control can be integrated into nematode management to reduce nematode populations below economic threshold level and avoiding yield losses. Thus, it is necessary to develop new and efficient control strategies with low toxicity to the environment and human.

Macrofungi are members of Basidiomycota, but few belong to Ascomycota. Basidiomycetous macrofungi such as bracket fungi, mushrooms and puffballs, play essential roles in maintaining forest ecosystems [8]. Many of the macro basidiomycetes produce several compounds which inhibit growth of bacteria, virus, and fungi and also recorded nematicidal and insecticidal properties [14]. A number of pharmaceutical substances with potent and unique characteristics have been extracted from Basidiomycetes. Macro basidiomycetes contain active polysaccharides in their fruiting bodies, cultured mycelia and cultured broth [15]. The application of culture filtrate and spawn of *Neonothopanus nimbis* suppressed root-knot disease incidence and root gallng in tomato plants in a greenhouse experiment [1, 2]. Sharma and Thorn *et al.* [13] proved that the toxin from the macro basidiomycetes, *Pleurotus* sp. could paralyzed the nematode. Several nematicidal-action substances were isolated from basidiomycetous fungi, including different fatty acids produced by species of *Pleurotus* [9]. Therefore, the present study was carried out to screen certain macro basidiomycetous fungi against *M. incognita* in vitro.
2. Materials and methods

2.1. Macro basidiomycetous fungi culture

In this study, eleven different macro basidiomycetous fungi were used for screening against root knot nematode *M. incognita*. *Ganoderma lucidum*, *Tremestes versicolor*, *Pycnosporus sp.*, *Schizophyllum commune*, *Pleurotus florida*, *P. eryngii*, *P. sajor caju*, *Lentinula edodes*, *Coprinopsis cinerea*, *Hypsizygus ulmaris* and *Auricularia auriculaea* were obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore - 641003. The cultures were sub-cultured in Potato Dextrose Agar medium and incubated in room temperature (28 ± 2°C).

2.2. Extractions of crude culture filtrate from macro basidiomycetous fungi

Mycelial disc of macro basidiomycetous fungi were inoculated to Potato Dextrose Broth. The macro basidiomycetous fungi were incubated for three weeks in an orbital shaker at the speed of 120 rpm at room temperature (28 ± 2°C). After three weeks filtrate were first filtered through Whatman No. 1 filter paper and then it was further filtered through 0.45μm syringe filter as per the method described by Heydari et al. [6].

2.3. In vitro screening of macro basidiomycetous fungi against *M. incognita*

The crude culture filtrates of macro basidiomycetous fungi were used for in vitro screening against *M. incognita*. The crude culture filtrates of macro basidiomycetous fungi used for an in vitro experiment at concentration of 100%. The crude culture filtrates (5ml) of each macro fungi was tested against one uniform sized egg mass which approximately contains 120 to 130 eggs placed in a small petri dish (6cm diameter) under room temperature (28 ± 2°C). Egg mass was placed in autoclaved broth and sterile distilled water served as control. Thirteen treatments with three replications were maintained. Observations were taken for every 24h up to 96h and the statistical design of this experiment was completely randomized design. Likewise, the crude culture filtrates of 5ml were tested against juvenile activity. 100 second stage juveniles (J2) were placed in a small petri dish (6cm diameter) under room temperature (28 ± 2°C). J2 which placed in autoclaved broth and sterile distilled water served as control. Thirteen treatments were replicated thrice. Observations were recorded at 24 h interval up to 96h. The nematodes were considered to be dead when they did not move on physical stimuli with a fine needle.

2.4. Statistical analysis

All data were subjected to statistical analysis of variance (ANOVA). The data were tested transformed by square root (egg hatching test) and arc sine (juvenile mortality test) transformation before being subjected to ANOVA. Means were separated using the Duncan’s multiple range test (DMRT) test at P≤0.01.

3. Results

3.1. Inhibitory effect on root knot nematode, *M. incognita* egg hatching

All the eleven macro basidiomycetous fungi showed nematostatic effect on egg mass of *M. incognita*. After 96 h, out of eleven macro basidiomycetous fungi, the lowest number of eggs hatched was recorded in crude culture filtrates of *Ganoderma lucidum* (15) (Table 1) followed by *Lentinus edodes* (19) at 100 percent concentration. The highest number of eggs hatched in control i.e., water (128) which is followed by autoclaved broth (104).

3.2. Effect on root knot nematode, *M. incognita* juvenile activity

All the eleven macro basidiomycetous fungi showed some nematicidal effect on juveniles of *M. incognita*. It was observed that the mortality of the juveniles was increased with increase in the time of exposure of crude culture filtrates. Highest mortality was observed in the crude culture filtrates of *Ganoderma lucidum* at 96 h (93.5%) (Table 2) at 100 percent concentration which was followed by *Lentinus edodes* (90.83%). The lowest mortality was recorded in control with water.

4. Discussion

An in vitro screening test was done to identify the effective macro basidiomycetous fungi against *M. incognita*. Several scientists had identified and exploited the biocontrol potential of various macro basidiomycetous fungi [5-4]. Culture filtrates of many fungi possess inhibitory activity against nematodes [13, 14], and the nematicidal action of these crude culture filtrates may involve the production of toxic metabolites produced by the fungi [9, 10]. In the current study crude culture filtrates of *G. lucidum* showed highest juvenile mortality rate and reduced egg hatching rate which was in accordance with the findings of Zhao et al. [16] in which lectin from *G. lucidum* evaluated against the soy bean cyst nematode *Heterodera glycines* and found to be effective against *H. glycines* with 43% juvenile mortality. Similarly, the culture filtrates of *Ganoderma resinaceum* inactivated the nematode by 37% within 2 h and it caused the highest inactivation of nematode after 24h and 48h by 84% and 99%, respectively [11]. They also found that the culture filtrates of *G. resinaceum* was very effective in affecting mobility of root-knot nematode *M. incognita* (J2), as well as mortality, killing more than 60% of second stage juveniles and reducing egg hatching by 40% [11]. In this, juvenile mortality rate is directly proportional to the time of exposure which is in agreement with the present investigation.

In the current study, the crude culture filtrates of *L. edodes* mitigated the hatching of eggs and caused mortality of juveniles which in agreement with the report of Hahn MH et al. [8]. They reported that *Lentinus edodes* (LEMID- Led01 and LEMID-Led02) had potential to cause mortality about 89.4 to 95.7% in *M. javanica* juveniles and also it caused immobilization of nematodes within 1h exposure and after 24h 100% nematodes were immobilized [3]. Antithetically, the culture filtrates of *L. edodes* showed 57.6% mortality of pine wilt nematode, *Bursaphelenchus xylophilus* at 72h exposure [4]. These studies were similar with the current study that the *L. edodes* had potential to inhibit the egg hatching and juvenile mortality of *M. incognita*. The mechanisms involved in increasing mortality of nematodes and reducing egg hatching can be either of biological or biochemical type. Bioactive compounds with nematicidal potency might be produced by macro basidiomycetous fungi, *G. lucidum* and *L. edodes*. It might have ruptured the gelatinous matrix of the egg mass of *M. incognita* and resulted in deformed eggs. The natural openings of the juveniles might have facilitated the toxin.
entry into the nematode body. This may be the presumable reason for the death of the infective juveniles.

5. Conclusion
This preliminary in vitro study has, however, explored that the macro basidiomycetous fungi have potential to control *M. incognita*. However, further studies should be developed to verify the efficiency of filtrates, extracts and possible interactions of the mycelium with nematodes under greenhouse and/or field conditions. Also, it is recommended to test if these macro basidiomycetous fungi have a supplemental potential for resistance induction or even plant growth.

6. Acknowledgement
I delivered my sincere gratitude to the Department of Nematology and Mushroom Research Lab, Department of Plant Pathology in Tamil Nadu Agricultural University, Coimbatore for providing macro basidiomycetous fungi culture and laboratory facilities to conduct the experiment successfully.

### Table 1: Effect of crude culture filtrates of macro basidiomycetous fungi on *M. incognita* eggmasses

| S. No. | Treatments             | Number of eggs hatched after an exposure* | 100% concentration |
|-------|------------------------|------------------------------------------|---------------------|
|       |                        | 24 h                                     | 48h                 | 72h | 96h |
| 1     | *Ganoderma lucidum*    | 2.33 (1.51)*                            | 5.67 (2.36)*        | 9.67 (3.10)* | 14.67 (3.82)* |
| 2     | *Pycnoporus spp*       | 5.67 (2.29)*                            | 27.33 (5.13)*       | 49.00 (6.99)* | 52.67 (7.25)* |
| 3     | *Schizophyllum commune*| 5.67 (2.36)*                            | 11.00 (3.31)*       | 19.67 (4.42)* | 26.00 (5.09)* |
| 4     | *Hypsicygus ulnaris*   | 18.00 (4.23)*                           | 44.00 (6.62)*       | 75.33 (8.67)* | 89.33 (9.44)* |
| 5     | *Pleurotus sajor caju* | 20.00 (4.46)*                           | 41.33 (6.42)*       | 90.67 (9.51)* | 91.33 (9.55)* |
| 6     | *F. florida*           | 21.33 (4.60)*                           | 46.67 (8.84)*       | 76.00 (8.71)* | 93.00 (9.63)* |
| 7     | *P. eryngii*           | 17.33 (4.15)*                           | 36.00 (5.99)*       | 71.00 (8.42)* | 95.00 (9.73)* |
| 8     | *Auricularia auriculata*| 14.00 (3.73)*                         | 15.33 (3.91)*       | 23.00 (4.78)* | 30.00 (5.47)* |
| 9     | *Trametes versicolor*  | 21.67 (4.64)*                           | 42.67 (6.52)*       | 88.00 (9.37)* | 101.67 (10.07)* |
| 10    | *Coprinopsis cinerea*  | 8.33 (2.87)*                            | 17.00 (4.12)*       | 27.33 (5.22)* | 31.67 (5.62)* |
| 11    | *Lentinula edodes*     | 3.67 (1.91)*                            | 7.00 (2.63)*        | 15.00 (3.87)* | 19.33 (4.38)* |
| 12    | Control (Broth)        | 27.00 (5.16)*                           | 67.67 (8.21)*       | 92.00 (9.58)* | 104.00 (10.18)* |
| 13    | Control (Water)        | 33.67 (5.78)*                           | 78.67 (8.86)*       | 97.67 (9.87)* | 128.00 (11.30)* |
| SEd   | 0.31                   | 0.33                                    | 0.17                | 0.18          |
| CD(P=0.01) | 0.86                 | 0.92                                    | 0.47                | 0.51          |

*values are mean of three replications, figures in parentheses are square root transformed value

In column means followed by a different letter are significantly different from each other at 1 percent level by DMRT

### Table 2: Effect of crude culture filtrates of macro basidiomycetous fungi on *M. incognita* juveniles

| S. No. | Treatments             | Number of juveniles dead after exposure of* | 100% concentration |
|-------|------------------------|------------------------------------------|---------------------|
|       |                        | 24h                                     | 48h                 | 72h | 96h |
| 1     | *Ganoderma lucidum*    | 86.50 (9.29)*                           | 89.50 (9.45)*       | 91.50 (9.04)* | 93.50 (9.66)* |
| 2     | *Pycnoporus spp*       | 19.83 (4.43)*                           | 22.17 (4.71)*       | 34.17 (5.84)* | 40.50 (6.36)* |
| 3     | *Schizophyllum commune*| 72.17 (8.49)*                           | 77.83 (8.81)*       | 81.38 (9.04)* | 83.50 (9.13)* |
| 4     | *Hypsicygus ulnaris*   | 18.50 (4.29)*                           | 17.50 (4.17)*       | 22.50 (4.73)* | 27.83 (5.26)* |
| 5     | *Pleurotus sajor caju* | 21.17 (4.59)*                           | 29.17 (5.39)*       | 32.50 (5.69)* | 41.43 (6.46)* |
| 6     | *F. florida*           | 19.50 (4.40)*                           | 27.50 (5.23)*       | 30.83 (5.55)* | 38.83 (6.22)* |
| 7     | *P. eryngii*           | 24.50 (4.92)*                           | 30.50 (5.52)*       | 34.50 (5.86)* | 38.17 (6.16)* |
| 8     | *Auricularia auriculata*| 52.17 (7.21)*                          | 60.83 (7.79)*       | 70.50 (8.39)* | 70.83 (8.41)* |
| 9     | *Trametes versicolor*  | 11.50 (3.38)*                           | 16.50 (4.05)*       | 20.50 (4.52)* | 22.40 (4.73)* |
| 10    | *Coprinopsis cinerea*  | 57.83 (7.6)*                            | 63.50 (7.96)*       | 73.50 (8.56)* | 77.50 (8.79)* |
| 11    | *Lentinula edodes*     | 80.50 (8.96)*                           | 85.17 (9.22)*       | 87.83 (9.36)* | 90.83 (9.52)* |
| 12    | Control (Broth)        | 2.17 (1.38)*                            | 10.83 (3.28)*       | 19.17 (4.36)* | 24.83 (4.97)* |
| 13    | Control (Water)        | 0.50 (0.70)*                            | 2.83 (1.64)*        | 6.17 (2.46)* | 11.30 (3.38)* |
| SEd   | 0.23                   | 0.15                                    | 0.14                | 0.15          |
| CD(P=0.01) | 0.63                 | 0.41                                    | 0.41                | 0.42          |

*values are mean of three replications, figures in parentheses are arc sin transformed values

In column means followed by a different letter are significantly different from each other at 1 percent level by DMRT

**References**

1. Bua-art S, Saksirirat W, Hiransalee A, Kanokmedhakul S, Lekphrom R. Effect of bioactive compound from luminescent mushroom (*Neonothopanus nambi* Speg.) on root-knot nematode (*Meloidogyne incognita* Chitwood) and non-target organisms. Asia-Pacific Journal of Science and Technology 2011;16(4):331-341.
2. Bua-art S, Saksirirat W, Hiransalee A, Kanokmedhakul S, Lekphrom R. Extraction of bioactive compounds from luminescent mushroom (*Neonothopanus nambi*) and its effect on root knot nematode (*Meloidogyne incognita*). Asia-Pacific Journal of Science and Technology 2010;15(8):726-737.
3. Chitwood DJ. Phytochemical based strategies for nematode control. Annual Review of Phytopathology 2002;40(1):221-249.
4. Dong JY, Li XP, Li L, Li GH, Liu YJ, Zhang KQ. Preliminary results on nematicidal activity from crude culture filtrates of Basidiomycetes against the pine wood nematode, *Bursaphelenchus xylophilus*
(Aphelenchoididae). Annals of Microbiology 2006;56(2):163.
5. Hahn MH, De Mio LLM, Kuhn OJ, Duarte HDSS. Nematophagous mushrooms can be an alternative to control Meloidogyne javanica. Biological Control 2019;138:104024.
6. Heydari R, Pourjam E, Goltapeh EM. Antagonistic effect of some species of Pleurotus on the root-knot nematode, Meloidogyne javanica in vitro. Plant Pathology Journal 2006;5(2):173-177.
7. Khun-in A, Sukhakul S, Chamswarng C, Tangkijchote P, Sasnarukkit A. Crude culture filtrates of Pleurotus ostreatus isolate Poa3 effect on egg mass hatching and juvenile of Meloidogyne incognita and its potential for biological control. Journal of ISSAAS (International Society for Southeast Asian Agricultural Sciences) 2015;21(1):46-54.
8. Lee WD, Lee H, Fong JJ, Oh SY, Park MS, Quan Y, Lim YW. A checklist of the basidiomycetous macro fungi and a record of five new species from Mt. Oseo in Korea. Mycobiology 2014;42(2):132-139.
9. Li G, Zhang K, Xu J, Dong J, Liu Y. Nematicidal substances from fungi. Recent Patents on Biotechnology 2007;1(3):212-233.
10. Liu JH, Wang L, Qiu JY, Jiang LL, Yan JY, Liu T et al. Nematicidal activity of Gymnoascus reesii against Meloidogyne incognita. African Journal of Microbiology Research 2011;5(18):2715-2719.
11. Mariam Al abed Al kader. In vitro Studies on Nematode Interactions with their Antagonistic Fungi in the Rhizosphere of Various Plants. Forest Botany Institute in Freiburg im Breisgau, Germany 2008.
12. Thorn RG, Jean-Marc Moncalvo, Reddy CA, Rytas Vilgalys. Phylogenetic analyses and the distribution of nematophagy support a monophyletic Pleurotaceae within the polyphylectic pleurotoid-lentinoid fungi. Mycologia 2000;92(2):241-252.
13. Sharma SG, Singh VK. Biological efficiency and cellulose activities of early and late fruiting Pleurotus spp. Mushroom Research 1999, 8(1).
14. Sivanandhan S, Khuro A, Paulraj MG, Ignacimuthu S, AL-Dhabi NA. Biocontrol properties of Basidiomycetes: an overview. Journal of Fungi 2017;3(1):2.
15. Wasser SP. Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. Applied Microbiology and Biotechnology 2011;89(5):1323-1332.
16. Zhao S, Guo YY, Liu QH, Wang HX, Ng TB. Lectins but not antifungal proteins exhibit anti-nematode activity. Environmental Toxicology and Pharmacology 2009;28(2):265-268.