

FIRST RECORD OF MYCETOPHAGOUS NEMATODE *APHELENCHUS*
AVENAE IN IRAQ WITH DESCRIPTION AND TESTING THEIR
PROPAGATION ON DIFFERENT FUNGUS CULTURE

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ABSTRACT

Aphelenchus avenae was isolated from the wheat crown in Summel distract- Duhok, Kurdistan region-Iraq infected by a crown rot disease which is caused by *Fusarium* spp; wheat's crown culturing on Potato Dextrose Agar (PDA) and incubating at 25°C *A. avenae* was found associated with fungal culture which meant that fungal nematode was parasitic on crown rot fungi on wheat crown, this species was described for the first time in Iraq. Fungal Nematode incubated with *Fusarium graminearum*, *F. oxysporum* and *Verticillium dahliae* reproduce in both solid and liquid media, best results of nematode reproduction were recorded on *F. graminearum* followed by *F. oxysporum* and *V. dahlia* respectively. The nematode *A. avenae* did not reproduce on the liquid media of these fungi.

Keywords: *Aphelenchus avenae*, Iraq, Mycetophagous, Nematode, Summel.

INTRODUCTION

Aphelenchus avenae Bastian, 1865 feeds on a variety range of fungi (Mankau and Mankau, 1963; Giannakis and Sanders, 1989; Okada and Ferris, 2001) and has a wide range of fungal host in both phyto-pathogenic and saprophytic species in different conditions. *A. avenae* is a mycophagous nematode which can't parasitize on higher plant (Barnes *et al.*, 1981), and there is no record of real parasitism, damage or feeding of this nematode on the plant, thus *A. avenae* is called fungal nematode. There are many studies on development, growth and feeding of *A. avenae* on fungi culture media, its growth on *Fusarium solani* and *Rhizoctonia solani* has been studied by Barnes *et al.* (1981). This nematode has been recorded in many countries worldwide in different soil types (Okada and Ferris, 2001). This nematode is capable to the growth and propagation on culture media of fungi grown on PDA in different amount of potato dextrose solution when incubated at 25°C; propagation of *A. avenae* in Semisolid substrate also studied (Ishibashi *et al.*, 2000). *A. avenae* was used as a bio-control agent against soil-born fungal pathogen in many studies, using the different population size of *A. avenae* in infested soil with different pathogenic fungi such as *R. solani*AG-4, *Fusarium oxysporum* f. sp. *lagenariae*, *Pythium* sp. and *Phytophthora nicotianae* var. *parasitica* showed that this nematode had a noticeable effect on the survival of cucumber plants, thus *A. avenae* is considered as a good biological control agent for those fungi (Barnes *et al.*, 1981).

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The attraction of *A. avenae* to plant roots also impedes colonization of plant-parasitic nematodes (Matsunaga *et al.*, 1996). The penetration of *Pratylenchus coffeae* and *Meloidogyne incognita* of cucumber transformed hairy roots on agar plate decreased the incidence of *A. avenae* (Ishibashi, 2005).

This study aims to indicate the first record and description of *A. avenae* in Iraq and to study its growth on different culture media and various soil born-fungi.

MATERIALS AND METHODS

Aphelenchus avenae is collected from wheat crown in Summel distract- Duhok, Kurdistan region-Iraq infected by crown rot disease and is caused by *Fusarium* spp. after culturing wheat's crown in potato dextrose agar (PDA) and incubated at 25 °C. Nematodes were extracted from culture media and collected according to Grewal *et al.* (2005) then added again to culture media for reproduction. Then nematode was picked and mounted on slide to study it's for identification. More than 30 slides were made for each of female, male and eggs. Following morphometrical measurements and body, ratios were calculated (Machado *et al.*, 2010):

L=total body length. V=distance from vulvae to posterior end. St= total stylet length.
F= total esophagus length. v-a= distance between vulvae and tail. T=total tail length.

$$a = \frac{\text{Body length}}{\text{Maximum body width}} \quad b = \frac{\text{Body length}}{\text{Oesphagal length}} \quad c = \frac{\text{Body Length}}{\text{Tail length}}$$

Study of *Aphelenchus avenae* reproduction in different fungus culture:

Three pure fungal cultures of *F. graminearum*, *F. oxysporum* and *V. dahliae* were used as host for fungal nematode, two type of medium, used both PDA solid medium and Malt extract liquid media were used with three petri dish for each replication. After calculating number of *A. avenae* in solution using counting dish, then 50 *A. avenae* was added by pipette to fully grow fungal culture and was incubated at 25°C for two weeks then nematodes was extracted again from each culture to calculate number of nematode to know the propagation rate of nematode on each fungal culture. Data were analyzed using SAS program and means were compared according to Duncan's Multiple Range test, P=0.05(SAS, 1999).

RESULTS AND DISCUSSION

Family of Aphelenchidae Fuchs, 1937, Steiner, 1949 Syn. Paraphelenchidae (Goodey, 1960) are characterized by large obvious median bulb (metacarpus) and occupied more than 75% of body width in the located part. Body of *A. avenae* usually large has plump lips, form flat cap, slightly offset, median bulb round slight elongate nerve ring just behind bulb, stylet long with or without small basal knobs, rectum lumen considerably widened just behind sphincter, anus and rectum can be seen very obviously near to the end of body so *A. avenae* has short tail. *A. avenae* fungal feeder nematode can grow and develop on different fungi (Okada and Ferris, 2001).

Aphelenchus avenae Bastian, 1865 description and identification:

Female: (Pl.1, A, B, E, G) body tapers slightly at head region narrowing abruptly behind vulva, there is no constriction or rarely constriction occurs behind anus. Tail short rounded rarely slightly flattened at terminal, tail is conical. Stylet knob less, junction conical parts is less than 1/2 of its length. Median bulb round to slight elongated, esophageal lumen narrows and widens into intestine, dorsal gland overlapping intestine, usually on left side as can be seen under compound microscope. Anus

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transverse 1/3 body width, anterior lip slightly protruding. Vulva oval, vagina has thick wall (Bastian, 1865).

Eggs: *A. avenae* eggs are similar to other nematode species and are oval shape to elongated transparent, embryogenesis process can observe inside eggs. Length and width of eggs were as follow: Eggs length= 98.75 μ m. Width=40.93 μ m

Female morphometrical measurements and body ratios:

L=1.2 mm. V= 220.95 μ m. St= 20.76 μ m. F= 54.91 μ m. v-a= 219.37 μ m.
T=56.49 μ m. C= 21.24. b= 21.85. a=35.03.

Male: Anterior part is similar to female body shape straight at rest (Pl.2, A B and C). It has two spicules cephalated near to tail. Gubernaculum V shape. Tail is taper; most studies mentioned that male rate is very rare (1:10000) or absent, but in our culture male ratio was approximately 1: 100 females (Bastian, 1865).

L= 0.6 mm. Spicules= 31 μ m. T=60.25 μ m. C=9.87. a= 19.59 μ m

***Aphelenchus avenae* propagation in different fungus culture:**

Diagram (1) illustrates that after two weeks of incubation *A. avenae* propagated and increased in number at the huge level on *F. graminearum* number of nematode reached to (114233) while *F. oxysporum* was secondly preferred by *A. avenae* and number of nematodes were (47013). This means that *A. avenae* was also propagating well on *F. oxysporum* and these results agreed with many researchers who stated that *A. avenae* could feed on different species of *Fusarium* (Okada, 2006; Karuri *et al.*, 2014). *A. avenae* incubated in *V. dahlia* increased in number to 15000, lower nematode reproduction on *V. dahlia* as compared with two other species of *Fusarium* is possibly due to the thinner width of *V. dahliae* hyphae, rather than the length of nematode stylet (Okada, 2006). These results illustrate that *A. avenae* can propagate very quickly and increase in number to reach a huge number in a short time when incubated on preferable fungi to feed on hyphal tissue (Pl. 3) which make it effective bio-control agents.

Number of *A. avenae* didn't increase and nematodes were dead while incubated on liquid culture of the same fungi after two weeks. This result agreed with the idea that *A. avenae* could not propagate and living in liquid substrate cause nematode need to perpendicularly attach their stylet to the hyphal tissue to feed (Grewal *et al.*, 2005). In all fungal culture nematode destroyed and hydrolyzed fungal mycelium during their feeding (Pl.4) which means that *A. avenae* secrete hydrolytic enzymes to dissolve and feed on hyphae tissues (Ishibashi *et al.*, 2005).

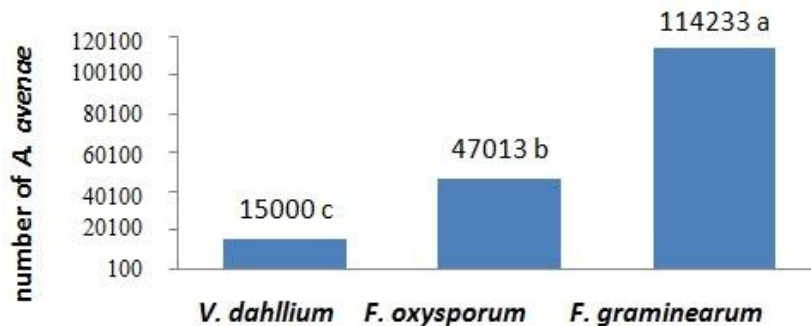


Diagram (1): Increasing in *A. avenae* population on *Fusarium graminearum*, *F. oxysporum* and *Verticillium dahlia* cultures grew on PDA after two week of incubation.

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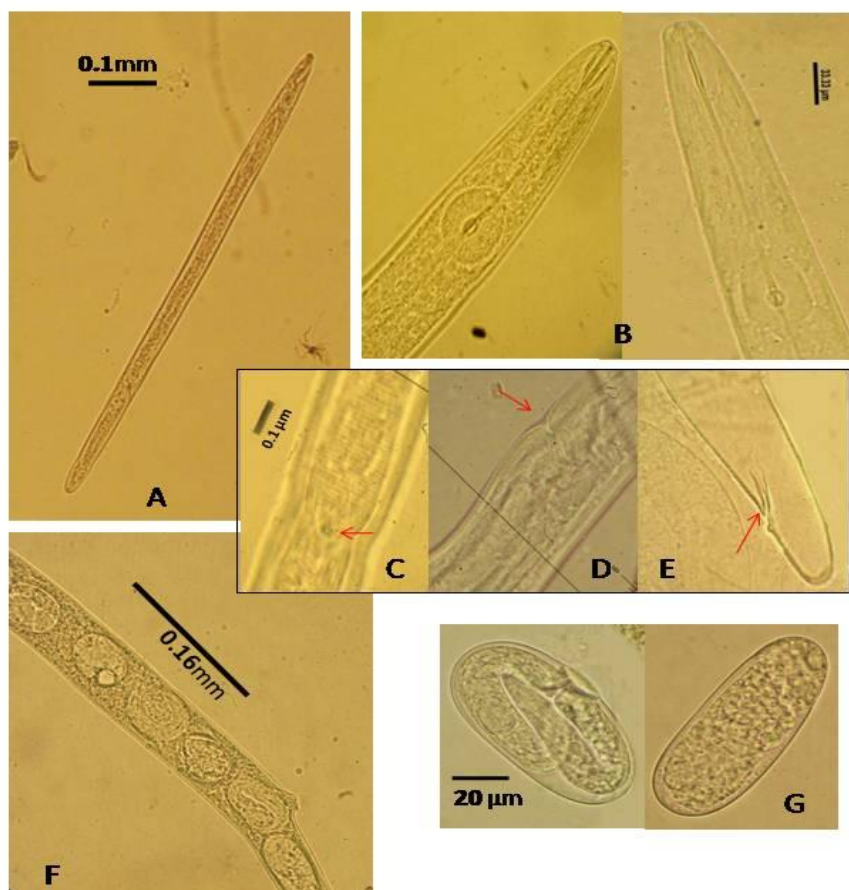


Plate (1): *Aphelenchus avenae*: A) Female; B) Anterior portion show esophagus, medium bulb and stylet. C and D) Vulva in ventral and lateral side. E) Tail region show rectum and anal opening. F) Female in egg laying stage, eggs can be seen inside body and vulva opened for egg laying. G) Eggs in different embryogenesis stage.

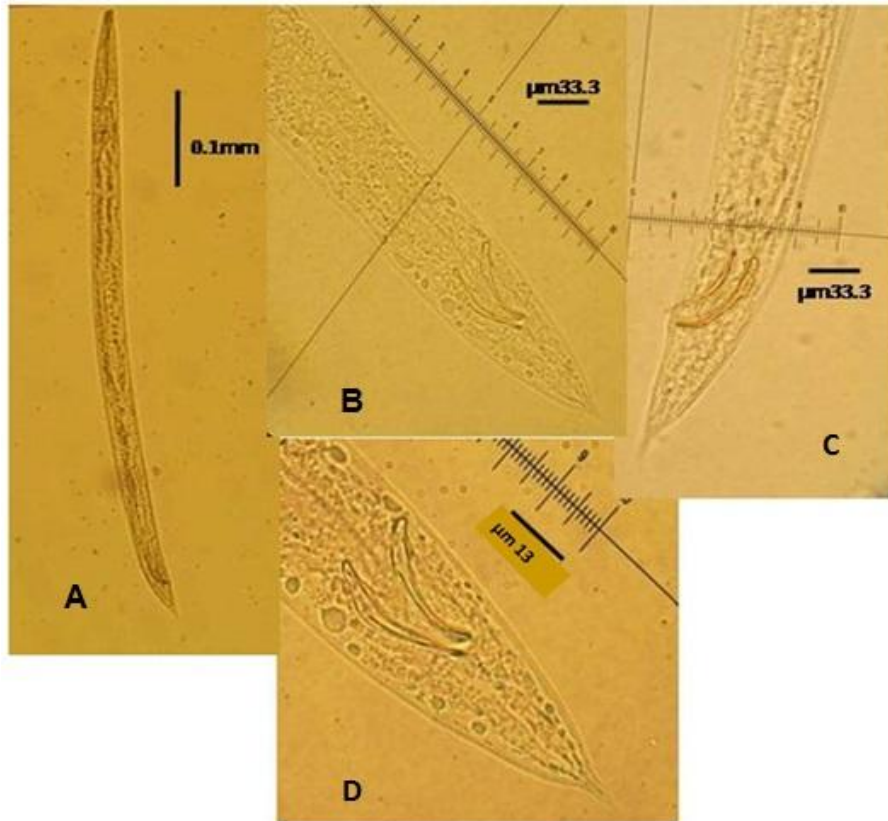


Plate (2): *Aphelenchus avenae* : A. male; B. C.D. posterior end of male show spicules and tail region.

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Plate (3): Show anterior portion of *A. avenae* and its stylet during feeding on fungi hayphae.

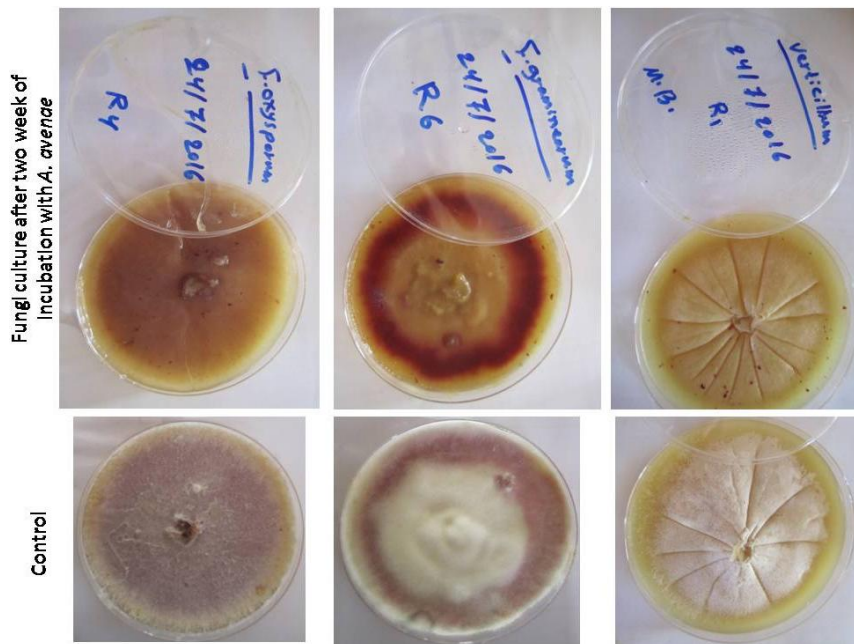


Plate (4): Fungi culture after two week on incubation with *A. avenae* and fungi culture without *A. avenae*, water added (control) shows hydrolysis of fungi mycelium.

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تسجيل اول للدودة الخيطية المتطفلة على الفطريات
Aphelenchus avenae في العراق
و وصفها ودراسة تكاثرها و تطفلها على اوساط زراعية مختلفة للفطريات

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الخلاصة

تم عزل الدودة الخيطية المتطفلة على الفطريات *Aphelenchus avenae* من تاج نباتات القمح المصابة بمرض تعفن التاج نتيجة اصابته بأنواع من *Fusarium spp.* في قضاء سميل - محافظة دهوك، اقليم كردستان- العراق. بعد زراعة تاج نباتات القمح في الوسط الغذائي PDA و تحضينه عند درجة حرارة ٢٥ م°، وجدت الدودة الخيطية *A. avenae* مصاحبة للفطريات مما يشير الى انها كانت متطفلة على الفطريات المصاحبة لتعفن تاج الحنطة، و اشارت النتائج الى انها تسجل لأول مرة في العراق.

حضنت *A. avenae* مع الفطريات *F. graminearum* و *F. oxysporum* و *Verticillium dahliae* لدراسة تكاثرها و تطفلها على هذه الفطريات في اوساط صلبة وسائلة، وجد بأن افضل تكاثر للدودة كان على الفطر *F. graminearum* ثم *F. oxysporum* و *V. dahlia* على التوالي. لم تتكاثر الدودة الخيطية *A. avenae* في المزارع السائلة لهذه الفطريات.