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***Orijinal araştırma* (*Original article*)**

The weevil, *Hypolixus pica* (F.) (Coleoptera: Curculionidae) as a potential biological control agent of *Amaranthus* species (Amaranthaceae) in Adana Province, Turkey

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Adana'da *Amaranthus* türleri (Amaranthaceae) üzerinde bulunan potansiyel bir biyolojik mücadele etmeni: *Hypolixus pica* (F.) (Coleoptera: Curculionidae)

Öz: *Amaranthus* türleri, Türkiye'de yazlık kültür bitkileri ile bahçelerde verimi etkileyen önemli yabancı otların başında yer almaktadır. Pestisitlerin çevre ve insan sağlığı üzerindeki zararlı etkileri ile *Amaranthus* türlerinin çeşitli herbisit etki mekanizmalarına karşı yüksek düzeyde dayanıklılık geliştirmesi, alternatif yabancı ot kontrol yöntemlerine ilgi duyulmasına neden olmuştur. Yabancı otlarla biyolojik mücadele son zamanlarda büyük önem kazandığından, bu çalışma Adana ilindeki *Amaranthus* türlerinin potansiyel olabilecek biyolojik mücadele ajanlarının belirlenmesi amacıyla yürütülmüştür. Bu amaçla Adana'nın üç ilçesinde (Yüreğir-Ceyhan-İmamoğlu) rastlantısal olarak 10 tarlada sörvey yapılmıştır. Her tarladan rastgele 10 adet *Amaranthus* bitkisi toplanmış, kesip açılmış ve farklı evrelerde bulunan böceklerin sayıları kaydedilmiştir. Bu çalışma ile, *Amaranthus* cinsine bağlı 3 yabancı ot türü tespit edilmiş olup *Amaranthus palmeri* L. en yaygın tür olmuş, bu türü *Amaranthus retroflexus* L. ve *Amaranthus spinosus* L. izlemiştir. *Amaranthus* bitkilerinde toplam olarak, 59 larva, 26 pupa ve 10 adet ergin birey tespit edilmiştir. Ergin bireylerin tamamı genital organlarına göre *Hypolixus pica* (F.) (Coleoptera: Curculionidae) olarak tanımlanmıştır. Sonuçlar, *H. pica*'nın *A. palmeri* ve *A. retoflexus*'un potansiyel biyolojik mücadele ajanı olabileceğini göstermiştir. Bu nedenle *H. pica* gibi potansiyel faydalı böcekler için kitle üretim yöntemleri geliştirmelidir. Ayrıca, ekolojileri ile hedef yabancı ot türleri üzerinde biyolojik mücadele etmeni olarak rollerini belirlemek için daha detaylı çalışmalara gereksinim vardır.

Anahtar kelimeler: Adana, *Amaranthus* spp., biyolojik mücadele, horoz ibiği, Türkiye

Abstract: *Amaranthus* species (Family) are among the most important weeds in summer crops and orchards in Turkey. The detrimental effects of pesticides on the environment and human health and the development of high levels of resistance to several herbicide classes by *Amaranthus* species have generate interest in alternative weed control methods. Since the biological control of weeds has gained great prominence recently, this study was carried

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out to determine the potential biological control agents of *Amaranthus* spp. in Adana Province. Turkey. For this aim, ten randomly selected fields randomly were in three districts (Yüreğir-Ceyhan-İmamoğlu). In each field, ten *Amaranthus* plants were collected randomly and dissected, and the numbers of the weevils and theirdifferent stages were recorded. In this study, three *Amaranthus* species were identified and *A. palmeri* was the most common, followed by *A. retroflexus* and *A. spinosus*. Fifty-nine larvae, 26 pupae and 10 adults were collected from the *Amaranthus* plants. All adult individuals were identified as *H. pica* according to their genital organs. Our results show that *H. pica* could be considered to be a potential biological control agent of *A. palmeri* and *A. retroflexus*. More detailed studies are needed to determine its ecology and role as a biological control agent of the target weed species. In addition, it is crucial to develop mass rearing methods for potential beneficial insects such as *H. pica*.

Keywords: Adana, *Amaranthus* spp., biological control, pigweeds, Turkey

Introduction

Turkey, which is located at the intersection of the continents of Asia and Europe, has different geographical regions in terms of climate and soil characteristics, and is especially rich in plant biodiversity. It has more than 12.000 plant species (Akbaş & Asav 2015). The highly diverse environment in Turkey also has many invasive plants that cause great productivity losses in agroecosystems.

Weeds, which are among these invasive plant species, are one of the major plant protection problems in cropping areas and can reduce the yields by more than 90% (Uygur 2002). Invasive weeds that cause harm in many parts of the world have been reported in different studies in Turkey (Önen 2015; Eren et al. 2016).

Seventy four amaranth species (*Amaranthus* spp., Amaranthaceae) which can become weeds have been described worldwide, mainly in the tropical and temperate zones (Mosyakin & Robertson 2004). *Amaranthus* species are among the most important weeds in summer crops and orchards in Turkey. With the listing of *Amaranthus crassipes* L. in the 'Flora of Turkey', the number of *Amaranthus* species increased to 15 and the number of taxa increased to 18 (Uygur et al. 2021). Moreover, *Amaranthus retroflexus* L. is one of the most important weed species in Europe because of the economic losses causes (Schroeder et al. 1993). Due to their wide ecological tolerance, their naturalization in Turkey was not difficult (Uygur et al., 2021). The first records in Turkey of *Amaranthus palmeri* L., a weed that is difficult to control, were from Adana, Osmaniye and Hatay Provinces of Turkey in 2014 (Eren et al. 2016).

Amaranthus palmeri is an invasive species that is quickly spreading across the western parts of Europe, Asia, Australia and North America (Steckel 2007). It is estimated that this weed was introduced to Turkey in animal manure, irrigation water and crop seeds. Although *A. palmeri* is seen especially in non-agricultural areas such as roadsides and irrigation canals, it can be seen in the citrus orchards, corn and peanut fields, which are important agricultural products of Turkey (Turhan 2017). Another *Amaranthus* species, *Amaranthus spinosus* L., which is native to tropical America is a noxious weed affecting 28 different crops in 44 countries (Waterhouse 1994). The presence of these weedy plant species was

reported in the Eastern Mediterranean Region of Turkey in 2000 (Gönen & Uygur 2000).

Herbicides are widely used to control weeds, including *Amaranthus* species, but their residues cause insidious environmental pollution (Uygun et al. 1994). Moreover, overreliance on herbicides has resulted in weak control of *Amaranthus* species due to high levels of resistance to several herbicides (Barralis & Gasquez 1987; Gossett et al. 1992; Horak & Peterson 1995; Steckel 2007; Vencill et al. 2008; Norsworthy et al. 2008). For these reasons, classical biological control methods are one of the most important strategies for sustainable agroecosystems. Although biological control requires intensive research and investment in the short term, it is the easiest, cheapest and sustainable method to apply for long term success.

Over the past 100 years, a total of 114 weed species have been targeted with 200 biological control agents. There are many studies on insect species with biological control potential on *Amaranthus* species in Europe; 241 insect species have been reported from 21 *Amaranthus* species (El Aydam & Bürki 1997; Winston et al., 2017). Among them, the Curculionidae (Coleoptera) family is the most important, including 48 beneficial species (Haseeb et al. 2006).

In this family, the genus *Hypolixus* Desbrochers, 1898 (Curculionidae: Lixinae) is distributed in the Palearctic, Afrotropical, Oriental and Australian regions (Alonso-Zarazaga & Lyal 2002). *Hypolixus truncatulus* (F.) (Coleoptera: Curculionidae) has been substituted for the use of herbicides in the control of *A. spinosus* in Thailand (Napompeth 1982). Moreover, *H. pica* was harmful to the roots and stems of *A. caudatus* L. in Egypt and the damage could reach 100% locally (Kolaib et al. 1986). Also, Gültekin & Korotyaev (2012) reported that *Cosmobaris discolor* (Boheman) (Coleoptera: Curculionidae) and *H. pica* may be useful as biological control agents against the *A. spinosus*.

Despite the increasing importance of biological control agents against weeds across the world, a very limited number of studies have been conducted in Turkey. Against that background, survey studies were carried out to determine potential biological control agents on *Amaranthus* species in Adana Province, Turkey.

Materials and Methods

Ten fields (3 corn, 2 cotton, 2 soybean, 2 peanut and 1 sunflower) were surveyed randomly in three districts (Yüreğir-Ceyhan-İmamoğlu) of Adana Province in 2021 (Figure 1).

Figure 1. Survey areas for *Hypolixus pica* on *Amaranthus* species in different cultivated crops in Adana Province, Turkey.

Sampling was generally carried out at the edge of the field where *Amaranthus* weeds were growing. In each field, ten plants were collected randomly and dissected, and the numbers of weevils and their different stages were counted (Gültekin & Korotyaev 2012) (Figure 2). Immature stages of the sampled weevils were cultured in a laboratory at 25 ± 1°C, %60 ± 10 relative humidity, and 14L:10D (L: light; D: dark) conditions, to obtain adults. All adult individuals were identified by the second author.

To determine the *Amaranthus* species, plant samples were wrapped in paper, put into labelled plastic bags and brought to the laboratory where they were identified by Prof. Dr. Sibel UYGUR and Selin TÜNK M.Sc. (Çukurova University, Plant Protection Department, Weed Science Laboratory, Adana, Turkey)

Data evaluation

The figure for surveyed areas was created on Google Earth with coordinated data. The ratios of different *Amaranthus* species were determined by dividing the number of *Amaranthus* identified in the same species by the total number of *Amaranthus* sampled. Different life stages of *H. pica* found within the plant tissues (galleries) of plants in the surveyed areas were determined. Ratios of the different life stages of *H. pica* in different *Amaranthus* species were calculated from the numbers of each biological stage of the weevil and the total number of the weevil in the same weed.

Figure 2. Damaged parts of *Amarantus* spp. due to the feeding activity of *Hypolixus pica* and different life stages of the weevil in Adana Province, Turkey in 2021.

Results and Discussion

Three *Amaranthus* species were identified and *A. palmeri* was the most common one, followed by *A. retroflexus* and *A. spinosus* (Figure 3).

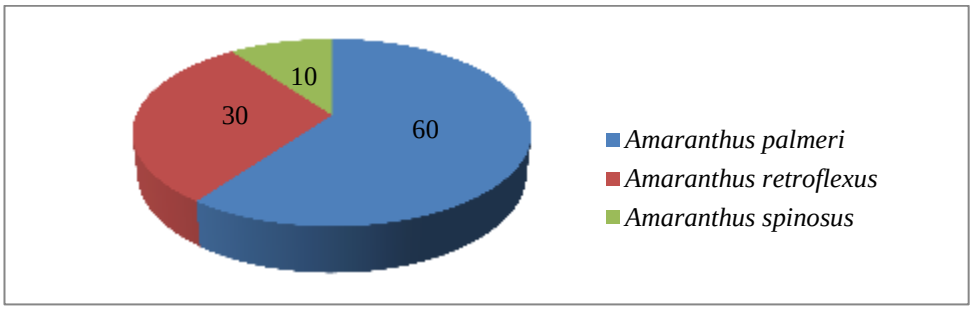

Figure 3. Proportions of three *Amaranthus* species sampled and identified in Adana Province, Turkey in 2021.

Three corn, two cotton, two soybean and two peanut fields, and 1 sunflower field, infested with *Amaranthus* plants, were surveyed. A total of fifty-nine larvae, 26 pupae and 10 adults were collected from the *Amaranthus* plants (Table 1).

Table 1. Total numbers of the different life stages of *Hypolixus pica* on *Amaranthus* species in in the sampling locations in Adana, Turkey in 2021

Sampling locations		*Amaranthus* species	Cultured plants with amaranths	Total number of *Hypolixus pica*		
				Larvae	Pupae	Adult
Yüreğir	1	*A. spinosus*	Corn	0	0	0
	2	*A. palmeri*	Corn	7	2	2
	3	*A. palmeri*	Cotton	1	2	0
	4	*A. retroflexus*	Cotton	6	2	3
Ceyhan	1	*A. retroflexus*	Soybean	3	2	1
	2	*A. palmeri*	Peanut	9	3	1
	3	*A. palmeri*	Corn	9	4	1
İmamoğlu	1	*A. retroflexus*	Peanut	13	2	0
	2	*A. palmeri*	Soybean	1	0	1
	3	*A. palmeri*	Sunflower	10	9	1
Amaranthus spp.			Total	59	26	10

All adult individuals were identified as *H. pica*, based on their genital organs (Figure 4). There were no individuals recorded from *A. spinosus*. More than half of the insects were collected from the *A. palmeri* and the rest were from *A. retroflexus* (Table 1)

Figure 4. Aedeagus: (a) dorsal view, (b) lateral view, (c) spermatheca and (d) spiculum ventral of the male of *Hypolixus pica*.

From *A. retroflexus*, a total of 32 *H. pica* individuals were collected; a substantial majority (68.75%) were larvae, followed by pupae (18.75%) and the rest were adults (12.50%). For *A. palmeri*, 58.73% were larvae of *H. pica*, followed by pupae (31.75%) and adults (9.52%) (Figure 5).

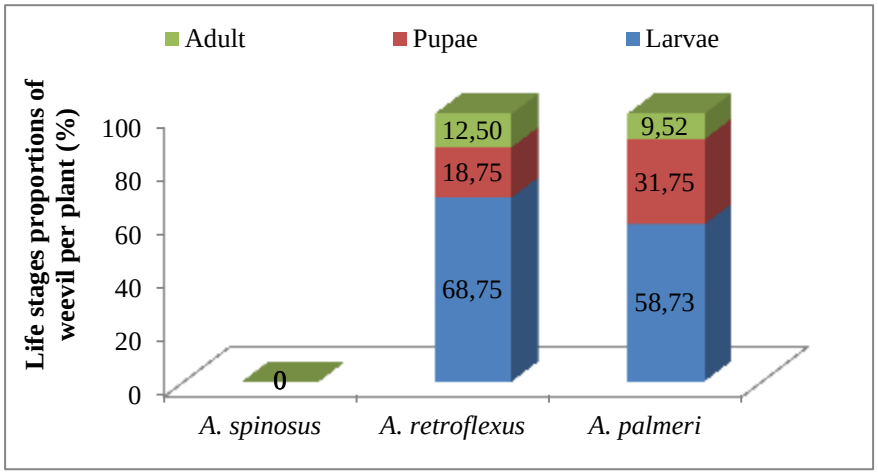

Figure 5. Ratios of different life stages of *Hypolixus pica* collected from three *Amaranthus* species in Adana Province, Turkey

Amaranthus species are some of the most important invasive weeds in crop fields and wastelands. In the United States of America, *A. palmeri* has been ranked as one of the most problematic weeds of crop plant species such as cotton, maize, and soybean (Ward et al. 2013). In the current research in 2021, *A. palmeri* was one of the most common species and it was detected in cotton, maize, peanut and soybean fields. In addition, *A. retroflexus* was collected from cotton, peanut and soybean fields (Table 1). Although *A. retroflexus* grows on a wide range of soil types and textures, it is less common on the acid soils in the south-eastern USA, where the other pigweed species, *A. palmeri*, is more abundant (Weaver & McWilliams 1980). The abundance and ecological impacts of some invasive plant species are much greater in their exotic ranges than in their native ranges (Hierro et al. 2013). Eren et al. (2016) reported that *A. palmeri* was an extremely aggressive species that had the potential to spread across large areas in Turkey.

Overreliance on herbicides to control weeds, including *Amaranthus* spp., and their detrimental effects on the environment and human health, and the risk of further deterioration of the natural balance, plus high levels of resistance to several herbicide classes, have generated interest in alternative weed control management such as biological control by arthropods and pathogens.

For more than a century, scientists have been interested in the potential use of insects for the control of *Amaranthus* spp. regarded as weeds in Europe; 241 species were reported from 21 *Amaranthus* species by El Aydam & Bürki (1997). *Amaranthus palmeri* was reported to be a host of some sucking insects, including *Aufeius impressicollis* Stål, 1870 (Hemiptera: Rhopalidae), *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Lygidae), *Polymerus basalis* (Reuter, 1876) and *Taylorilygus pallidulus* (Blanchard, 1852) (Hemiptera: Miridae) (Snodgrass et al. 1984; Jones & Allen 2012). Moreover, *A. retroflexus* has been determined to be a host plant for many insects such as *Ceutorrhynchus floralis* (Paykull), *Lixus subtilis* Boheman, *Rhinoncus perpendicularis* (Reich), *Sitona lineatus* Linnaeus, *Sitona hispidulus* (Fabricius) and *Tychius picirostris* (Fabricius) (Coleoptera:

Curculionidae) (Burki 1997). Also, several authors have suggested *Disonycha glabrata* (Tisler, 1990) (Coleoptera: Chrysomelidae) as a potential control agent of *Amaranthus* species (Hamenway & Whitcomb 1968; Vogt & Cordo 1976; Burki et al., 1997). *Disonycha glabrata* (Fabricius) (Coleoptera: Chrysomelidae), which is native to South America, feeds on *A. retroflexus*, and lays eggs internally (Hamenway & Whitcomb 1968). Balsbaugh et al. (1981) reported that adults of this species were released to suppress amaranths in North Dakota (USA) in 1979-80. Also, Tisler (1990) noted that the same species was being successfully used in the biological control of *A. retroflexus* in the warm regions of the USA.

Although many studies have reported different insect species on amaranth plants, in this study only *H. pica* was collected from *A. palmeri* and *A. retroflexus* growing naturally at the edges of, or inside fields of five different cultivated crops in Adana Province (Table 1). Napompeth (1982) noted that the Curcolionidae species, *Hypolixus truncatulus* (F.), has been substituted for the use of herbicides in the control of *A. spinosus* in Thailand. Moreover, some *Amaranthus* species have been determined to be host plants of *H. pica* in different countries (Tawfik et al. 1976; Kolaib et al. 1986; Pourtaherzarei et al. 2010). Kolaib et al. (1986) reported that *H. pica* was harmful to the roots and stems of *A. caudatus* plants in Egypt, and its damage could reach 100%. In Iran, adults of *H. pica* fed on leaves and seeds of *A. retroflexus* and laid their eggs inside stems and the larvae tunneled in the stem. New generation adults, which made holes when emerging from stems, produced three offspring per year (Pourtaherzarei et al. 2010). More recently, *H. pica* and *C. discolor* were determined to be potential biological control agents of *A. spinosus* in Turkey (Gültekin & Koryataev 2012). Overall, the results of the current study corroborated the findings of earlier research in Turkey.

In conclusion, with increasing public pressure to use more environmentally friendly and sustainable crop production, interest in alternative weed control strategies is rising. Biological control could be one of the most important tools for controlling weedy *Amaranthus* spp. However, controlling the weeds in the initial stages of crop growth limits the potential use of classical biological control due to the slow build-up of effective populations. For this reason, it is crucial to develop mass rearing methods for potential beneficial insects such as *H. pica* and to understand their ecology and interactions with the target weed species.

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