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Biodiversity of Nematode Antagonistic Fungi under Vegetation of Province Khulais, Western of Saudi Arabia Kingdom

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Abstract

The diversity and community structure of nematode trapping fungi (NTF) were investigated in thirteen sites located in province of Khulais, western of Kingdom of Saudi Arabia with different vegetation. NTF were isolated by using sprinkle technique. The soil pH value, soil texture and organic matters percentages of each soil sample were analyzed to investigate the relationship between these environmental factors and the occurrence of nematode trapping and parasitic fungi. Three species of nematode antagonistic fungi genera were found and identified in this study. The Hyphomycetes fungus *Dactylaria brochopaga* Drechsier, was isolated from 9 sites by rate 69.2% from total collected sites. The fungus was found naturally trapping of second stage juveniles (J_2s) for 3 nematode orders distributed between 6 plant species in soil samples containing organic matters more than 1.5%. The occurrence of *D. brochopaga* Drechsier was reduced when amount of organic matter in soil samples was less than 1%. The optimal pH value for growth of *D. brochopaga* Drechsier in natural habitat was ranged from 6.5 to 7.1 in sandy loamy soil texture. In addition, two species of the Chytridiomycetous fungi *Catenaria* were observed in 11 sites by rate 84.6% of total sites. One of them was identified as *C. anguillulae* Sorokin, which found parasitized on nematode body causing the breakdown of the nematode cuticle in 8 sites with different plants. While, the another specie was identified as *C. auxiliaris* (Kühn) Tribe, which cause destroying nematode egg in 6 sites by rate 46.15% from total sites. The suitable pH value for the two species of Chytridiomycetous fungi was ranged from 7.0 to 8.0. *C. auxiliaries* (Kühn) Tribe was found in 3 soil types. While the fungus *C. anguillulae* Sorokin was found in 2 soil types. On the other hand, the organic matters haven't any effects on the growth of *C. auxiliaries* (Kühn) Tribe or the ability of parasitism, but they have a great effect on *C. anguillulae* Sorokin. The present study is considered as preliminary survey on nematode trapping fungi for the first time in province of Khulais and second study on predacious fungi in Saudi Arabian soils.

Key word: Ecology**,** Nematode, trapping fungi, *Dactylaria, Catenaria*

1. Introduction

Nematode antagonistic fungi are common soil inhabitants and have been found in many types of soil. The role of these fungi is controlling nematode populations in the soil. Nematode antagonistic fungi have been studied worldwide for their potential as biocontrol agents and their unique predatory habits (Bird & Herd, 1995; Fox, 2001). Many fungi have been isolated from nematode body and egg masses during the past two decades throughout the world (Rodriguez-Kabana & Morgan-Jones 1988; Crump 1991; Arnold 2003). At least 168 species of fungi have isolated from nematode (Carris & Glawe 1989). These fungi can be divided into four categories: endoparasitic fungi, nematode trapping fungi, fungi which parasitize on nematode eggs and females, and toxin producing fungi (Barron & Thorn 1987;

Dackman *et al.* 1992). Nematode trapping fungi are unique in their morphological adaptation to the predacious habit and in their ability to capture and consume nematode. Different types of hyphal structures form adhesive nets, knobs, branches or hyphae, constricting rings or non constricting rings in order to capture nematodes (Barron 1977). *Dactylaria brochopaga* is a nematophagous fungus, which dramatically captures and kills saprophytic and parasitic nematodes *In Vivo* and *In Vitro* by producing three celled trapping rings. *D. brochopaga* is a common fungus in agricultural soils, decaying plant materials and old decayed root-galls (Bandyopadhyay 1998; Kumar 2003; Singh *et al.* 2007; Kumar *et al.* 2010; Kumar & Singh 2010; Saadabi 2010). The bioefficacy of the fungus *D. brochopaga* as a nematode antagonist was enhanced when its spore suspensions and mass culture were applied with amount of organic matter such as cow dung manure which causing reduce in nematode number in soil (Kumar & Singh 2011). Abdelmoneim (2006) recorded the fungus of *Catenaria* sp. infected and destroyed egg masses of some plant parasitic nematode in root samples collected from three fields cultivated with vegetables. Also Vaish *et al.* (2010) found five isolates of fungus *Catenaria anguillulae* causing 100% mortality with full sporangial development in second stage juveniles of nematode *Anguina tritici* and Saadabi (2010) recorded ten species of nematode trapping fungi in some Saudi Arabia soils. The present study was undertaken to 1-isolate and identifies the nematode trapping fungi with different plant communities for the first time in Khulais province western of Saudi Arabia Kingdom, to fill the gap of knowledge about nematode trapping fungi in this area. 2- Study some factor effects on fungal occurrence and activity, such as pH of the soil and its structure, different vegetation and percent of organic matter.

2. Material and Methods

2.1. Area description

Khulais province is located in western Saudi Arabia kingdom, 90 km away from Jeddah to the north-east. It is about 30 km from the coast of the Red Sea. It is to the east on the latitude of 22 degrees, longitude of 39 degrees. The average annual temperature in this area is 26° C, with a high of 31° C and low of 21° C. The Average annual rainfall is about 0.79mm. There are famous valleys such as Khulais, Grand, Wadi Keded, Abu Hlevae and Marwani. Valley Khulais, it is one of the most fertile valleys in the Kingdom and is famous for crops such as dates of agricultural, vegetable and other fruits. Its climatic characteristics are consistent throughout the year.

2.2. Sampling:

Samples were collected from thirteen sites located in province of Khulias, Kingdom of Saudi Arabia from different vegetation (field crops, vegetables and some wild plants). Three samples collected from each site (39 soil samples). Soil sample was composed of about 250 gm, and it was taken by digging the soil to a depth of 15-25 cm with a trowel. Samples were kept in polyethylene bags, labeled and sent directly to the laboratory to be stored in a refrigerator on 5 ±2 °C until processed for examination.

2.3. Nematode Panagrellus redivius culture (bait culture):

The nematode *Panagrellus redivius* (free-living nematode) was obtained as a monoxenic culture from Microbiology laboratory of Agricultural Botany, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. Nematode *P. redivius* were extracted from culture medium following modified Baerman's funnel technique (Barron 1977) suspended in sterile distilled water, and then used as a bait for capture nematode trapping fungi from soil samples.

2.4. Isolation of nematode trapping fungi from soil:

The soil samples from parasitic nematode infested fields were collected and air-dried till the water content was less than 10%. Two gm of the soil sample was added into 1% corn meal agar (CMA) plate containing approximately 500 second stage juveniles (J_2s) of nematode *Panagrellus redivius*.

Each sample was cultured on three different petri dishes, resulting in a total of 117 plates form 39 soil samples. The plates were incubated at room temperature (25±2°C) in the dark condition for one week, and then observed under a dissecting microscope for detect the nematode trapping fungi. The fungi were identified and recorded according to the taxonomic keys provided by Li *et al.* (2000).

2.5. Isolation of nematode antagonistic fungi from nematode eggs:

Nematode eggs (*Meloidogyne* sp.) were extracted from the infected roots by 0.5% NaOCl and collected by the wet sieving and centrifuged by using sugar flotation method according to Hussey & Barker (1973). The suspension of eggs was poured into two conical centrifuge tubes (*Vol.* 15ml) and centrifuged for 4 minutes at 3000 rpm. The supernatant was discarded and 10ml sucrose solution was poured into each tube to re-suspend the eggs. Tubes were centrifuged for 2.5 minutes at 3000 rpm to separate eggs from other debris. The supernatant was poured into a 25mm pore sieve, rinsed thoroughly with tap water to remove the sucrose solution, washed into sterile centrifuge tubes. The egg surface disinfected methods as described by Abdelmoneim (2006). Eggs (3500 eggs/50× in 8cm Petri dish) were distributed evenly over the surface of water agar supplemented with 2gm KH_2PO_4 and 12.5mg of Chlortetraycline and 300 g of Streptomycin sulfate per liter. Then 0.5 g of the soil sample was added into egg plate, the plates were incubated at 25±1°C for 3-7 days on dark condition. Each plate was inspected daily at 50× magnification with a stereoscopic microscope.

2.6. Soil chemical analyses:

The organic carbon and pH was determined according to Page *et al.* (1982). The soil mechanical analysis was determined according to the method described by Piper (1950).

3. Results

Soil samples were examined to detect the nematode antagonistic fungi, which associated with plant parasitic and free living nematodes in the rhizospheres of the surveyed plants. Data presented in Table1, shown that the Hyphomycetes fungus *Dactylaria brochopaga* Drechsier 1937 (Nematode trapping fungi by forming constricting ring) was isolated from nine sites by rate 69.2% from total collected sites (13 sites). *Dactylaria brochopaga* was found naturally trapping of second stage juveniles (J_2s) for three nematode orders (Tylenchida, Rhabditida and Dorylaimida) distributed between 6 plant species. The fungus mycelium branched, septated hyphae and the trap mechanism is constricting rings. Each ring consists of three curved cells, which capture nematodes through a mechanical positive action (Fig. 1A-D). In addition to two species of Chytridiomycetous fungus *Catenaria* (parasitic fungus on nematode and their eggs) were observed in eleven sites by rate 84.6% of total sites. One of them was identified as *C. anguillulae* Sorokin 1876, which found parasitized on nematode body causing the breakdown of the nematode cuticle in eight sites (61.5% from total collected sites) with different plants. The nematode order Dorylaimida is the most infected order with *C. anguillulae* (Fig. 1E-K). While, the another specie was identified as *C. auxiliaris* (Kühn) Tribe 1977, which cause destroying nematode egg in 6 sites by rate 46.15% from total sites (Fig. 1L-N). The vegetative thallus of *C. auxiliaris* consists of a chain of swollen cells delimited by septa at maturity the swollen cells from precursor sporangia. In addition to some others groups of saprophytic fungi were observed in seven sites by rate 53.8% with different modes of parasitism.

The soil pH value, soil texture and organic matters source were analyzed for each soil sample to investigate the relationship between these environmental factors and the occurrence of nematode trapping fungi in province of Khulais Table 2. The fungus *D. brochopaga* Drechsier, was found in soil samples containing organic matters more than 1.5%, while, the distribution of this fungus reduced when amount of organic matter was less than 1% in all collected soil samples. The best result was observed when the organic matter added to soil in form poultry droppings (fertilization treatment in some vegetables farms).

The optimal pH value for growth of *D. brochopaga* Drechsier in natural habitat was ranged from 6.5 to 7.1in sandy loamy soil texture. The suitable pH value for the two species of fungus *Catenaria* was ranged from 7.0 to 8.0. *Catenaria auxiliaries* (Kühn) Tribe was found in three soil types sandy, sandy loamy and loamy sandy soil. While *C. anguillulae* Sorokin was found in two soil types loamy sandy and sandy loamy soil. On the other hand, the organic matters haven't any effects on the growth of *C. auxiliaries* (Kühn) Tribe or the ability of parasitism, but they have a great effect on *C. anguillulae* Sorokin. *Dactylaria brochopaga* Drechsier was give the highest value of isolation frequency percentage 23.07% in all collected soil samples (39 samples) followed by fungus *C. anguillulae* Sorokin 15.38% then fungus *C. auxiliaris* (Kühn) Tribe (Fig. 2).

4. Discussion:

The present study is considered as preliminary survey on nematode trapping fungi for the first time in province of Khulais and second study on predacious fungi in Saudi Arabian soils. The results indicated that there are different suppressive fungi to nematodes in study area. *Dactylaria brochopaga* (Hyphomycetes) was found naturally trapping of second stage juveniles for three nematode orders in agriculture soil samples (Bandyopadhyay 1998; Kumar 2003; Singh *et al.* 2007; Kumar *et al.* 2010; Kumar & Singh 2010; Saadabi 2010). The isolation frequency percentage of *D. brochopage* was affected by present of organic matter and it's percentage in soil samples. The organic matter in some soil samples may be enhanced the fungal spore germination and increased trapping nematode number in some soil samples. This result was agreement with Kumar & Singh (2011) they found the application of *D. brochopage* with caw dung manure as a source of organic matter causing increase the fungus bioefficacy to decrease nematode number in soil. The soil pH value and soil texture are important factors for distribution of *D. brochopage* in some study sites of Khulais province. This result was agreement with Saadabi (2010) who record *D. brochopage* as a restricted fungus to specific areas. The Chytridiomycetous fungus *Catenaria* was recorded in almost of collected samples in two species. The species of *Catenaria* were identified as *C. anguillulae* Sorokin and *C. auxiliaris* (Kühn) Trib. It's the first record for *C. auxiliaris* (Kühn) Trib in Saudi Arabian soils on vegetation of study area comparing with the publication data for the preliminary survey of predacious fungi in Saudi Arabia by Saadabi (2010). On the other hand the organic matters percentages haven't any effects on the growth of *C. auxiliaries* (Kühn) Tribe or the ability of parasitism, but it has a great effect on *C. anguillulae* Sorokin. This result might be due to direct effect of organic matter on increasing of nematode population especially free living nematode. Consequently increasing in the number of nematode prays to zoospores of *C. anguillulae* Sorokin, which parasitized on nematode body.

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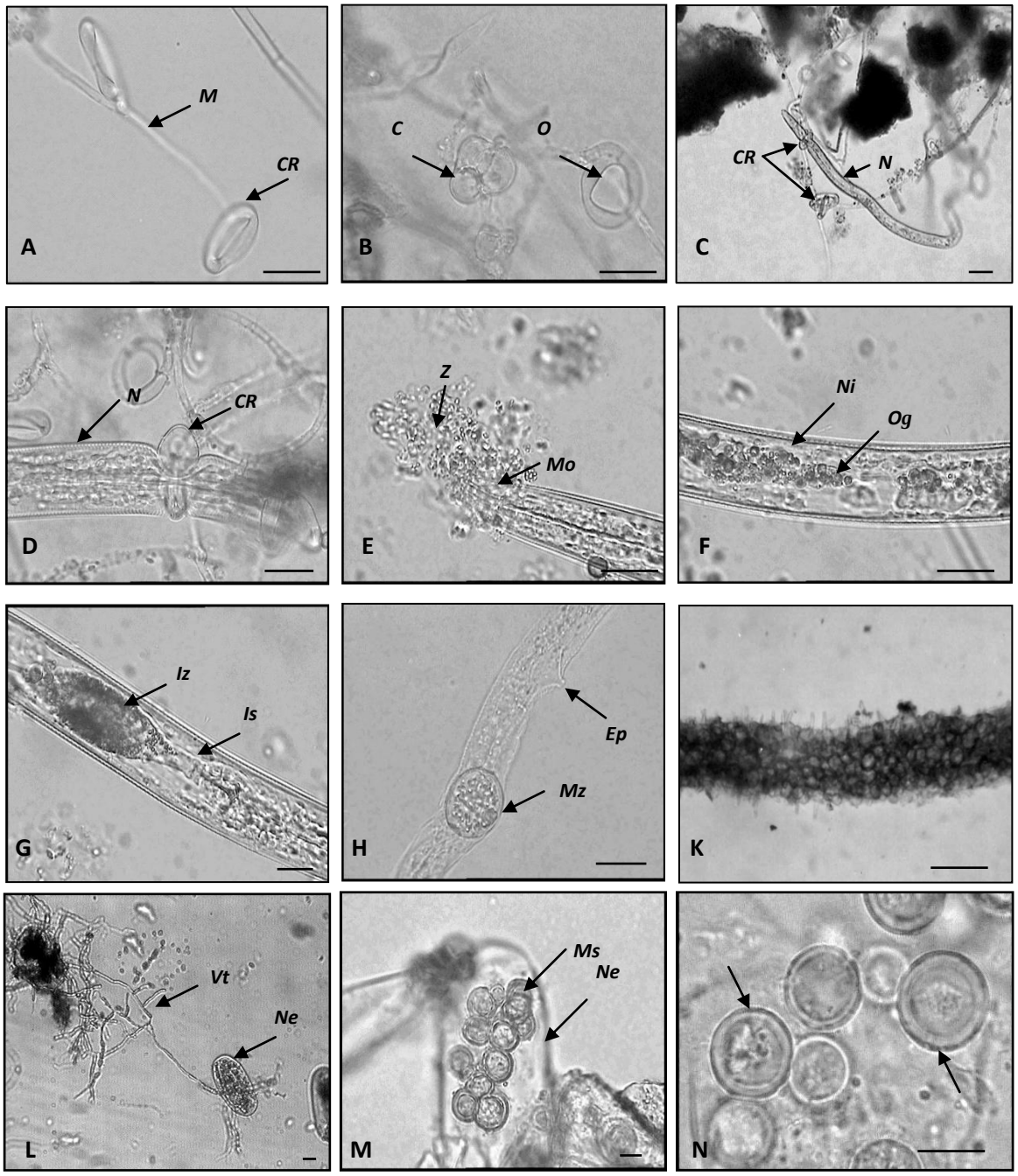

Figure1. Trapping organs of the fungus *Dactylaria brochopaga* Drechsier
A: The fungal forming constricting ring (*CR*) and mycelium (*M*). B: The fungal constricting ring opening (O) and other close (C). C: J_2 of nematode from order Tylenchida (N) was trapped by the fungus constricting ring (CR). D: Nematode *Panagrellus redivius* in bait culture (*N*) trapping by the fungal constricting ring (CR). (Bar=30µm)
-Parasite stages of the fungus *Catenaria anguillulae* Sorokin on nematode body
E: Zoospores *(*Z*)* was attracted to the nematode mouth opening *(*Mo*)*. F: The fungus forming oil globules leaking *(*Og*)* into nematode intestine *(*Ni*)*. G: Immature zoosporangium (Iz) forming into nematode intestine and fungus forming isthmus *(*Is*)* between zoosporangium and other. H: Mature zoosporangium *(*Mz*)* forming into nematode body and the zoospores were released from escape pore (*Ep*) of zoosporangium. K: Hyper parasitism of the fungus on the nematode body. (Bar=30µm)-Parasitism of the nematode eggs by the fungus *Catenaria auxiliaries* (Kühn) Tribe L: The vegetative thallus (Vt) of fungus *C. auxiliaris* consists of a chain of swollen cells grown from nematode egg (Ne). M: Mature spore (Ms) of *C. auxiliaries* in a nematode egg (Ne) on water Agar medium. N: High magnification of mature spores of the fungus [arrows] (Bar=20µm)

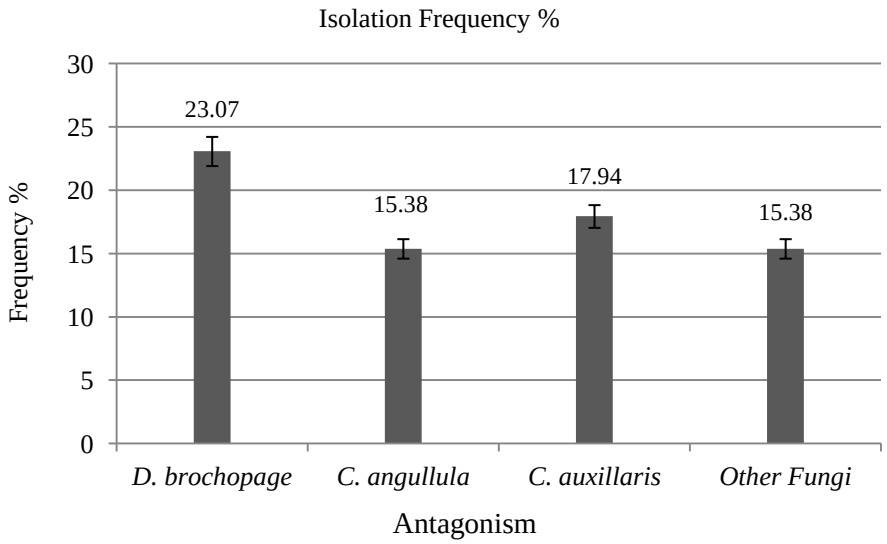

Figure 2. Isolation frequency percentage of nematode trapping fungi (antagonism) in all soil samples collected from study area.

Table 1. The presence of nematode trapping fungi in thirteen sites on vegetation of province Khulais with different nematode order

Site No.	Fungi species	Plant	Nematode Order	Parasite mode
1,2,4,5,6,7, 8, 10 and 12	*Dactylaria brochopaga* Drechsier	*Solanum melongena*	Tylenchida	Constricting rings
		Lycopersicum esculentum	Tylenchida & Rhabditida	
		Pennisetum americanum	Rhabditida	
		Zea maize	Rhabitida	
		Cucurbita pepo	Tylenchida & Rhabditida	
		Calotropis procera	Dorylaimida	
3,4,5,6,8,9, 10 and 11	*Catenaria anguillulae* Sorokin	*Abelmoschus esculentus*	Dorylaimida & Tylenchida	Endoparasitic by uniflagellate zoospores
		Citrullus colocynthis	Dorylaimida	
		Vigna sinensis	Dorylaimida & Rhabditida	
		Abutilon grandifolium	Dorylaimida	
		Cucurbita pepo	Dorylaimida	
1,2,3,8, 9 and 13	*Catenaria auxiliaris* (Kühn) Tribe	*Citrullus colocynthis*	Dorylaimida	
		Cucurbita pepo	Tylenchida & Rhabditida	
		Solanum melongena	Dorylaimida	
1,3,4,5,10, 12 and 13	Other Fungi	*Lycopersicum esculentum*	Tylenchida & Rhabditida	Variable
		Abutilon grandifolium	Dorylaimida	
		Zilla spinosa	Dorylaimida	
		Calotropis procera	Dorylaimida	

Table 2. The occurrence of nematode trapping fungi in collect soil samples at different sites with different soil properties

Site No.	Sample No.	Trapping fungi	UFC*/1g soil	pH	Soil type	Organic matter source	
						Source	%
1	1	*Dactylaria brochopaga* Drechsier	9.13±1.20	6.5	Sandy loamy	PD***	3.5
	2	*Catenaria auxiliaris* (Kühn) Tribe	8.13±1.85	7.0	Sandy loamy	PD***	2.0
	3	Other fungi	12.8±1.64	7.1	Sandy loamy	PD***	2.3
2	4	*Dactylaria brochopaga* Drechsier	8.20±1.08	6.6	Sandy loamy	PD***	2.9
	5	ND**	--	7.0	Sandy	--	0.9
	6	*Catenaria auxiliaris* (Kühn) Tribe	7.66±1.47	7.2	Loamy sandy	--	0.8
3	7	*Catenaria anguillulae* Sorokin	1.70±0.75	7.0	Loamy sandy	--	0.3
	8	*Catenaria auxiliaris* (Kühn) Tribe	2.28±0.94	7.1	Loamy sandy	--	0.6
	9	Other fungi	20.8±2.43	7.0	Sandy	--	0.7
4	10	*Catenaria anguillulae* Sorokin	4.20±1.08	8.0	Sandy loamy	PD***	2.0
	11	*Dactylaria brochopaga* Drechsier	8.36±0.63	6.9	Sandy loamy	PD***	3.0
	12	Other fungi	23.4±2.95	7.0	Sandy loamy	PD***	1.7
5	13	*Catenaria anguillulae* Sorokin	9.66±1.47	7.9	Sandy loamy	--	2.3
	14	*Dactylaria brochopaga* Drechsier	7.06±1.81	7.1	Sandy loamy	PD***	3.0
	15	Other fungi	8.46±1.30	7.3	Sandy loamy	--	0.9
6	16	*Catenaria anguillulae* Sorokin	5.20±1.08	7.9	Sandy loamy	--	2.0
	17	*Dactylaria brochopaga* Drechsier	4.40±1.24	6.6	Sandy loamy	--	2.5
	18	ND**	--	7.2	Sandy	PD***	1.9
7	19	ND**	--	7.1	Sandy	--	2.8
	20	*Dactylaria brochopaga* Drechsier	7.40±1.24	6.7	Sandy loamy	--	3.0
	21	ND**	--	7.1	Sandy	PD***	2.0
8	22	*Dactylaria brochopaga* Drechsier	8.00±0.91	6.9	Sandy loamy	--	3.0
	23	ND**	--	7.6	Sandy	PD***	2.5
	24	*Catenaria auxiliaris* (Kühn) Tribe	6.40±1.24	7.3	Sandy	--	1.7
9	25	ND**	--	7.1	Sandy	--	0.9
	26	*Catenaria auxiliaris* (Kühn) Tribe	2.03±0.86	8.0	Sandy loamy	--	0.3
	27	*Catenaria auxiliaris* (Kühn) Tribe	6.40±1.24	7.1	Sandy	--	0.4
10	28	*Dactylaria brochopaga* Drechsier	3.06±0.30	7.0	Sandy loamy	PD***	2.0
	29	*Catenaria anguillulae* Sorokin	1.7±0.75	8.0	Sandy loamy	--	1.7
	30	Other fungi	14.3±2.04	7.3	Sandy	--	1.2
11	31	ND**	--	7.0	Sandy	--	1.2
	32	*Catenaria anguillulae* Sorokin	5.20±1.08	7.5	Sandy loamy	--	0.2
	33	ND**	--	7.1	Sandy	--	1.8
12	34	*Dactylaria brochopaga* Drechsier	2.53±0.55	7.0	Sandy loamy	--	1.6
	35	Other fungi	22.3±2.04	7.0	Sandy	--	0.8
	36	ND**	--	7.7	Sandy	--	0.6
13	37	ND**	--	7.3	Sandy	--	0.3
	38	*Catenaria auxiliaris* (Kühn) Tribe	3.06±0.30	7.2	Sandy	--	0.9
	39	Other fungi	15.0±0.91	7.0	Sandy	--	0.2

- UFC*: Unite forming colony.
- ND**: Not detectable Nematode antagonisms
- PD***: Poultry droppings