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ISOLATION OF NEMATOPHAGOUS FUNGI FROM DECAYED DEBRIS OF SOME ORCHARD PLANTS AROUND ZARIA, NIGERIA

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SUMMARY

Nematophagous fungi are natural enemies of nematodes. They are currently being used for biological control of plant parasitic nematodes with resounding success. In view of their potential as biocontrol agents against plant-parasitic nematodes, a survey was carried out in some orchard plants around Zaria with the view to identifying nematophagous fungi that can be deployed for bio-control of plant parasitic nematodes in Nigeria. Samples of decayed debris were collected from underneath mango, cashew and orange trees. Five samples collected from different locations under each tree were thoroughly mixed together and packaged into labelled polyethylene bags. From each sample, little debris was sprinkled on prepared water agar medium in 9 cm diameter Petri-dishes. Three pellets of goat excreta were added to boost the nutrients of the medium. Each sample was replicated four times. After 7 days, the debris was found to harbour nematophagous fungi. The isolated fungi were introduced into cavity blocks and about 100 juveniles of *Meloidogyne incognita* were pipetted into the cavities. After 6 days, 92% and 66% of the nematode juveniles were captured in the debris collected from Botanical Garden, ABU, Samaru and Wusasa area, respectively. The fungi were identified to be Arthrobotrys species based on their morphological structure and trapping devices (three-dimensional adhesive nets and constricting rings). These nematophagous fungi will be utilized to test their performance as bio-control agents for the control of plant-parasitic nematodes.

Key words: Nematophagous fungi, isolation, *Meloidogyne* species.

Biological control which is an alternative to nematicides has been gaining ground and becoming important in recent years, most especially, the use of natural enemies within the same environment to control plant parasitic nematodes and such natural enemies of nematodes that their population is abundant in all types of soils is the predacious fungi. These fungi have a significant contact with nematodes in their vicinity and thus, can constantly destroy nematodes in nearly all soil at different geographical areas (17). Nematophagous fungi have been the subject of research over several decades in fundamental studies of their ecology, distribution, systematic and as potential biological control agents of nematode pathogens of plants and animals (11, 13, 8;

(3). The primary function of these fungi appears to be that of plant decay to obtain carbon and hence they are cellulolytic or lingo-cellulolytic fungi (5). However, predation of nematodes by these fungi adds extra protein (nitrogen) to their system and reduces the carbon to nitrogen ratio to low proportion (5). (4) Suggested that the parasitic habit of nematode-trapping fungi has evolved among cellulolytic fungi as a response to nutrient deficiencies. In such environment where plant debris is in abundance with high carbon, nematodes might serve as an important source of nitrogen during growth on carbohydrate containing substrates. These predacious fungi are commonly found in natural soils, agricultural soils and all kinds of decaying manures. Many species in the genera *Pleurotus* and *Hohenbuehelia* are nematoph-agous that is they derive nutrition by consuming nematodes. This is made possible by hyphae that may have adhesive knobs that attach to passing nematodes and secret nematoxic compounds (18 and 10). In the case of *Arthrobotrys* species, they are known to produce a range of nematicidal compounds, including Linoleic acid (3) and Oligosporons (4', 5'-dihydrooligosporon, hydroxyoligosporon, and 10', 11', -epoxyoligosporon) (2). Although, many of the trap-forming and egg-parasitic fungi can survive in soil saprophytically, the endoparasites are mostly more dependent on nematodes for nutrients source. Aiming at improving the bio-control process and to have alternative to chemical nematicides, nematophagous fungi which is effective, safe, sustainable and having no deleterious effect on the environment as well as the plant was isolated in some orchard plants in Zaria. The objective of this research work involved the isolation of some nematophagous fungi in Zaria.

MATERIALS AND METHODS

From four different locations, leaf debris were collected in separate polyethylene bags from four locations in Zaria environs. The town is about 80km north of Kaduna and located between longitude 7° 44" East, and latitude 11° 6" North of the equator (Global positioning system 2010). The research work was carried out between July and September, 2010 and repeated between the same periods in 2011. The locations were, Department of Biological Science Botanical Garden, Ahmadu Bello University, Samaru, Zaria, an orchard farm along Kano express way, Dogarawa, an orchard farm along new Jos road, Dambo, and an orchard farm in Wusasa, from underneath mango, cashew, and orange trees. Underneath each tree, sites were randomly selected for collection of samples. About 500 gm of decayed leaf debris in contact with the soil surface were collected into a labelled clean polyethylene bag using hand trowel. All the samples from all the locations were transported to the Department of Crop Protection, Nematology Laboratory for isolation of nematophagous fungi.

Preparation of Agar Media and Inoculation with Samples of decayed leaf debris

Sterilized water agar medium was prepared as follows (agar 20gm all in 1000ml distilled water). The content was autoclaved and

poured into several sterilized Petri-dishes covering nearly 2/3rd area of a plate and allowed to solidify. During the course of preparation, streptomycin was added to prevent bacterial growth. Also, sheep or goat excreta pellets (3) were added to serve as nutrient in each Petri-dish. Each sample collected was thoroughly mixed and the pH of the leaf debris was determined. From each sample, 2g was sprinkled over the prepared water agar medium in four replicates in the replicated Petri-dishes. The inoculated Petri-dishes were left to incubate for 72 hours for the induction of capturing devices

Four Petri-dishes which served as replicates were properly labelled and inoculated at room temperature of 25-30°C. The inoculated Petri-dishes were observed for 10 days under stereoscopic binocular microscope for the presence of nematophagous fungi.

Pure Culture, Single Spore Culture isolates.

Sterilized corn agar medium prepared as follows (maize 20gm, agar 20gm all in 1000ml distilled water) were poured into several Petri-dishes. From the identified nematophagous fungi from different leaf debris above, clusters of conidia and captured nematodes were picked using a fine needle (sterilized) into sterilized Petri-dishes containing corn meal agar medium and left to incubate for 10 days. After 10 days, spores of each isolate was sub-cultured into another Petri-dishes containing CMA medium and the culture of each isolate maintained at 29+1°C for collection of pure culture.

Induction of Capturing Devices and Capturing of Nematodes

Freshly hatched second stage juvenile of *Meloidogyne* sp. were obtained from egg masses of root-knot of infected *Lycopersicon lycopersicum* culture. The egg masses were allowed to hatch in sterilized water for twenty four hours. Small drop containing about 100 juveniles were pipetted into each cavity block containing the isolated nematophagous fungi from each location. The cavity blocks were respectively inoculated for 24 hours for trapping or capturing and killing of nematodes. The cavity blocks were observed at 2 days interval for 8 days

RESULTS

After 7 to 10 days of inoculation, it was observed that the sprinkled decayed leaf debris from different locations within Zaria inoculated in water agar medium was found to harbour nematophagous fungi. The nematophagous fungi were identified based on their morphological structures as described by (1, and 9). On the second day of inoculation, the Petri-dishes were observed under the microscope. Nematodes and free living nematodes that are non-parasitic were in abundance. These nematodes' presence might have stimulated the induction of the capturing devices of the nematophagous fungi.

Isolate from each location were subjected to their ability to capture nematodes by introducing *Meloidogyne* sp. juveniles into each isolate in a separate cavity block. The isolates from all the locations were found to be more of *Arthrobotrys* species than others

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... sp. and *Nematoctonus* sp.
...identified nematophag-ous
...ght conidiophores and bear
...of 5-20 two celled, 16-30μm
... μm broad conidia clearly
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...wice as large as the proximal cell
...elow are some micro-photograph depicting
...he morphological structures and capturing
...evices of *Arthrobotrys* species isolated
...rom the used debris (Plates 1 – 7)
...fter 6 days, it was observed that nematopha-
...ous fungi isolated from mango decayed leaf
...ebris from Botanical garden, Samaru
...aptured 92% of the nematodes introduced
...nd the least was 66% from the isolate of
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...able 2. High numbers of nematodes were
...ecorded captured in mango, followed by
...ashew and orange tree respectively as

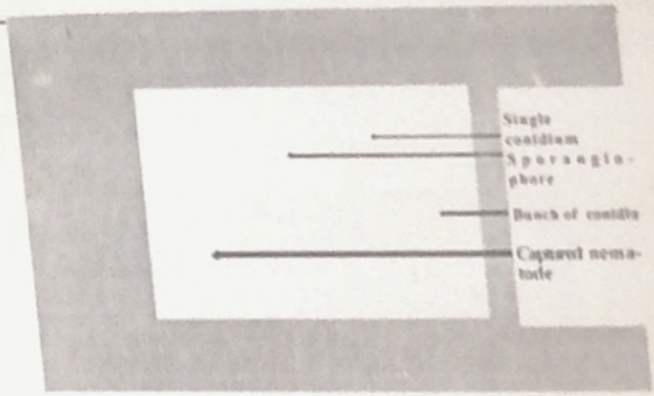

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Plate 2: *Arthrobotrys oligosporal* and a captured nematode at both ends

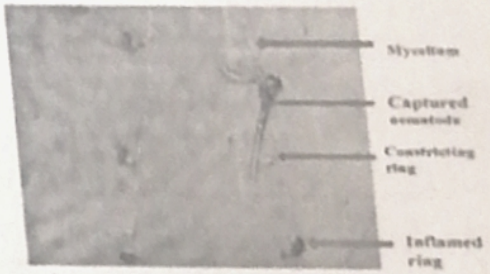

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Plate 3: Nematode captured by *Arthrobotrys oligosporal*

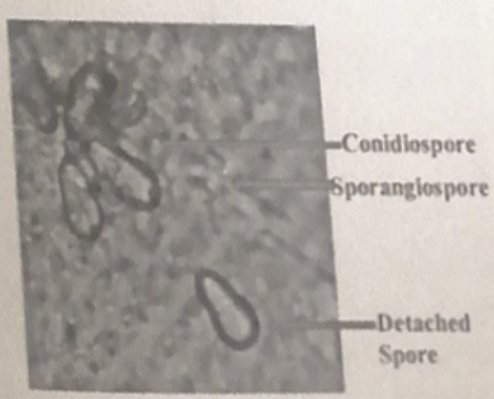

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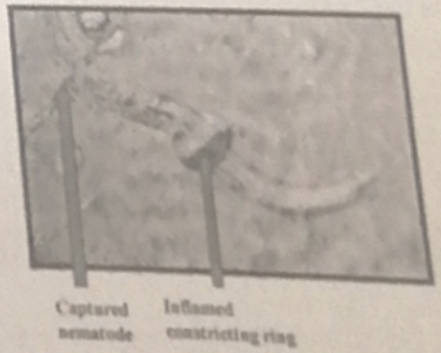
Plate 1: Nematophagous fungi (*Arthrobotrys oligosporal*) with a detached spore

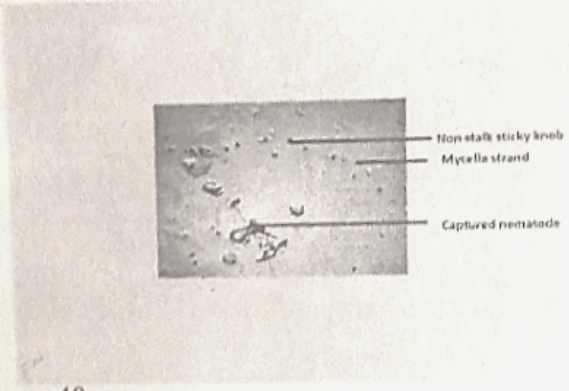


x100

Plate 4: Captured plant parasitic nematode in an inflamed constricting ring

x 40

Plate 5: A single non stalk constricting ring arising from mycelium



x 40

Plate 6: Non stalk sticky knob and non constricting ring produced by the same fungus

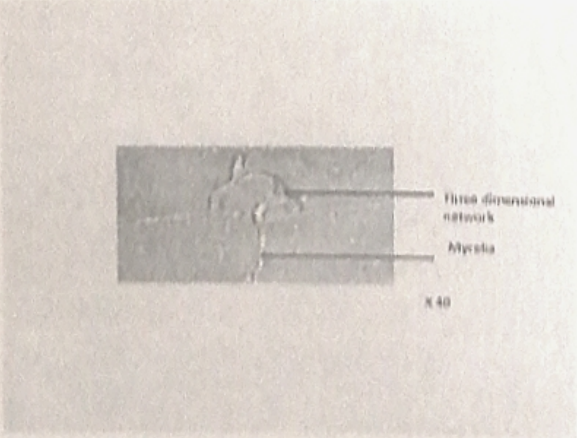


Plate 7: Three dimensional network ring of *A. oligosporal*

shown in Table 1. There were significant ($P<0.05$) differences between the leaf debris of the three orchard trees irrespective of the locations.

Number of Nematode Captured by Nematophagous Fungi in different locations in Zaria

High numbers of nematodes were captured in mango leaf debris, followed by cashew and orange tree respectively (Table 1). Significant difference was observed between the debris collected from all the orchard trees after 8 days. The mango tree nematophagous fungi captured 77.75 nematodes,, followed by cashew (74.56) and orange (71.31) tree respectively (Table 1). However, with respect to location or point of collection, it was observed that there was a significant difference among locations after 4 days, with Samaru (58.50) having the highest number of nematodes captured and lowest in samples collected from Jos road (21.50). After 6 and 8 days of observations, there was a significant difference between Samaru and Kano road. The nematodes captured were highest in Samaru location at both 6 and 8 days (82 and 92) as indicated in Table 2. In respect of locations and orchard tree types, and at 4 days, it was observed that there were no significant differences between cashew and mango decayed leaf debris in Jos road location as well as Samaru location, but there was a significant difference between the two tree debris and orange debris in these two locations. However, there was no significant difference between the number of nematodes captured in orange debris in Jos road and Wusasa, as well as

Table 1: Average number of nematodes captured bynematophagous fungi in different leaf debris in Zaria

Treatment (Tree)	Sampling period (days)			
	2	4	6	8
Mango	31.00a	55.19a	76.06a	77.75a
Cashew	28.75a	48.82b	71.31b	74.56b
Orange	22.44c	31.88c	63.88c	71.31c
SE±	0.468	0.607	0.480	0.546

Means in column followed by different letter are significantly different ($P \leq 0.05$), using (LSD) least significant difference.

Table 2: Average number of nematodes captured by nematophagous fungi in different locations in Zaria

Treatment(Location)	Sampling period (days)			
	2	4	6	8
Samaru	31.92a	58.50a	81.75a	92.17a
Kano road	30.25b	53.00b	73.67b	75.08b
Wusasa	25.92c	37.08c	63.75c	65.50c
Jos road	21.50d	32.58d	62.50c	65.42c
SE±	0.541	0.701	0.554	0.630

Means in column followed by different letter are significantly different ($P \leq 0.05$) using (LSD) least significant difference.

between Kano road Samaru (Table 3). Similarly, it was observed that nematodes captured in leaf debris of cashew and orange from Jos road and Wusasa location, were not significantly different. However, nematophagous fungi of leaf debris collected from cashew and mango trees from Botanical garden, Samaru captured more nematodes than the other locations (Table 3). After 8 days of incubation, more than 90% of the nematodes captured from the isolate of the leaf debris of cashew and mango collected from Samaru location showed no significant differences between the trees and that of orange. For all the orchard trees, nematophagous fungi from Samaru tends to be more

Table 3: Interactive effect of nematodes captured by nematophagous fungi after 4 days in different locations and types of trees in Zaria

Treatment(Location)	Type of trees		
	Cashew	Mango	Orange
Jos road	33.75e	36.25ea	27.75f
Kano road	58.50c	63.75b	36.75e
Samaru Wusasa	68.75a34.25e	70.75a50.00d	36.00e27.00f
SE±		1.21	

Means in column followed by different letter are significantly different ($P \leq 0.05$) using (LSD) least significant difference.

Table 4: Interactive effect of nematode captured by nematophagous fungi after 8days in different locations and type of trees in Zaria

Treatment(Location)	Types of trees		
	Cashew	Mango	Orange
Jos road	65.25d	65.50d	65.75d
Kano road	74.00c	86.50b	64.75d
Samaru Wusasa	94.50a64.50d	93.00a66.66d	89.00b65.75d
SE±		1.09	

Means in column followed by different letter are significantly different ($P \leq 0.05$) using (LSD) least significant difference

virulent in capturing nematodes based on the number of nematodes cannibalised by these fungi. Table 4 also indicated that, there was no significant difference between Jos road and Wusasa locations for all the three orchard trees.

DISCUSSION

The nematophagous fungi isolated from the leaf debris were found to developed sophisticated hyphal trapping structures such as hyphal nets, in three-dimensional adhesive nets, constricting rings, and adhesive knobs to capture nematodes.

However, there are more than 160 species of predacious fungi that are able to capture and kill nematodes in soil and plant debris (7, 17 and 18). The high number of nematodes captured by the nematophagous fungi isolated in Samaru compare to other locations may be as result of high concentration of organic waste piled up over the years or a different virulent strain of *Arthrobotrys* sp. (14)

reported that *A. oligosporal* and *A. dactyloides* which produce constricting ring are common fungi that were frequently detected in soils amended with organic matter. Similarly, the type of nematode-trapping structures formed depends on species or even strains of species as well as on environmental conditions, both biotic and abiotic factors. The most important biotic factor is living nematodes, which not only induce the formation of trapping structures by touching the mycelium but also serve as food source for the fungi after they have been invaded (15). Thus, the nematodes induce the formation of the structures in which they are later consumed and serve as an additional food source. The nematophagous fungi isolated from these different locations were similar to some reports on the isolation and characterization of nematophagous fungi from various sources including soil, dung, compost and fresh faeces of some animal species at different geographical areas (6 and 16). Nematophagous fungi are said to be carnivorous and specialized in trapping and digesting nematodes, and there exist both species that live inside the nematodes from the beginning and others that catch them mostly with glue traps or in rings and some species possess both types of traps. Another technique employed by the fungi is to stun the nematodes using toxins which is a method usually employed by *Coprinus comatus* and the family *Pleurotaceae* (18)

In conclusion, use of chemicals for the control of nematodes which nomarlly requires applications of large amounts to control root-knot nematodes on various crops should be discouraged to check the effect of pollution on the environment which may eventually result to climate changes. Besides, these chemicals target the nervous system of both nematodes and human beings and could lead to death. In view of this, there is need to replace highly toxic and potentially polluting chemicals used for the management of plant parasitic nematodes with some of these nematophagous fungi that are non-phytotoxic and commonly found within the rhizosphere of the nematodes.

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