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ISSN 2146-0035-E-ISSN 2548-1002 ***Orijinal araştırma* (*Original article*)**

Morphological diagnosis of *Aelia rostrata* Boh. (Heteroptera: Pentatomidae) parasitized by *Hexamermis* sp. (Nematoda: Mermithidae) in Ankara, Turkey

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Ankara'da *Hexamermis* sp. (Nematoda: Mermithidae) tarafından parazitlenmiş *Aelia rostrata* Boh. (Heteroptera: Pentatomidae)'nın morfolojik teşhisi

Öz: Buğday üretim alanlarında kaliteyi ve maliyeti etkileyen faktörlerden birisi zararlı böceklerdir. Kımıl, *Aelia rostrata* Boh. (Heteroptera: Pentatomidae) Türkiye'nin Orta Anadolu Bölgesi'nde buğdayın ekonomik olarak en önemli zararlılardan birisidir. Bu zararlının erginleri yaklaşık dokuz ay süreyle ergin dönemde kışlak alanlardaki kuru yapraklar altında veya kök çevresindeki toprak içerisinde kışı geçirir. Bu dönemde, kışlak alanlardaki *Hexamermis* sp. (Nematoda: Mermithidae) zararlının önemli bir doğal düşmanıdır. Zararlının mücadelesinde bu nematod, biyolojik kontrol etmeni olarak önemli bir potansiyele sahiptir. Nematoddan ari alanların bulaştırılmasında, kışlama alanlarından toplanan bulaşık ergin kımılların çok büyük önemi vardır. Bu yöntemin başarısı mermithidlerle bulaşık bireylerin doğru morfolojik teşhisinin yapılmasına bağlıdır. Çalışma Ankara ilinde Oyaca kışlağından 2015 yılında toplanan örnekler ile yürütülmüştür. Çalışma sonuçlarına göre bulaşık kımıl bireylerinin vücutları ıslak ve yağlı bir görünüme sahiptir. Bulaşık kımılın hareketi nematodun konukçunun vücudundan çıkmasına yakın zamanda yavaşlar. Nematod ile bulaşık böceğin boynu ileri doğru uzamakta ve nematodlar genellikle konukçu vücudunu göğüs ve başın birleştiği yerden terk etmektedir. Nematod konukçuyu terk etmeden önce başın göz seviyesi ile göğüs arasındaki ortalama mesafe 355.3 ± 113.17 µm (n = 11) olarak ölçülmüştür.

Anahtar kelimeler: *Aelia rostrata, Hexamermis*, Mermithidae, morfolojik teşhis, buğday, Ankara

Abstract: One of the factors affecting the quality and cost of the wheat is harmful insects in wheat producing areas. The wheat stink bug (WSB), *Aelia rostrata* Boh. (Heteroptera: Pentatomidae) is one of the most economically important pests of wheat in the Central Anatolia Region of Turkey. Adults of this pest overwinter in the adult life stage under dry leaves or in soil around roots for about nine months in overwintering areas. During this period, *Hexamermis* sp. (Nematoda: Mermithidae) is an important natural enemy of this pest in overwintering areas and has considerable potential as a biological control agent.

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Infected WSB adults collected from overwintering areas are of great importance for the infection of nematode-free areas. The success of this method depends on the morphological diagnosis of individuals infected with mermithids. The study was carried out by collecting overwintering adults from Oyaca in Ankara Province in 2015. The bodies of infected WSB individuals have a wet and greasy appearance. The movement of infected WSB is slowed when the time is near for nematodes to leave the host's body. The cervix of the infected insect was prolonged and the nematodes usually left the host's body from the junction of the thorax and head. Before leaving the host, the mean distance between the head at eye level and the thorax was 355.3 ± 113.17 μm (n = 11).

Keywords: *Aelia rostrata*, *Hexamermis*, Mermithidae, morphological diagnosis, wheat, Ankara

Introduction

Wheat, *Triticum aestivum* L. (Poales: Poaceae), is the world's most important crop in terms of total harvested weight and use for both human and animal nutrition. In wheat production, one of the factors affecting the quality and cost is harmful insects. Among them, the wheat stink bugs (WSBs) of the genus *Aelia* (Hemiptera: Pentatomidae) are the economically important pests of wheat in Central Anatolia Region of Turkey.

The *Aelia* genus contains about 25 species in the Palearctic region (Wagner 1960; Brown 1962; Lodos 1981). They cause significant damage to wheat in Greece, Hungary, France, Spain, Sicily, Austria and the old Yugoslavia in some places and years (Grigorov 1960; Tadic 1970; Campanella et al. 1978; Gallego & Sanchez-Boccherini 1980; Salis et al. 2013). Although there are a total of 12 species belong to this genus in Turkey, *Aelia rostrata* Boh. is a very serious pest in the major wheat-growing areas of Turkey, especially in Central Anatolia (Lodos 1981).This species occurs mostly in the Near Eastern countries such as Turkey, the southwestern part of the newly independent states of the former U.S.S.R., and northeastern Iran (Putschkov 1961, Brown 1962; Panizzi et al. 2000). It attacks wheat, barley, rye, oats and a number of other graminaceous plants, with wheat being the preferred host. A 34% - 93% loss in wheat production due to *A. rostrata* was reported in Ankara Province from 1989 to 1991 (Memisoglu et al. 1994). This major pest of grain could cause crop losses of up to 100% in some outbreak years (Memisoglu et al. 1996). *Aelia rostrata* completes one generation per year (Putschkov 1961; Dikyar 1981; Panizzi et al. 2000). This pest spends about 3 months of its life cycle in wheat and for the rest of the time they mostly live inactively in the adult life stage under dry leaves or in the soil around the roots of stubs of weeds on the mountains and hills around wheat fields during hibernation and aestivation. During this period, climatic conditions, adult parasitoids, nematodes and entomopathogenic fungi appear to play an important role in reducing their populations. The total mortality rate reached 97.62% in the overwintering areas in some years but the individual contributions of these factors

have not been determined (Memisoglu et al. 1996). Some studies have dealt with the relationship of the mermithids and WSB in overwintering areas. Many mermithids have been reported from species of the closely related hemipterans. Examples of these were *Mermis* sp. (Nematoda: Mermithidae) and *Hexamermis* sp. (Nematoda: Mermithidae) parasitizing *A. rostrata* and *Eurygaster maura* L. (Heteroptera: Scutelleridae) in Turkey (Dikyar 1981; Memisoglu & Ozer 1994; Memisoglu et al. 1994; Tarla et al. 2011; 2012; 2015) and *Hexamermis* sp. parasitizing *Rhaphigaster nebulosa* Poda (Heteroptera: Pentatomidae) in Italy (Manachini & Landi 2003). In addition, *Pentatomimermis pentatomiae* (Rubtzov) (Nematoda: Mermithidae) was reported from *Elasmostethus interstinctus* (L.) (Heteroptera: Acanthosomatidae) at Novosibirsk, Russia (Rubstov 1978). Also, the genus *Hexamermis* was discovered infesting *Piezodorus guildinii* (Westwood) and *Acrosternum hilare* (Say) (Heteroptera: Pentatomidae) in the United States (Kamminga et al. 2012). In recent years, a new species was obtained from *Eurygaster integriceps* Put. (Heteroptera: Scutelleridae) in Turkey and named *Hexamermis eurygasteri* Tarla, Poinar & Tarla (Tarla et al. 2011). Tarla et al. (2012) reported parasitism data relating to this pest under natural conditions in Gaziantep Province, Turkey. In addition, mermithid species have been reported from some other insect species in Turkey (Yaman et al. 2002; Mennan & Erturk 2006; Yaman et. al. 2009).

The parasitism rate of the mermithids attacking *A. rostrata* reached 36.1% in overwintering areas at Haymana in Ankara Province, Turkey (Tarla et al. 2012). The results of that study suggest that *Hexamermis* sp. is a major mortality factor for WSB populations under natural conditions. The earliest studies on the overwintering and migration of WSB were conducted in Turkey (Brown 1965; Memisoglu et al. 1994). Adults of this pest become inactive during hibernation and aestivation for about nine months in overwintering areas. These areas are very important for the biological control of this pest because adults can be easily collected from there and sent to infect the nematode-free overwintering areas. The success of this method depends on true morphological diagnosis of the individuals infected with mermithids which are potential biological control agents of this pest.

The aim of this study was to diagnose the morphological characteristics of *A. rostrata* parasitized by *Hexamermis* sp..

Material and methods

Before the migration of *A. rostrata* to cereal fields, overwintering adults were collected by hand under dead leaves of *Quercus* spp. at Oyaca in Ankara Province, Turkey (39° 30' 27" N, 32° 38' 32" E; 1315 m) on March 15, 2015. Insects were taken to the laboratory in transparent plastic bags and separated by gender (141 females and 149 males). After that, a pair was released onto wheat plants in plastic

petri - dishes (90x16 mm) lined with blotting paper, according to the method described by Tarla et al. (2012). These were placed in a climate controlled room maintained at 26 ± 2 °C and 65 ± 10% relative humidity (RH), and under a light: dark (L: D) cycle of 16: 8 hours. Wheat plants were replaced by fresh ones once every 2 - 3 days. The blotting paper was moistened with distilled water once every two days to provide humidity. The samples were checked every day. It was thought that the insect that not laying eggs may be parasitized with entomoparasitic nematodes. It was reached a final decision after post-parasitic juvenile nematode left the body of *A. rostrate* (Figure 1). Morphological differences were observed in the adult individuals of *A. rostrata* for diagnosis of those parasitized by *Hexamermis* species, until all males and females in the trial had died.

Figure 1. Post-parasitic juvenile of *Hexamermis* sp. after leaving the body of *Aelia rostrata*

The taxonomic differentiation of *A. rostrata* species was completed according to characteristics reported by Wagner (1960; 1966), Brown (1962), Lodos (1958) and China & Lodos (1959). The genus identification of nematodes was made by the author. The photographs were acquired by using an Olympus SZX10 microscope with an integrated Olympus SC30 camera.

Results and discussion

Many adults of *A. rostrata* were found to be parasitized by *Hexamermis* species. Morphological observations were done on them under laboratory conditions. Some important differences were determined. The movement of the infected insect slowed down before the entomoparasitic nematodes leave the body of the insect. The outer surfaces of their bodies have a wet and greasy appearance. The heads of the individuals infected with the nematode were prolonged and the cervix was expanded. Similar symptoms in *A. rostrata* infected with *Agamermis* sp. were reported by Memisoglu & Ozer (1994). The mermithids usually left the body from the cervix of *A. rostrata*. Before the nematodes left their *A. rostrate* hosts, the mean distance between the head at eye level and the prothorax (Figure 2 A) was 355.3 ±

113.17 µm (n = 11). There was no distance between the head and the prothorax of uninfected insects (Fig. 2 B).

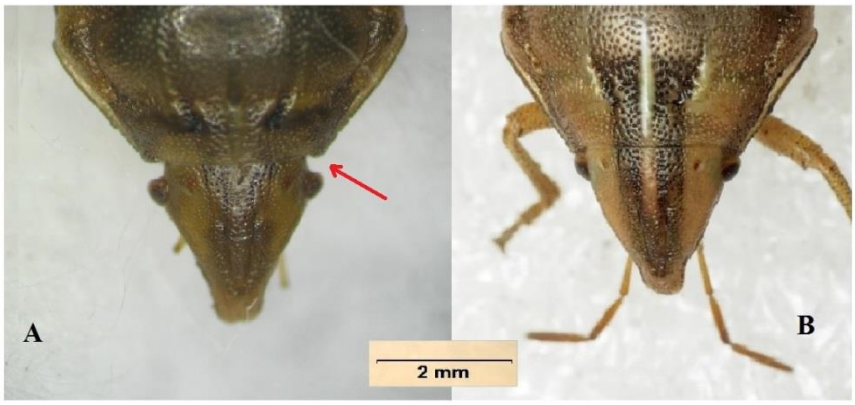

Figure 2. Distance between the head at eye level and the prothorax of infected (A) and uninfected *Aelia rostrata* (B)

Aelia rostrata overwinters in the adult life stage under dry leaves or in soil covered with leaves of *Pinus* spp., *Acantholimon venustum* Boiss., *Quercus* spp., *Astragalus acicularis* Bunge, *A. microcephalus* Wild. and *Thymus* sp. on mountains or hills. It can be found on all aspects of the hills, but it prefers the north side. Generally, it is found more densely at 1500-1700 m altitudes (Memisoglu et al. 1996). These areas are very important because overwintering adults can be easily collected and sent as a biological control agent to infect nematode-free overwintering areas. The success of this method depends on the morphological diagnosis of individuals infected with mermithids.

Morphological observations done on adult individuals of these *Hexamermis* sp. revealed that this species may be different from *H. eurygasteri*. For this reason, a study is being conducted to identify this nematode species with biotechnical methods.

Hexamermis sp. is one of the most important parasites of *A. rostrata* in overwintering areas. Parasitism rates in WSB females and males by *Hexamermis* sp. were 24.7 and 33.6% in 2010 and 21.4 and 36.1% in 2011 in Ankara province of Turkey (Tarla et al. 2012). The parasitism rate of the nematode *Agamermis* sp. attacking *A. rostrata* reached 45.9% in overwintering areas in Ankara province of Turkey in 1981 (Memisoglu et al. 1994). In another study, the infection rate of *A. rostrata* by *Mermis* sp. was 40.0% in the same province in 1966 (Dikyar 1981). On the basis of the information provided earlier, the need for detailed studies on the identification of the species and biology of this nematode became apparent. Understanding the ecological and biological relationships between the nematode and *A. rostrata* should result in its most appropriate use as a biological control

agent contributing to an integrated approach to WSB management. This mermithid species could be important candidate for use in the biological control of *A. rostrata* in the future.

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