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Population development and Parasitoids of *Acanthiophilus helianthi* (Rossi) (Diptera: Tephritidae) On Five Different Safflower Varieties in Van, Turkey

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Abstract: *Acanthiophilus helianthi* (Rossi, 1794) (Diptera: Tephritidae) also known as safflower fly, is an oligophagous species that cause significant damage to safflower plants Safflower, *Carthamus tinctorius* (L.) (Asterales: Asteraceae), which is among its hosts, is an important energy plant that is drought resistant and has high adaptability. This study investigated the population growth of *A. helianthi* on five different safflower varieties (Asol, Ayaz, Balcı, Dinçer and Göktürk) in Van Yuzuncu Yıl University, Faculty of Agriculture for two years (2019-2020) in Van province. Result of the study, it was determined that the pest showed a similar population change in both years, the adult individuals were seen at the end of June - mid-August, and their pupae from this date to the beginning of September. It was seen that the insect density was statistically different between the varieties at different sampling dates and the highest adult density was found in variety of Asol pupae in both years, and in Göktürk variety. During sampling with sweep net, 4 parasitoids, *Ormyrus* sp., *M. annulatus, E. acroptilae* and *Bracon* sp., were detected and it was understood in the literature that they were parasitoids of *A. helianthi*.

Keywords: Acanthiophilus helianthi, parasitoids, population development, safflower

Acanthiophilus helianthi (Rossi) (Diptera: Tephritidae)'nin Van (Türkiye) İli'nde Beş Farklı Aspir Çeşidinde Popülasyon Gelişimi ve Parazitoitleri

Öz: Acantiophilus helianthi (Rossi, 1794) (Diptera: Tephritidae) Aspir sineği olarak bilinen aspir bitkilerinde önemli zararlara neden olan oligofag bir türdür. Konukçuları arasında bulunan aspir, *Carthamus tinctorius* (L.) (Asterales: Asteraceae) bitkisi, kuraklığa dayanıklı ve adaptasyon yeteneği yüksek önemli bir enerji bitkisidir. Bu çalışma Van Yüzüncü Yıl Üniversitesi Ziraat Fakültesi deneme alanlarında iki yıl boyunca (2019-2020) Van ilinde *A. helianthi* 'nin farklı aspir çeşitleri üstünde (Asol, Ayaz, Balcı, Dinçer ve Göktürk) populasyon gelişimi incelenmiştir. Çalışma sonucunda zararlının her iki yılda da benzer bir populasyon değişimi gösterdiği, ergin bireylerin haziran sonu - ağustos ortası arasında ve bu tarihten eylül başına kadar pupalarının görüldüğü belirlenmiştir. Farklı örnekleme tarihlerindeki çeşitler arasında böcek yoğunluğunun istatistiksel olarak farklı olduğu ve her iki yılda da en yüksek ergin yoğunluğu Asol çeşidinde, pupada ise Göktürk çeşidinde olduğu görülmüştür. Atrapla örnekleme esnasında 4 parazitoit tür *Ormyrus* sp. (Hymenoptera: Ormyridae), *Microdontomerus annulatus* (Spinola, 1808) (Hymenoptera: Torymidae), *Eurytoma acroptilae* (Zerova, 1986) (Hymenoptera: Eurytomidae) ve *Bracon* sp. (Hymenoptera: Braconidae) elde edilmiş ve bunlarını literatürde *Acantiophilus helianthi*'nin parazitoitleri olduğu anlaşılmıştır.

Anahtar Kelimeler: Acanthiophilus helianthi, aspir, parazitoitler, populasyon gelişimi

1. Introduction

Safflower is a plant species of the genus *Carthamus*, belonging to the Asteraceae family. There are 25 safflower species determined in the world. (Singh et al., 2006). Safflower *Carthamus tinctorius* (L., 1753) (Asterales: Asteraceae), which is grown today, was cultured from *Carthamus lanatus* (Saffron thistle) (L., 1753) (Asterales: Asteraceae) and *Carthamus oxyacantha* (Wild safflower) (L., 1753) (Asterales: Asteraceae) (Taşlıgil & Şahin, 2016). Safflower, which was brought to Turkey by immigrants from Bulgaria in the first years of the Turkey Republic, was first registered as Yenice in 1931, followed by Dincer in 1977 and Remzibey-05 in 2005. Among these three registered varieties, it is traditionally grown in a few provinces such as Dincer and Remzibey-05, Balıkesir, Eskişehir, Isparta and Konya (Kayaçetin et al., 2012). Safflower, a hot and drought region plant, is an annual plant grown for its oilseeds. It is about 80 - 120 cm tall, very branched, and in the form of a shrub. The narrow and long leaves are dark green, with saw-toothed edges, and thorny in some species (Kayaçetin et al., 2012). Safflower flowers consist of yellow, red, orange, or a mixture of these colors. Although the color of the flowers changes according to the variety, these features also add market value to the safflower. Safflower seeds

are white or cream, and some may have dark stripes (Ekin, 2005). Today, there are more than 200 varieties, and their oil ratio varies between 38% and 71.7% (Taşlıgil & Şahin, 2016).

Safflower is susceptible to many fungal, bacterial, and viral diseases, some of which can cause significant damage (Singh & Nimbkar, 2006), and fungal diseases are the most common among them. When the safflower plant is irrigated, diseases become much more common than those grown with rain and cause significant damage to the product (Nimbkar, 2008 & Mirshekari et al., 2013). Many pests cause product economic losses in the safflower plant (Nimbkar, 2008; Saeidi & Adam, 2011 & Esfahani et al., 2012, Lotfalizadeh & Gharali 2014). These are Acanthiophilus helianthi, Chaetorellia carthami, Terellia luteola, Urophora mauritanica (Diptera: Tephritidae), Uroleucon carthami, U. jaceae (Hemiptera: Aphididae), Thrips sp. (Thysanoptera: Thripidae), whitefly, Agrotis sp., Helicoverpa sp., Heliothis peltigera, Spodoptera littoralis, S. exigua (Lepidoptera: Noctuidae), Empoasca decipiens (Hemiptera: Cicadellidae), **O**xycarneus pallens (Hemiptera: Lygaeidae), Tetranychus urtica (Acari: Tetranychidae), Tropinota (*Epicometis*) hirta (Coleoptera:Scarabeidae). Acanthiophilus helianthi (Safflower fly), is a key pest of safflower which causes substantial yield losses in every season (Hand & Ro, 2018; Khuhro et al., 2021). It is a harmful species that feeds on various plant species belonging to the Cardueae (Asteraceae) family. Although the safflower fly A. helianthi is an oligophagous pest, it is one of the major pests limiting crop production in many countries (Talpur et al., 1995; Sabzailian et al., 2010; Saeidi & Adam, 2011; Damkacı, 2013; Basheer et al., 2014; Riaz et al., 2014). In addition to the oligophag harmful safflower, it also causes damage to 24 wild plants belonging to the Compositae family. It is the larvae that do the damage. After damage, the seeds usually dry before they are fully developed and take on a brown color. As a result of the damage, the oil rate in the seeds decreases, and their germination power is lost (Şengonca, 1983).

As a result of the researches, it has been observed that there are very few studies on the determination of the species that cause damage to the safflower plant. In this study, the population development of *A. helianthi*, which causes severe damage to *C. tinctorius* which has an important place among energy plants, was investigated. In the study, Turkey's most commercially preferred safflower varieties considered to host plants for safflower fly. As a result of the study, parasitoids of *A. helianthi*, the density of adults and pupae and the effects of cultivars on them were determined.

2. Materials and Method

In this study, population growth of *Acantiophilus helianthi* on five different safflower plants (Asol, Ayaz, Balcı, Dinçer and Göktürk) was investigated in field conditions in 25 March-30 September 2019 and 2020. The study area consists of a total of 25 parcels, 5 for each variety. The parcels were prepared with a length of 5 m x 5 m, leaving a 2 m gap between the parcels. The row spacing was 25 cm and 5 cm intervals were formed on the rows. Each plot is arranged in 12 rows. The field experiment was prepared according to the randomized plot design in the experimental areas of Van Yuzuncu Yıl University, Faculty of Agriculture, and was carried out along two years as five replications.

2.1. Density of adult and pupa

Adults and parasitoids of *A. helianthi* (Figure 1) were collected by sweep net at weekly (50 sweep net in each plot, a total of 250 sweep net for one variety) just after the plants started to form the flower bed. Weekly observations were made from the date the plants started to form the flower head, and every flower head that was damaged from the moment the sign of *A. helianthi* was seen (Figure 2) was recorded by making pupa counts under the binocular (Figure 2).

After the plants started to develop, all insects collected with sweep net every week were transferred to jars (kill bottle) containing ethyl acetate. After it was sorted and counted in the laboratory, the necessary information was written and left in eppendorf tubes. The scraping process was carried out for 2 years in a row, a total of 16 samples were collected. Photographs of the obtained insect species were taken under binoculars. Hymenoptera were identified by Hossein Lotfalizadeh (Iranian Research Institute of Plant Protection), Diptera Saeed Mohamadzade Namin (Islamic Azad University, Braconidae were identified by Konstantin Samartsev (Zoological Institute RAS, St. Petersburg).

2.2 Evaluation of data

The difference in the number of adults and pupae collected from different cultivars by weekly sampling was tested using the Twosex MSChart (Chi, 2021) program according to the Bootstrap (Paired, 100,000 B) technique (Chi & Liu 1985; Chi, 1988). Graphs related to the data were prepared using SigmaPlot (ver. 12) and MS Excel package programs.





Figure 1. Acanthiophilus helianthi (Kına E. Original). Şekil 1. Acanthiophilus helianthi (Kına E. Orijinal).



Figure 2. Damage pattern and pupa appearance of *Acanthiophilus helianthi* in safflower (Kına E. Original). *Şekil 2*. *Acanthiophilus helianthi'nin aspirde zarar şekli ve pupa görüntüsü (Kına E. Orijinal)*

3. Result and Discussion

It was determined that *Acantiophilus helianthi* formed a population with different densities on five different safflower cultivars discussed in the study.

3.1. Population density of *Acanthiophilus helianthi* on five different safflower varieties

The densities of adults and pupae of the pest on safflower varieties at the weekly were determined with the samples made in the experimental area in 2019 and 2020. The results are shown in (Figure 3) according to the years. Adult and pupa densities of the pest determined on cultivars at each sampling were compared statistically, and the results are given in Table 1. The climate data of both years in which the study carried out are shown in (Figure 4). In the first year of sampling, adult individuals were found on June 30 in all varieties (Table 1). When the samples collected on this date were compared according to their densities on different cultivars, it was seen that they were not statistically different (P>0.05) the average densities varied between 0.60-1.40 individuals/sweep net (Table 1). Adult densities were found to be statistically different in all samples except the densities determined on August 4 and July 7 in the samples made until the harvest period after this date (P<0.05).

The first pupae were found on Asol, Ayaz, and Göktürk cultivars on August 17 (Table 1). It was determined that pupa densities were not statistically different in all samples except the densities determined on August 24 in the samples made until the harvest period after this date (P>0.05) (Table 1). According to the data, the highest pupa density was found in Asol cultivar (4.40 individuals/sweep net) on August 24, and statistically, differences were found between cultivars.

Adult individuals were seen on 29 June in the second year samplings. When the samples collected on this date were compared according to the densities of being on different varieties, it was seen that they were statistically different in all samples except the densities determined on August 3 (P<0.05) (Table 1). When we look at the pupa density data of the second year, the inflorescences were found on August 17 in other cultivars, except for Balcı and Dinçer, as in the first year. (P<0.05). Averages densities rate were changed from 0.40-4.80 individuals/ Sweep net. It was determined that pupa densities were not statistically different in all samples except the densities determined on August 24 in the samples made until the harvest period after this date (P>0.05) (Table 1). When looking the data, it was found that Balcı cultivar (2.60 individuals/ Sweep net) on 24 August was lower than other cultivars on average. It was determined that the averages were in the range of 1.40-1.60 on September 8, close to the harvest period, and there was no difference between the varieties (P>0.05 Table 1).

It was observed that there were fluctuations in adult density in all varieties during the sampling dates for two years (Figure 3). Until August 17, when the last individual was seen, the highest adult was found in the Asol variety for both years compared to other types, followed by the Balc1 and Göktürk variety (Figure 3 and Table 1). When we look at the 2019 data, the pupa density showed a rapid increase on August 17-24, followed a stable course on August 31 and September 8, showed a rapid decrease until September 15, and ended entirely in the following week (Figure 3). It was observed that the adult density followed a similar course to the first year in the second year but was higher in terms of the number of individuals (Figure 3). The highest values were obtained from the Asol variety for both years, followed by the Göktürk ve Balc1 variety (Figure 3 and Table 1).

Table 1. The adult and pupa density of *Acanthiophilus helianthi* on five different safflower cultivars in 2019 and 2020 (Mean \pm SE)

(Ortalama \pm SH)	<i>Çizelge 1.</i> Acanthiophilus helianthi	nin 2019 ve 2020 yıllarında	ı beş farklı aspir çeşidine	de ergin ve pupa yoğunluğu
	$(Ortalama \pm SH)$			

$(01tatatita \pm 511)$	NI	AGOT	A \$7 A 77	DALCI	DINCED	CÖVTÜDV D	
2019	N	ASOL	AYAZ	BALCI	DİNÇER	GÖKTÜRK P	
Adult 30 June	5	0.60 ± 0.36	0.60 ± 0.36	1.00 ± 0.28	1.40 ± 0.22	0.80 ± 0.33	> 0.05
7 July	5	0.80 ± 0.52	1.40 ± 0.46	0.60 ± 0.35	0.20 ± 0.18	0.60 ± 0.22	> 0.05
14 July	5	$1.20\pm0.33~b$	0.60 ±0.36 b	2.60 ± 0.22 a		$0.20\pm0.18~b$	< 0.05
21 July	5	1.00 ± 0.28 bc	1.40 ± 0.35 bc	1.80 ± 0.33 ab	2.20 ± 0.33 a	$0.80\pm0.17~c$	< 0.05
28 July	5	2.40 ± 0.22 a	0.40 ±0.22 c	$1.40\pm0.22\ b$	$0.80\pm0.33\ bc$	$0.20\pm0.18\ c$	< 0.05
4 August	5	0.20 ± 0.18			0.40 ± 0.36	0.60 ± 0.22	> 0.05
11 August	5	1.60 ± 0.36 a				$0.20\pm0.18\ b$	< 0.05
Total	7	1.11 ± 0.25 a	0.63 ± 0.20 ab	$1.06\pm0.33~ab$	$0.71\pm0.29~ab$	0.48 ± 0.09	< 0.05
Pupae 17 August	5	2.40 ± 1.04	1.20 ± 0.71			0.40 ± 0.36	> 0.05
24 August	5	$4.40 \pm 1.28 \text{ ab}$	$1.40\pm0.22\ b$	$2.20\pm0.52~ab$	$2.00\pm0.69~ab$	2.80 ± 0.99 ab	< 0.05
31 August	5	3.00 ± 0.80	3.40 ± 0.88	2.60 ± 0.60	2.20 ± 0.66	4.20 ± 1.42	> 0.05
8 September	5	3.00 ± 0.80	1.40 ± 0.22	1.40 ± 0.22	1.60 ± 0.36	1.20 ± 0.18	> 0.05
Total	4	3.20 ± 0.37 a	2.55 ± 0.46 ab	$1.85\pm0.54\ b$	$1.60\pm0.46~b$	$3.40\pm0.88~ab$	< 0.05
2020							
Adult 29 June	5	2.20 ± 0.33 a	$0.60\pm0.21~b$	$0.60\pm0.35~b$	$0.20\pm0.17~b$	$0.60\pm0.22~b$	< 0.05
6 July	5	$0.60\pm0.36\ b$	$0.60\pm0.22\ b$	$1.40 \pm 0.61 \text{ ab}$	$0.40\pm0.22\ b$	1.40 ± 0.22 a	< 0.05
13 July	5	2.20 ± 0.33 a	$0.40\pm0.18\ b$	$0.40\pm0.22\ b$	$0.40\pm0.22\ b$	$0.80\pm0.18\ b$	< 0.05
20 July	5	$0.40\pm0.35\ b$	1.00 ± 0.28 bc	$1.20\pm0.18\ b$	$1.40\pm0.22\ b$	2.20 ± 0.18 a	< 0.05
27 July	5	$0.60\pm0.22~b$	$0.80\pm0.33\ b$	$0.80\pm0.17~b$	2.60 ± 0.53 a	$0.80\pm0.17~b$	< 0.05
3 August	5	0.80 ± 0.18	1.20 ± 0.33	1.20 ± 0.17	0.60 ± 0.36	0.80 ± 0.34	> 0.05
10 August	5	0.60 ± 0.22 bc	1.00 ± 0.00 ab	1.40 ± 0.22 a	$0.60\pm0.22~bc$	$0.20\pm0.18\ c$	< 0.05
Total	7	1.08 ± 0.27	0.88 ± 0.08	0.79 ± 0.13	0.88 ± 0.29	1.05 ± 0.20	> 0.05
Pupae 17 August	5	1.40 ± 0.53	1.40 ± 0.53			0.40 ± 0.35	> 0.05
24 August	5	$4.20\pm0.33~ab$	$3.20\pm0.33~ab$	$2.60\pm0.45~b$	$3.40\pm0.45~a$	4.60 ± 0.66 a	< 0.05
31 August	5	2.80 ± 0.86	3.20 ± 0.82	2.20 ± 0.52	2.80 ± 0.90	4.80 ± 1.24	> 0.05
8 September	5	1.40 ± 0.21	1.60 ± 0.35	1.40 ± 0.21	1.60 ± 0.35	1.40 ± 0.21	> 0.05
Total	4	2.45 ± 0.58	2.35 ± 0.43	1.55 ± 0.50	1.95 ± 0.65	2.80 ± 0.97	> 0.05

In population sampling of *A. helianthi* on different safflower cultivars, *Ormyrus* sp., *Microdontomerus annulatus*, *Eurytoma acroptilae* and *Bracon* sp. including parasitoids species were found (Figure 7). It has been reported that these species feed on the larvae

and pupae of *A. helianthi* (Saeidi et al., 2011; Lotfalizadeh & Gharali 2014). Images of 4 parasitoids *Ormyrus* sp., *M. annulatus*, *E. acroptilae* and *Bracon* sp. on different safflower varieties of *A. helianthi* (Figure 5).

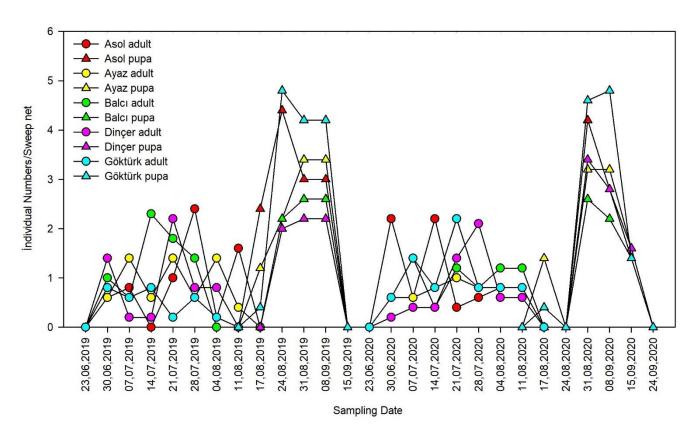
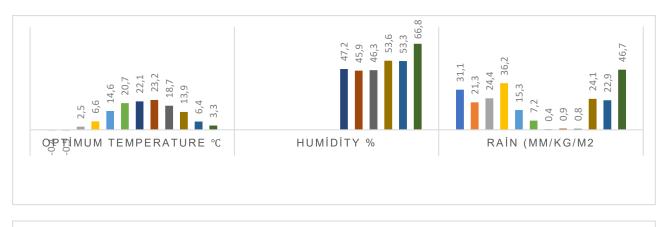


Figure 3. Adult and pupa densities of *Acanthiophilus helianthi* in 2019 and 2020 (number of adult individuals/ Sweep net, pupa number/plate).

Şekil 3. Acanthiophilus helianthi'nin 2019 ve 2020'deki ergin ve pupa yoğunlukları (ergin birey sayısı/atrap, pupa sayısı/plaka).



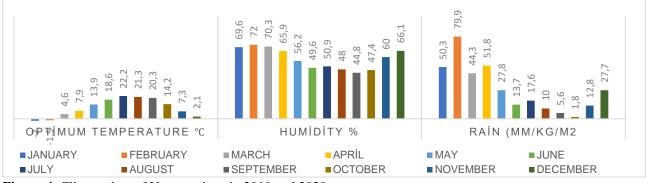


Figure 4. Climate data of Van province in 2019 and 2020. *Sekil 4*. *Van ili 2019 ve 2020 iklim verileri*.



(c) (d) **Figure 5**. (a) Ormyrus sp. (b) Microdontomerus annulatus (c) Eurytoma acroptilae (d) Bracon sp. **Şekil 5**. (a) Ormyrus sp. (b) Microdontomerus annulatus (c) Eurytoma acroptilae (d) Bracon sp.

It has not any research found on population growth, variety preference, and determination of parasitoids on Asol, Ayaz, Balcı, Dinçer, and Göktürk cultivars of A. helianthi. However, it has been observed that there are studies examining the population fluctuations of A. helianthi on different safflower cultivars (Al-Ali et al., 1977; Dusty et al., 2013). It was determined that the first adults of A. helianthi became active on 29-30 June and it has been different densities on five varieties until 10-11 August in safflower plants that started to be planted in April. It was determined that pupae started to appear on August 17, when the adult densities reached zero individuals, and continued until September 8. The observations of adults at the time when the flower heads begin to form and the density of pupae in the period when the harvest time support this information. Ormyrus sp., M. annulatus, E. acroptilae and Bracon sp. it was observed that the densities of the parasitoids increased at the time of pupae, and the number of individuals and the time of emergence differed over five species. Lotfalizadeh & Gharali (2014), determined Hymenopterous parasitoids of safflower seed pests in Iran. These arae Pronotalia carlinarum (Szelényi & Erdos 1951) (Hymenoptera: Eulophidae), Aprostocetus

(Hymenoptera: Eulophidae), E. acroptilae, sp. Sycophila submutica (Thomson, 1876) (Hymenoptera: Eurytomidae), Ormyrus gratiusus (Förster, 1832), Ormyrus orientalis (Walker, 1835) (Hymenoptera: Ormyridae), Pteromalus albipennis (Walker, 1835), Colotrechnus viridis (Masi, 1921) (Hymenoptera: Pteromalidae), M. annulatus, Adontomerus crassipes (Boucek, 1982) (Hymenoptera: Torymidae), Bracon luteator (Spinola, 1808), B. brevicornis (Wesmael, 1838) ve B. hebetor (Say, 1836) (Hymenoptera: Braconidae) many parasitoids have been identified. When compared, it was determined that all 4 species identified in our study were the same. In another study conducted by Saeidi et al. (2011), B. hebetor, B. luteator, C. viridis, M. annulatus, O. orientalis, E. acroptilae, P. carlinarum, Pteromalus (Hymenoptera: sp. Pteromalidae) species were found to be related to A. helianthi. When compared with our study, it was observed that the detrmined species were common. In a survey study for the determination of pupa parasitoids was conducted in Iran and were determined *Antistrophoplex* conthurnatus. Microdontomenus annulatus, Bracon hebetor, B. luteator, Pronotalia carlinarum, Ormyrus orientalis, Colotrechnus viridis,

Pteromalus sp., Eurytoma acroptilae and *Isocolus tinctorious* as pupal parasitoids of *A. helianthi*. It has been determined that *M. annulatus* play an active role in pupa density of *A. helianthi* and creates differences in parasitism rate among species. The presence of the same parasitoids and their densities at different rates support our study (Saeidi et al., 2016).

Considering the adult densities, the highest values were determined in the Asol variety in both years, and the lowest values in Göktürk for the first year and Balcı varieties for the second year. The highest value in pupa densities was found in the Göktürk variety in both years and the lowest values were obtained from the Dincer variety in the first year and the Balcı variety in the second year. When the results obtained with a study similar to this study were compared, it was determined that adults and pupae were encountered in the weekly samplings between 19 June and 15 July and that they formed different densities on seven cultivars (Goldasht, Padideh, Zarghan, Varamin, PI, Acataria, Mec163) showed similarity with our study (Dustiy et al., 2013). In another study carried out on the Gina variety of safflower, it was determined that adults emerged between 12 May and 31 July, and 79 adults were reached in 80 days. It has been concluded that the safflower fly is the larvae that cause the damage that it spends its life on the flower bed until it reaches adult (Al-Ali et al., 1977). When evaluated together with our study, it was observed that the adults emerged much earlier, and similarly, the damage symptoms caused by the larvae in the seeds were observed in all cultivars.

4. Results

As a result of two years of observation and data, it has been observed that A. helianthi has grown in the fields of five safflower cultivars planted. During sampling with sweep net, 4 parasitoids, Ormyrus sp., M. annulatus, E. acroptilae and Bracon sp., were detected and it was understood in the literature that they were the pupal parasitoids of A. helianthi. At the same time, it is thought that the color diversity of the flower heads in orange, red and yellow tones, the differences in the vegetative development of the plants depending on the climate and the density of the parasitoids may have affected the population density of A. helianthi. It is foreseen that these results will provide some information to the producers who will produce safflower in the future, and in addition to this information, more detailed studies will be an important step in minimizing product losses.

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