Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(6): 641-644

Received: 12-09-2020
Accepted: 15-10-2020

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

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DOI: https://doi.org/10.22271/j.ento.2020.v8.i6i.7919

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 nimbi* suppressed root-knot disease incidence and root galling 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 paralyze 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. erynjii, P. sajor caju, Lentinula edodes, Coprinopsis cinerea, Hypsizygus ulmaris* and *Auricularia auriculata* 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 ± 2^0C).

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 orbiter shaker at
the speed of 120 rpm at room temperature (28 ± 2^0C). 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 ± 2^0C). 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 (J_2) were placed in a small petri dish (6cm diameter) under room temperature (28 ± 2^0C). J_2 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 resinacium* 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.* [5]. 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 [5]. Antithetically, the culture filtrates of *L. edodes* showed 57.6% mortality of pine wilt nematode, *Bursaphelenchus xylophillus* 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)^{a}	5.67 (2.36)^{a}	9.67 (3.10)^{a}	14.67 (3.82)^{a}
2	*Pycnosporus spp*	5.67 (2.29)^{bc}	27.33 (5.13)^{e}	49.00 (6.99)^{f}	52.67 (7.25)^{e}
3	*Schizophyllum commune*	5.67 (2.36)^{bc}	11.00 (3.31)^{bc}	19.67 (4.42)^{c}	26.00 (5.09)^{c}
4	*Hypsizygus ulmaris*	18.00 (4.23)^{de}	44.00 (6.62)^{fg}	75.33 (8.67)^{g}	89.33 (9.44)^{f}
5	*Pleurotus sajor caju*	20.00 (4.46)^{e}	41.33 (6.42)^{fg}	90.67 (9.51)^{h}	91.33 (9.55)^{f}
6	*P. florida*	21.33 (4.60)^{ef}	46.67 (6.82)^{g}	76.00 (8.71)^{g}	93.00 (9.63)^{f}
7	*P. eryngii*	17.33 (4.15)^{de}	36.00 (5.99)^{f}	71.00 (8.42)^{g}	95.00 (9.73)^{fg}
8	*Auricularia auriculata*	14.00 (3.73)^{d}	15.33 (3.91)^{c}	23.00 (4.78)^{d}	30.00 (5.47)^{cd}
9	*Trametes versicolor*	21.67 (4.64)^{ef}	42.67 (6.52)^{fg}	88.00 (9.37)^{h}	101.67 (10.07)^{gh}
10	*Coprinopsis cinerea*	8.33 (2.87)^{c}	17.00 (4.12)^{d}	27.33 (5.22)^{e}	31.67 (5.62)^{d}
11	*Lentinula edodes*	3.67 (1.91)^{ab}	7.00 (2.63)^{ab}	15.00 (3.87)^{b}	19.33 (4.38)^{b}
12	Control (Broth)	27.00 (5.16)^{fg}	67.67 (8.21)^{h}	92.00 (9.58)^{hi}	104.00 (10.18)^{h}
13	Control (Water)	33.67 (5.78)^{g}	78.67 (8.86)^{h}	97.67 (9.87)^{i}	128.00 (11.30)^{i}
	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 DM

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)^{a}	89.50 (9.45)^{a}	91.50 (9.04)^{a}	93.50 (9.66)^{a}
2.	*Pycnosporus spp*	19.83 (4.43)^{e}	22.17 (4.7)^{e}	34.17 (5.84)^{de}	40.50 (6.36)^{e}
3.	*Schizophyllum commune*	72.17 (8.49)^{b}	77.83 (8.81)^{b}	81.83 (9.04)^{b}	83.50 (9.13)^{b}
4.	*Hypsizygus ulmaris*	18.50 (4.29)^{e}	17.50 (4.17)^{f}	22.50 (4.73)^{f}	27.83 (5.26)^{f}
5.	*Pleurotus sajor caju*	21.17 (4.59)^{de}	29.17 (5.39)^{d}	32.50 (5.69)^{de}	41.83 (6.46)^{e}
6.	*P. florida*	19.50 (4.40)^{e}	27.50 (5.23)^{d}	30.83 (5.55)^{e}	38.83 (6.22)^{e}
7.	*P. eryngii*	24.50 (4.92)^{d}	30.50 (5.52)^{d}	34.50 (5.86)^{d}	38.17 (6.16)^{e}
8.	*Auricularia auriculata*	52.17 (7.21)^{c}	60.83 (7.79)^{c}	70.50 (8.39)^{c}	70.83 (8.41)^{d}
9.	*Trametes versicolor*	11.50 (3.38)^{f}	16.50 (4.05)^{f}	20.50 (4.52)^{fg}	22.50 (4.73)^{g}
10.	*Coprinopsis cinerea*	57.83 (7.6)^{c}	63.50 (7.96)^{c}	73.50 (8.56)^{c}	77.50 (8.79)^{c}
11.	*Lentinula edodes*	80.50 (8.96)^{a}	85.17 (9.22)^{a}	87.83 (9.36)^{a}	90.83 (9.52)^{a}
12.	Control (Broth)	2.17 (1.38)^{g}	10.83 (3.28)^{g}	19.17 (4.36)^{g}	24.83 (4.97)^{fg}
13.	Control (Water)	0.50 (0.70)^{h}	2.83 (1.64)^{h}	6.17 (2.46)^{h}	11.50 (3.38)^{h}
	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 sine transformed values
In column means followed by a different letter are significantly different from each other at 1 percent level by DMRT

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