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BIOCIDAL EFFICACY OF *AGARICUS BISPORUS* ON ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA*

Akinlabi RUFAI[1], Suwebat AKINYEMI[2*], Temitope ADUROJA[2]
[1]Department of Zoology, Osun State University, Osogbo, Osun State, Nigeria
[2]Department of Plant Biology, Osun State University, Osogbo, Osun State, Nigeria
**Corresponding author's e-mail: suwebat.akinyemi@uniosun.edu.ng*
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ABSTRACT
The root damage caused by nematodes is a major challenge in the cultivation of agricultural and horticultural crops worldwide. The aim of this study was to evaluate the efficacy of aqueous extracts of Agaricus bisporus for the control of the root-knot nematode, Meloidogyne incognita. 0, 5, 10, 15 and 20% concentrations of the stock solution of Agaricus bisporus were applied to test the nematicidal activities of the mushroom. The root-knot nematode was extracted using centrifugal floatation method. Application of mushroom extracts on the extracted nematodes were done by placing soil into beakers labelled A, B, C, D and E. This was further divided by weight into five equal portions labelled A1 through A5, B1 through B5, C1 through C5, D1 through D5 and E1 through E5 respectively. Observation was made through microscope to distinguish between the motile and dead nematodes. The result shows the death rate of the nematode is proportional to the concentrations of the extracts of Agaricus bisporus and the duration of application. The study suggests that extracts of Agaricus bisporus are efficient for the extermination of Meloidogyne incognita at the application of 15% concentration for 3 days and 20% concentration for 2½ days.
KEY WORDS: *Agaricus bisporus, Meloidogyne incognita, biocontrol*

INTRODUCTION

Plant-parasitic nematodes have been in existence for decades and continuously pose threat to the cultivation of crops (Williamson *et al*., 2003; Bernard *et al*., 2017). Root-knot nematodes belonging to the genus, *Meloidogyne* are found in a wide range of agricultural crops (Gaziea *et al*., 2020). Unlike other diseases and pests, root-knot nematodes survive by feeding directly off the nutrients pumped through the roots of plants. They form galls that can reach up to an inch wide where they hide and reproduce, causing a number of symptoms that point to problems in infected plants' transport systems (Davis *et al*., 2000).

Pesticides have been in use for several years for the control of parasitic nematodes of crops (Manzanilla-López *et al*., 2002). However, the use of pesticides

has resulted in increased environmental pollution and resistance of several species of nematodes (Torres-Acosta *et al*., 2012; Pérez-Cogollo *et al*., 2018) which has led to the interest in seeking alternatives for the control of plant-parasitic nematodes worldwide.

Certain reports suggest that mushrooms have antihelminthic, antiviral, antibacterial and antifungal properties (Jasrotia *et al*., 2012; Wang *et al*., 2012). Antioxidant and antitumor activities have also been recorded (Ding *et al*., 2012; Abol Hassan *et al*., 2015). Currently, many substances are derived from mushrooms and used as dietary supplements (Silva *et al*., 2012; DeAssuncao *et al*., 2012) and to lower cholesterol (Jayakumar *et al*., 2007; Regina *et al.*, 2008). Mushrooms are considered an important source of chemicals that could be used for plant protection or as organic compounds with insecticidal properties (Wang *et al*., 2002). Several researchers have demonstrated that mushrooms and spent mushroom compost contain a number of active compounds, including phenolic compounds, flavonoids, alkaloids, organic acids, flavones, anthocyanins, polyketides, terpenoids and steroids (Elmastas *et al*., 2009; Jayakumar *et al*., 2011; Keles *et al*., 2011; Aslam *et* al., 2013; Fpa *et al*., 2013; Mondal *et al.*, 2013).

Nematophagous fungi refer to a diverse group of fungi which colonize and parasitize nematodes for exploitation of nutritious substances. Some of them are obligate parasites of nematodes, but the majority are facultative saprophytes. They are usually regarded as soil inhabitants, however, they can be found in aquatic environment. Nematophagous fungi are found in most fungal taxa such as *Ascomycetes (anamorphic Orbiliaceae and Clavicipitaceae), Basidiomycetes (Pleurotaceae), Zygomycetes (Zoopagales), Chytridiomycetes and Oomycetes*. It is suggested that the nematophagous habit evolved from lignolytic and cellulolytic fungi in different fungal taxonomic groups, as an adaptation to conquer competition for nutritious substances in soil. Nematophagous fungi are reportedly used in the biological control of nematodes (Poveda *et al*., 2020). These fungi produce antagonistic substances that are capable of killing the nematodes (Tranier *et al*., 2014).

Okorie *et al*. (2011) reported that *Pleurotus spp*. was able to control *Meloidogyne incognita* in soybean, evident from reduction in number of galls and promotion of plant growth. In the study conducted by Luo *et al*. (2017), the root-knot-nematode, *Meloidogyne arenaria* was exposed to *Coprinus comatus* mycelia. The findings showed that nematodes were paralyzed by 95.8% in an 8 h exposure period. The aqueous extracts of *Pleurotus ostreatus, P. ostreatoroseus, P. citrinopileatus, P. sajor-caju* and *P. pulmonarius* showed some nematostatic and nematicidal activity against the eggs and second juvenile stage of *M. incognita* found on lettuce (Wille *et*

al., 2019). A study evaluated the extracts obtained from the fruiting body of *P. ostreatus* against the nematode *Ditylenchus dipsaci*, during 1 h exposure. High nematode mortalities (95%) were reported for the acetonic and methanolic extracts respectively (Aldaz-Merchán *et al*., 2018).

There are numerous studies on the use of different species of edible mushrooms, especially those belonging to the Genus, *Pleurotus*, for the biological control of nematodes (Castañeda-Ramírez, 2020). However, there is dearth of information about the roles of *Agaricus bisporus* in controlling the root-knot nematodes of crops. This study aimed to evaluate the potential of extracts of *Agaricus bisporus* on *Meloidogyne incognita*.

MATERIAL AND METHODS

The mushrooms were sourced from Agrikk Matas, Osogbo, Osun State and root-knot nematode were collected from the pot of a tomato plant in the botanical garden of Osun State University. The quantity of the materials used are presented in table 1.

Extraction of mushroom. The harvested mushrooms were transferred to a wire mesh within 30 minutes of harvest and squeezed using manual force. The filtrate was collected into a sterilized container and covered to prevent the intrusion of impurities.

Extraction of nematodes. A pot of tomato plant suspected to be infested with nematodes was obtained from the botanical garden and taken to the laboratory. The Baermann Funnel method and Centrifugal Flotation method were used for this study (Bezooijen, 2006). The Baermann funnel was used to verify the presence and potency of *Meloidogyne incognita* in the soil sample while the centrifugal flotation method was then used for the main extraction of the nematode after the application of the mushroom to the soil sample.

The Baermann funnel method. Infected soil material sample was placed in water to allow nematodes swim out of the soil sample and sink. A glass funnel was placed into a perforated wooden beam so that the funnel can pass into the beam through its narrow base, and sit on the circumference of the hole in the beam through the wider top of the funnel. A flexible tube was then tightly fitted to the tip of the funnel. The end of the tube was tightly clamped using a clip so that water does not pass through the tube unless the clip is opened. Water was poured into the funnel to about halfway of the conical part of the funnel and a circular wire mesh was placed into the cone so that it fits into it, for mechanical support of the soil load. Filter paper was then

placed on the wire mesh upon which the sieved soil was placed. The soil was placed in a manner whereby the water only gets halfway of the soil height, so that the soil absorbs the water gradually. In the event that the water does not get high enough, the filter paper was gently moved, and water was introduced from the top through a narrow tube. When the nematodes in the soil came in contact with the water, they swam downwards through the filter paper, and were collected by opening the clamp gradually.

TABLE 1: Quantity of materials used for the study

Samples	Mass of Soil (g)	Percentage of Mushroom Extract (%)	Volume of Extract (ml)	Volume of Distilled Water (ml)
A1	25	0	0.0	250.0
A2	25	0	0.0	250.0
A3	25	0	0.0	250.0
A4	25	0	0.0	250.0
A5	25	0	0.0	250.0
B1	25	5	12.5	237.5
B2	25	5	12.5	237.5
B3	25	5	12.5	237.5
B4	25	5	12.5	237.5
B5	25	5	12.5	237.5
C1	25	10	25.0	237.5
C2	25	10	25.0	237.5
C3	25	10	25.0	237.5
C4	25	10	25.0	237.5
C5	25	10	25.0	237.5
D1	25	15	37.5	212.5
D2	25	15	37.5	212.5
D3	25	15	37.5	212.5
D4	25	15	37.5	212.5
D5	25	15	37.5	212.5
E1	25	20	50.0	200.0
E2	25	20	50.0	200.0
E3	25	20	50.0	200.0
E4	25	20	50.0	200.0
E5	25	20	50.0	200.0

Application of mushroom concentrates to the nematodes. Five soil samples were collected from the pot into beakers and labelled A, B, C, D and E. Each of them was thoroughly mixed to achieve homogeneity of the sample, and then each sample was further divided by weight into five equal portions labelled A1 through A5, B1 through B5, C1 through C5, D1 through D5 and E1 through E5 respectively. The first

division was used for the application of the mushroom extract at 0%, 5%, 10%, 15%, and 20% while the second division was used for the timed observation. The time for observations was governed by the equation: $t = 12x: 2 \leq x \leq 6$. The nematodes were observed under an electronic microscope to distinguish between the motile and dead nematodes. The observation was done after every twelve hours for five iterations, and the death rate was recorded. The living and dead nematodes were distinguishable through their motility and colour, as dead nematodes were brown while the live nematodes were white in colour.

Statistical analysis. The data obtained from this research and the inferences thereof were subjected to Descriptive Statistics including mean and standard deviation and Inferential Statistics including correlation and test statistics using Microsoft Excel Software and Statistical Package for Social Sciences (SPSS).

RESULTS AND DISCUSSION

The findings obtained in this study are represented in tables 2-6 and figures 1-2.

TABLE 2: The death rate of nematodes over a course of time (hours) at different concentrations of mushroom extracts

Concentration (%)	Nematodes count	Inspection time (hours)									
		24		36		48		60		72	
		Extermi nated	Dead fraction	Extermi nated	Dead fraction	Extermi nated	Dead fraction	Extermi nated	Dead fraction	Extermi nated	Dead fraction
20	**7**	6	86%	0	86%	0	86%	1	100%	0	100%
15	**6**	4	67%	1	83%	0	83%	0	83%	1	100%
10	**6**	3	50%	0	50%	1	67%	2	83%	0	100%
5	**5**	2	40%	0	40%	1	60%	1	80%	1	100%
0	**6**	0	0%	2	33%	1	50%	1	67%	1	83%

TABLE 3: Mathematical Model of nematodes' death based on concentrates of mushroom extracts

% Concentrate	Model of Nematodes Deaths	(R^2)	Mean	Standard Deviation
0	$y = 5E-05t^3 - 0.0079t^2 + 0.505t - 8.2$	$R^2 = 0.999$	6.40	0.49
5	$y = -5E-05t^3 - 0.0079t^2 - 0.03383t + 6.2$	$R^2 = 0.9979$	5.00	0.63
10	$y = -1E-04t^3 + 0.0213t^2 + 0.901.8t + 14.4$	$R^2 = 0.986$	4.40	1.36
15	$y = -1E-04t^3 - 0.0139t^2 + 0.6528t - 5$	$R^2 = 1$	3.20	1.17
20	$y = -5E-05t^3 - 0.0074t^2 - 0.3323t + 10.4$	$R^2 = 0.8929$	2.80	1.72

TABLE 4: Mathematical Model of nematodes' death based on time of application of mushroom extracts

Time (Hours)	Model of Nematodes Deaths	R^2
24	$y = 0.0013\mathrm{c} - 0.04c^3 + 0.5667c - 3E - 13$	R^2=1
36	$y = 0.0086c^2 + 0.0486c + 1.8286$	R^2=0.9654
48	$y = 0.0057c^2 + 0.0457c + 2.8857$	R^2=0.9832
60	$y = 0.0007c^4 - 0.0287c^3 + 0.3417c^2 - 1.0833c + 4$	R^2=1
72	$y = 0.0029c^2 + 0.00429c + 4.9429$	R^2=0.9184

TABLE 5: Mathematical Model of nematodes' death rate based on concentrates of mushroom extracts

Concentration (%)	Model of Death Rate
0	$y = 15E - 05t^2 - 0.0158t - 0.505$
5	$y = -15E - 05t^2 + 0.0158t - 0.3383$
10	$y = -1E - 12t^2 - 0.0416\mathrm{t} + 0.9018$
15	$y = -1E - 12t^2 - 0.0278\mathrm{t} - 0.6528$
20	$y = -5E - 15\mathrm{t} + 0.01486t - 0.3323$

TABLE 6: Mathematical Model of nematodes' death rate based on time of application of concentrates of mushroom extracts

Time (Hours)	Model of Nematode Death
24	$y = 0.0039c^2 - 0.08\mathrm{c} + 0.566c$
36	$y = 0.0172c + 0.048c$
48	$y = 0.0104c + 0.0914$
60	$y = 0.0028c^3 - 0.0861c^2 + 0.682$
72	$y = 0.0058c + 0.00429$

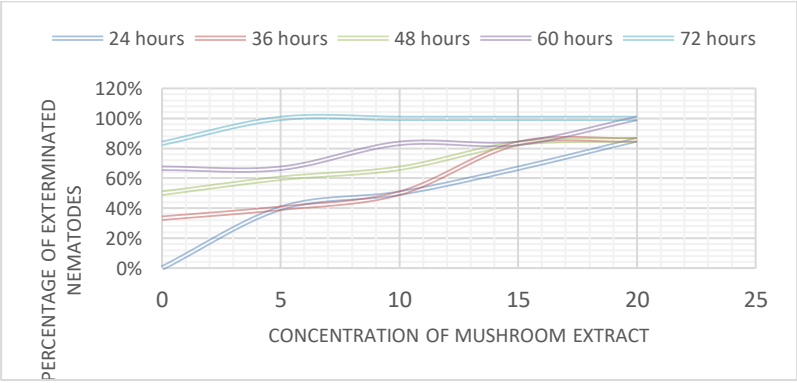

FIGURE 1: Death rate of Nematodes Relative to Concentration of Mushroom Extract

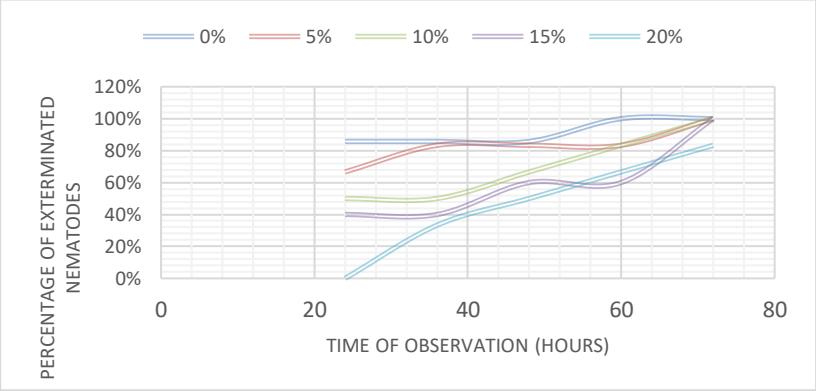

FIGURE 2: Death rate of Nematodes Relative to Time of Application of the Concentrates

The results presented in table 2 indicate that the nematodes generally reduced in number with increase in concentrations of the mushroom extracts over time. The death rate of the nematodes relative to increase in the concentration of the mushroom extracts, as well as relative to time are presented in figures 1 and 2. The trend shows that at 0% of the concentrates, 83% of the nematodes were found to have been exterminated after 72 hours. The *Meloidogyne* life cycle consists of six developmental stages: egg, four larval stages (juvenile) and adult, with an average life span of 25 days (Maleita *et al.*, 2012). The nematodes were observed to be fully matured; hence, their deaths could have occurred from natural causes at 0% concentration. Upon application of the concentrates, the study found that 100% of the nematodes were exterminated after 72 hours of application of the mushroom extracts for every concentration. The death count of the nematode occurred proportionally with time, and the death rate was observed to be greatest at 20 % concentrate. The nematicidal activities of *Agaricus bisporus* are generally under-reported; however, Muhai *et al.*, (2015) reported that the mixed use of *Agaricus bisporus* and a biological agent yielded a better control of the root-knot nematode of cucumber. Several studies have confirmed the nematicidal activities of other mushrooms. The effect of the mycelium of *Stropharia* sp. on juveniles of the second stage (J_2) of *Meloidogyne incognita*, recorded a 100% mortality of nematodes after 36 hours of exposure (Chuixu *et al*., 2013). Aqueous extracts were obtained from the fruiting bodies of several edible mushrooms such as *Amanita muscaria, Pleurotus ostreatus, P. pulmonarius, P. citrinopileatus, Lactarius deliciosus, Boletus sp.*, *Russula amethystina* and *Suillus* sp. and tested for nematicidal activities against *Meloidogyne incognita* (Wille *et al*., 2019). The mushrooms were reported to

show high nematicidal activities, with 90.7 to 100.0% mortality rate after 24 hours. *Pleurotus ostreatus* significantly reduced the infecting number of peanut root-knot nematodes, *Meloidogyne arenaria* and was effective at an application time of 20 days before sowing (Xiang *et al*., 2000). Junxianke, a product of *Beauveria bassiana* has been reported to have nematicidal activity against *Meloidogyne* spp. (Akshaya *et al*., 2021). The findings of the aforementioned research studies are similar to this study as they show that mushroom extracts have nematicidal activities.

The arithmetic means of the death rate, standard deviation and the coefficient of correlation of the data are presented in table 3. The results corroborates the discussions from figures 1 and 2 as the mean death rate of 6.4 nematodes per half day was recorded with a standard deviation of 0.49. Mathematical models of the death count of the nematodes with concentration and time as independent variables were generated in the form: $y = g(c)$, and $y = k(t)$ respectively and presented in tables 3 and 4 which can be used to extrapolate the deaths of nematodes for any desired length of time in hours and extract concentration (where y represents the expected death count, t represents the independent variable of time, $t: t = 12x: 2 \leq x \leq 6$, and c represents the independent variable of concentration, c: $c = 5x: 0 \leq x \leq 4$). These inference equations are supported by the corresponding coefficients of correlation which were found to be very strong ($0.88 \leq R^2 \leq 1$). Mathematical models of the expected death rates of the nematodes relative to time and concentration of extracts are also presented in tables 5 and 6.

This study established that the extracts of *Agaricus bisporus* concentrates are effective for the control of *Meloidogyne incognita*, and the death rate of the nematodes is proportional to the concentration of the extract and time of application. The study also revealed that the death rate of the nematodes varies for different concentrations and different length of time of application. The models presented in tables 5 and 6 may be used for the determination of nematode death rates with up to 88% to 100% accuracy. The study suggests that extracts of *Agaricus bisporus* is efficient for the extermination of *Meloidogyne incognita* at 15% concentration when applied for 3 days and 20% of the concentrates may also be used to exterminate the pest when applied for a period of 2½ days.

CONCLUSIONS

The results of this study revealed that the extracts of *Agaricus bisporus* are highly effective for the control of *Meloidogyne incognita* and thus can be explored for use as nematicides in addition to the species of *Pleurotus* that have been used over the

years. The study recommends that further research should be carried out on the effect of *Agaricus bisporus* on different life cycle stages of *Meloidogyne incognita*. A research study can also be carried out to examine the additive effects of *Agaricus bisporus* with the extracts of other mushrooms and in relation to the geophysical properties of soil.

COMPETING INTERESTS. The authors declare that there are no competing interests.
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