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Morphometric Characterization of Nematophagous Fungi Isolated From Some Orchards around Zaria, Nigeria

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Abstract. 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. Debris from each sample was plated on water agar supplemented with goat excreta. Each sample was replicated four times. Nematophagous fungi grew from the debris in culture between 10-14 days. The isolated fungi were sub-cultured in corn meal agar medium enriched with goat excrete and about 100 juveniles of *Meloidogyne incognita* were pipetted unto the medium. After 7 days, all the petri dishes were examined under the stereoscopic microscope for morphological identification of the fungi from the debris collected from Botanical Garden, ABU, Samaru, Wusasa area, Jos road and Kano road around Zaria metropolis respectively. The fungi identification was done on the basis of their morphological structure and trapping devices (three-dimensional adhesive nets and constricting rings), non-stalk constricting ring and adhesive sticky knob. Fungi identified from the cultures were *Arthrobotrys*, *Dactylella*, *Nematoctonus* and *Monacrosporium* species.

Keywords: *Arthrobotrys* spp., *Dactylella* spp., *Nematoctonus* spp., Nematophagous fungi, *Meloidogyne* spp., *Monacrosporium* spp., isolation

INTRODUCTION:

Nematodes are obligate plant parasites, most of them are pathogens that destroy the plant on which they feed. The most important plant parasitic nematodes include root-knot nematodes (*Meloidogyne spp*.,), cyst nematodes (*Heterodera spp*., and *Globodera spp*.,), root lesion nematodes (*Pratylenchus spp*.,) stem nematodes (*Ditylenchus dipsaci*), burrowing nematodes (*Radopholus similis*) etc. Nematode population densities can be controlled by several measures. First and foremost is the use of synthetic nematicides. A short coming in their use is that nematicides are toxic to human beings, cause environmental hazards, too costly for use especially by our local farmers and the developing world.

Biological control which is an alternative to nematicides has been gaining ground and is becoming important in recent years, most especially the use of natural enemies within the same environment to control plant parasitic nematodes and such natural enemy 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 soils at different geographical areas (Siddiqui and Mahmood, 1996). 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 (Barron, 2003). 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.

Nematophagous fungi have been the subject of research over several decades in fundamental studies of their ecology, distribution and systematic, and as potential biological control agents of nematode pathogens of plants and animals (Li *et al*., 2000; Liu and Zhang, 2003; Dong *et al*., 2004; Li *et al*., 2005). Many species in the genera *Pleurotus* and *Hohenbuehelia* are nematophagous. This is made possible by hyphae that may have adhesive knobs that attach to passing nematodes and secret nematoxic compounds (Thorn *et al*., 2000 and Koziak *et al*., 2007). The predacious hyphomycetes in *Arthrobotrys*, *Corda* and related genera, some with teleomorphs in *Orbilia* Fr. (*Ascomycota*, *Orbiliaceae*), destroy nematodes using several kinds of trapping devices: stalker and sessile adhesive knobs, two- or three-dimensional adhesive nets, and constricting and non-constricting hyphal rings (Scholler *et al*., 1999).

Since the discovery of predacious activity in *Arthrobotrys oligosporal* (Zopf, 1888), the nematode-trapping fungi have attracted much interest amongst mycologists probably due to the spectacular trapping method, in the physiology involved with it and, last but not the least, in their potential economic importance as biocontrol agents. *Arthrobotrys* species are known to produce a range of nematicidal compounds, including Linoleic acid (Anke *et al*., 1995) and Oligosporons (4', 5'-dihydrooligosporon, hydroxyoligosporon, and 10', 11', -epoxyoligosporon) (Anderson *et al*., 1995).

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 to improve on the

biocontrol process and to have alternative to chemical nematicides that are toxic to human beings and cause environmental hazards, there is need to control these plant parasitic nematodes using some eco-friendly techniques and biocontrol agents such as predaceous and endoparasitic fungi that are safe, sustainable and have no deleterious effect on the environment as well as the plant (Goswami and Uma, 1995).

MATERIALS AND METHODS

Collection of Samples and Isolation of Nematophagous Fungi

Isolation of different isolates of the fungi was carried out using the method of Bandyopadhyay and Singh (2000). From different locations 250gm of decayed leaf debris were collected in separate labelled polyethylene bags from different locations within Zaria town, which is about 80 km north of Kaduna. Zaria is located between longitude 7º 44' East, and latitude 11º 6' North of the equator (Duze and Ojo, 1990).

The locations were Samaru, Wusasa, Sabon-Gari and Basawa. In each location, three economic orchard trees were selected for sampling, *Mangifera indica* (Mango), *Anacardium occidentale* (Cashew) and *Citrus* species (Orange). Underneath each tree, samples of decayed leaf debris were randomly collected at five different points and mixed together in a labelled polyethylene bags to represent a sample from each tree. All the samples collected were transported to Department of Crop Protection, nematology laboratory, ABU, Zaria and stored in the cool room for future use.

Preparation of Agar Medium and Inoculation with decayed Leaf Samples

Twenty grammmes of agar-agar was dissolved in a conical flask in 1,000 ml tap water on a hot plate for 15 to 20 minutes. The mixture was continuously stirred with a clean glass rod to dissolve the agar-agar. Few drops of streptomycin was added the resulting solution to prevent bacteria growth before autoclaving at 15psi pressure and 121ºC for 15 minutes. Afterwards it was allowed to cool for a few minutes and dispensed gently into 9 cm Petri-dishes to cover about 2/3rd area of each plate. All the Petri-dishes were labelled to correspond with each tree sample. From each sample, 2 g of decayed leaf debris were carefully sprinkled onto the solidified medium and 3 g of ground goat excrete pellets were added. Each selected sample had four replicates inoculated for the sporulation of nematophagous fungi. The inoculated plates were arranged on laboratory bench to incubate and routinely observe for two weeks for the presence of nematophagous fungi under stereoscopic binocular microscope.

Isolation of Nematophagous fungi and nematodes from Decayed Leaf Debris of Mango, Cashew and Orange

Corn meal agar was prepared by adding 20 g each of maize powder and agar-agar in 1,000 ml distilled water, streptomycin was added to prevent bacteria growth. The sterilized CMA was poured into several Petri-dishes and allowed to solidify. Similarly, goat excrete agar was prepared and thin layer poured over the CMA to boost the nutrient level of the medium. Subcultures were made from the conidial heads of individual sporulated fungi into each Petri-dish of CMA. Nematodes observed to be trapped by fungi were picked with the help of a sterilized needle into Petri-dishes containing corn meal agar

medium (CMA).All the Petri-dishes were incubated on the laboratory bench in the clean room, for 10 days.

Identification of the fungi

For identification of different isolates of the nematophagous fungi, conidia, conidiophores, hyphae, trapping devices formed on the growing mycelium and directly formed on the spores as well as nematodes captured were recorded and compared with the original description given by Drechsler (1937) and Cook and Godfrey (1963). Isolates from each location were tested for their ability to capture nematodes by introducing *Meloidogyne* spp. juveniles into separate CMA plates.

Inoculation of *Meloidogyne incognita* and observation of fungal trapping devices

Freshly hatched second stage juveniles of *M. incognita* were collected in large numbers from egg masses of root galls of *Solanum lycopersicum.* The juveniles were washed five times with sterilized water. A 10 µl drop containing 100 nematodes was transferred into each CMA plate containing nematophagous fungi. All the Petri-dishes were incubated for 24 hours for trapping or capturing and killing of nematodes. After 24 hours, the Petri-dishes were observed under the stereoscopic microscope for nematodes captured, predaceous fungi, capturing organs, fungi mycelia, conidia and spores. The observation was routinely carried out daily for 8 days. All the morphological futures that can help in the identification of the fungi were recorded.

RESULTS

After 14 days of inoculation, it was observed that the sprinkled decayed leaf debris from different locations within Zaria inoculated on water agar medium harboured nematophagous fungi. On the second day of inoculation, the Petri-dishes were observed under the microscope. Nematodes were in abundance in the samples collected. The presence of nematodes might have stimulated the induction of capturing devices of the nematophagous fungi.

The isolates from all the locations had more of *Arthrobotrys* species than *Dactylella* spp., *Nematoctonus* spp., and *Monacrosporium* spp.

At the species level the type of trapping organ is considered to have high taxonomic significance. As a rule, a species can only form one type of trapping organ. Three modifications of this rule were noted in this study:

a) Non-constricting rings may be accompanied by stalked knobs.
b) Within the knob-forming fungi a variation in the stalk can be observed. The proliferation of knobs, as seen in *M. parvicolle*, sometimes resembles the hyphal branches of *M. gephyropagum*.
c) The simple hyphal branches (*M. cionopagum*) tend to fuse, forming a two-dimensional network (*M. gephyropagum*).

Below are some pictures depicting the morphological structure and capturing devices of nematophagous fungi isolated from these plant debris (Plates 1 – 9).

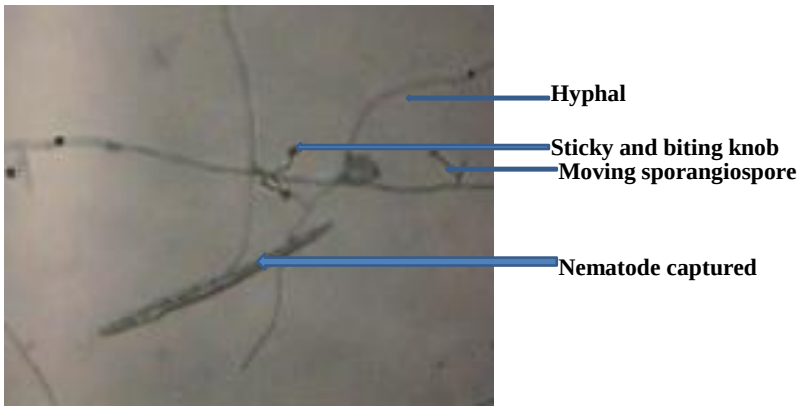


Plate 1. ***Monacrosporium*** **species that captured nematode**

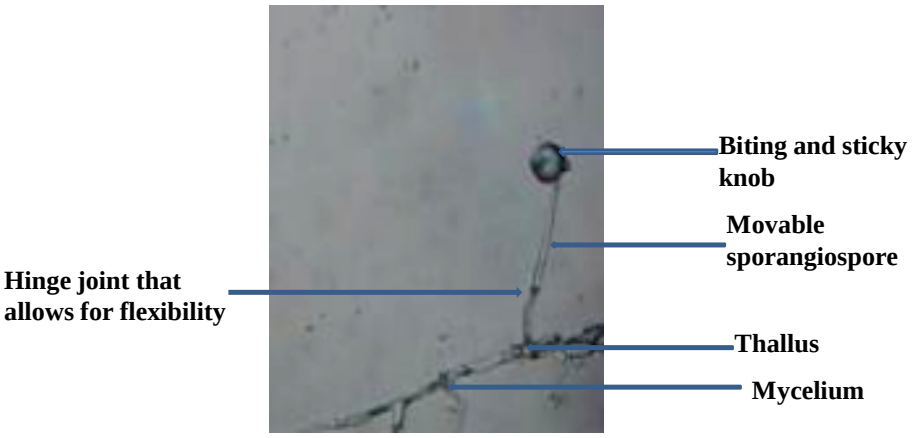


Plate 2. Matured predaceous fungi: ***Monacrosporium*** **spp**

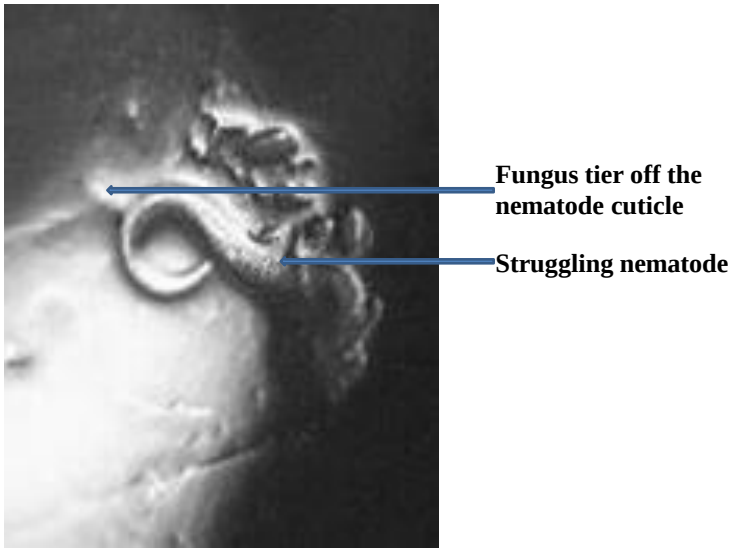
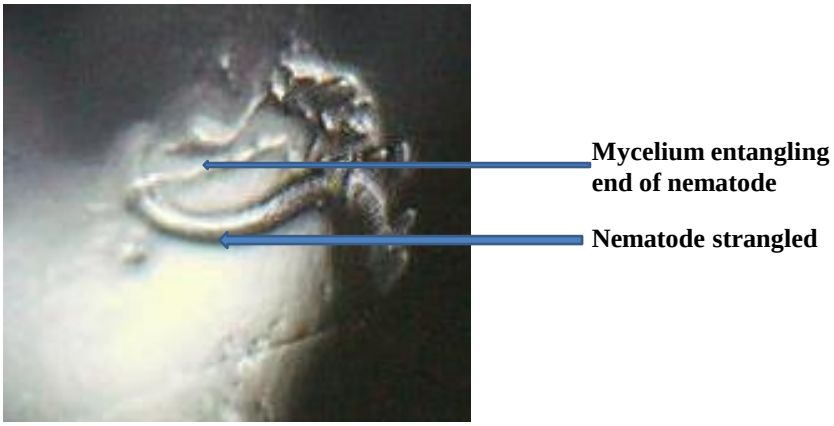


Plate 3. ***Monacrosporium*** **spp. destroyed the nematode cuticle**



Plare 4. ***Monacrosporium*** **spp. entangling the nematode after tearing off the cuticle**

Figure 3 and 4 are not quite clear and do nor refer to any specific funi, i think it should be deleted

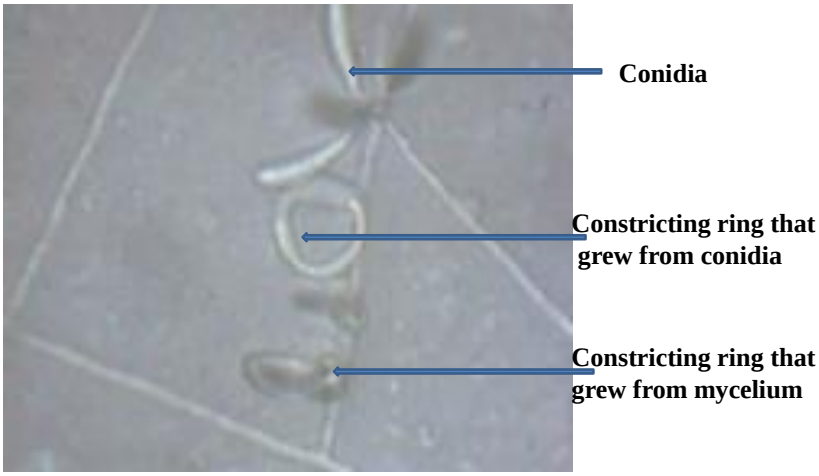


Plate 5. *Dactylella* spp.

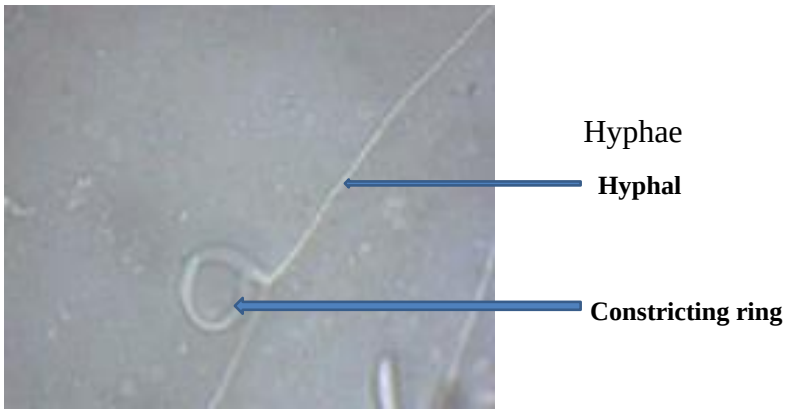


Plate 6. Constricting ring of *Dactylella* species

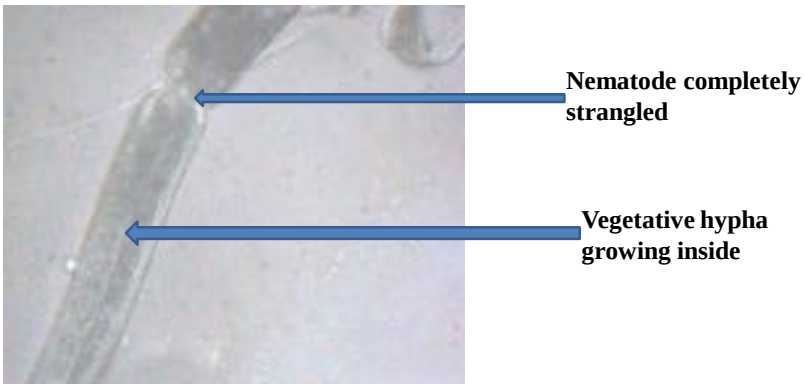


Plate 7. Nematode strangled with vegetative hypha *Dactylella* spp. growing inside the nematode

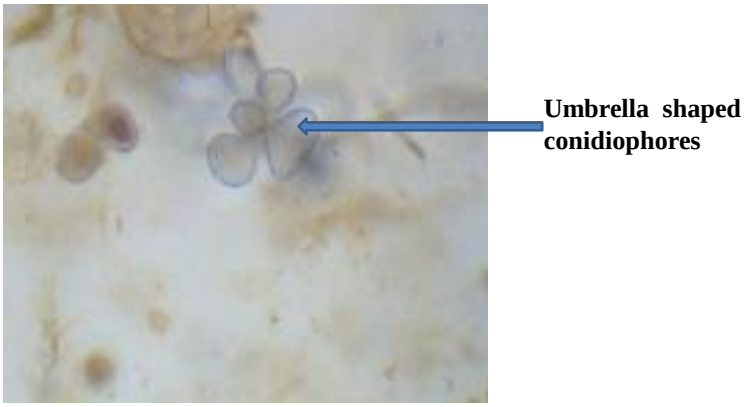


Plate 8. *Arthrobotrys oligosporal* isolated from decayed leaf of mango

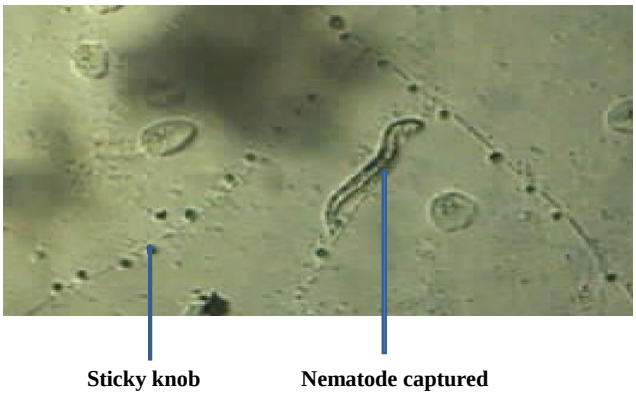


Plate 9. **Nematode captured by *Nematoctonus* spp.**

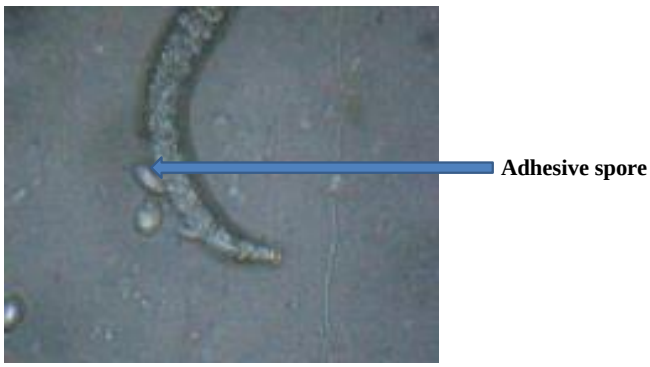


Plate10. **Spores of *Nematoctonus* species attached to nematode**

After 8 days of observation, it was noted that nematophagous fungi isolated from decayed mango leaf debris from Botanical garden, Samaru captured about 90 juveniles of the nematodes introduced and the least was 66 juveniles from the isolate of mango leaf litter from Wusasa in an average of 100 juveniles introduced as indicated in Table 2

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

High numbers of nematodes were recorded captured in mango, followed by cashew and orange tree respectively as shown in Table 1. There was a significant difference at ($p \leq 0.05$) between the leaf debris of the three orchard trees irrespective of their locations. Significant difference was observed between the debris collected from all the orchard trees after 8 days. The mango tree has the highest number of nematodes (77.75) captured, 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 between all the locations after 4 days, 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 after 6 and 8 days. The nematodes captured were highest in Samaru location at both 6 and 8 days (82 and 92) as indicated in Table 2.

Table 1. Average Number of Nematode Captured by Nematophagous Fungi in different leaf debris in Zaria

	Sampling period (Days)			
Treatment(Tree)	2	4	6	8
Mango	31.00[a]	55.19[a]	76.06[a]	77.75[a]
Cashew	28.75[b]	48.82[b]	71.31[b]	74.56[b]
Orange	22.44[c]	31.88[c]	63.88[c]	71.31[c]
LSD(≤0.05)	1.34	1.74	1.34	1.57

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

Table 2. Average Number of Nematode Captured by Nematophagous Fungi in different locations in Zaria

	Sampling period(Days)			
Treatment(Location)	2	4	6	8
Samaru	31.92[a]	58.50[a]	81.75[a]	92.17[a]
Kano road	30.25[b]	53.00[b]	73.67[b]	75.08[b]
Wusasa	25.92[c]	37.08[c]	63.75[c]	65.50[c]
Jos road	21.50[d]	32.58[d]	62.50[c]	65.42[c]
LSD(P≤0.05)	1.54	2.00	1.58	1.80

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

In respect of locations and orchard tree types, at 4 days of observation, 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 nematodes captured in orange debris in Jos road and Wusasa, as well as between Kano roads Samaru (Table 3). Similarly, it was observed that nematodes captured in leaf debris of cashew, orange from Jos road and Wusasa location, there was no significant difference between the two locations. However, leaf debris collected from cashew and mango trees from Botanical garden Samaru captured more nematodes than the other locations (Table 3).

Table 3. Interactive effect of Nematode Captured by Nematophagous Fungi after 4 days in different locations and types of trees in Zaria

	Orchard Trees		
Treatment(Location)	Cashew	Mango	Orange
Jos road	33.75[e]	36.25[ea]	27.75[f]
Kano road	58.50[c]	63.75[b]	36.75[e]
Samaru	68.75[a]	70.75[a]	36.00[e]
Wusasa	34.25[e]	50.00[d]	27.00[f]

Means in column followed by different letter are significantly different (P≤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

	Types of trees		
Treatment(Location)	Cashew	Mango	Orange
Jos road	65.25d	65.50d	65.75d
Kano road	74.00c	86.50b	64.75d
Samaru	94.50a	93.00a	89.00b
Wusasa	64.50d	66.66d	65.75d

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

After 8 days of incubation, more than 90 out of the 100 nematodes introduced into the CMA petri dishes were captured from the isolate of the leaf debris of cashew and mango collected from Samaru location, however there was a significant difference between these trees and orange debris. For all the orchard trees, nematophagous fungi from Samaru tends to be more virulent in capturing nematodes based on the number of nematodes cannibalised by these fungi. These predaceous fungi were mostly *Arthrobotrys species*. Table IV 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 this leaf debris were found to develop sophisticated hyphal trapping structures such as hyphal nets, 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 (Dijksterhuis *et al*., 1994; Siddiqui *et al*., 1996; Thorn *et al.*, 2000). 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 years or a different virulent of *Arthrobotrys* spp. Mankau (1968) reported that *A. oligosporal* and *A. dactyloides*, which produce constricting ring are common fungi that were frequently detected in soils amended with organic amended soils. Drechsler (1937) also reported that *Arthrobotrys oligosporal* is the most widespread. This fungi has been isolated from plenty of

different substrates, e.g. from compost, decomposing wood and animal excrements. The upright conidiophores is said to bear 20-30 groups of 5-20 two celled, 16-30µm long and 8-16 µm broad conidia clearly indented at the septa, whose distal cell is about twice as large as the proximal cell (Haard, 1968).

Similarly, the type of nematode-trapping structure 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 when it touches fungal mycelium but also serve as food source for the fungi after they might have been invaded by the fungi (Nordbring-Hertz, 2004). Thus the nematodes induce the formation of the structures in which they are later consumed, serving 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 (Chandrawathani *et al*., 2001; Sanyal, 2000). Nematophagous fungi are said to be carnivorous fungi that specialize 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. 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* (Thorn *et al*., 2000).

In conclusion, use of chemical in the control of nematodes, which requires application of large amounts of chemicals and knowledge of special equipments to control root-knot nematodes on various crops should be discouraged. If successfully done, it would also check the effect on climate change. In view of this, there is need to replace highly toxic and potentially polluting chemicals used for the management of plant parasitic nematodes. Some of these nematophagous fungi that are non-phytotoxic and commonly found within the rhizosphere should be put into use. Farmers should be encouraged to use organic amendments in the production of their crops in order to facilitate the growth of these nematophagous fungi, hence control of plant-parasitic nematodes.

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