ONLINE ISSN 2651-3579

Journal of the Entomological Research Society

Volume: 24 Part: 1 2022

JOURNAL OF THE ENTOMOLOGICAL RESEARCH SOCIETY

Published by the Gazi Entomological Research Society

Subscription information

Published by GERS in single volumes three times (March, July, November) per year. The Journal is distributed to members only. Non-members are able to obtain the journal upon giving a donation to GERS.

Papers in *J. Entomol. Res. Soc.* are indexed and abstracted in Science Citation Index Expanded (SCIE), Zoological Record, Biological Abstracts, BIOSIS Previews, Scopus, Essential Science Indicators, ProQuest, Scimago Journal & Country Rank (SJR), IPIndexing Portal, Resurchify, ORES, Academic Accelerator, CiteFactor, scinapse, Acarindex, Publons, TR Dizin.

Publication date: March 21, 2022

J. Entomol. Res. Soc., 24(1): 01-06, 2022
Doi: 10.51963/jers.v24i1.1994

Research Article
Online ISSN:2651-3579

Description of a New Species of *Kiotina* Klapálek, 1907(Plecoptera: Perlidae) from Southern China

Zhiteng CHEN

School of Grain Science and Technology, Jiangsu University of Science and Technology,
Zhenjiang 212004, CHINA
e-mail: 741208116@qq.com, ORCID ID: 0000-0002-6331-8978

ABSTRACT

A new species of the perlid genus *Kiotina* Klapálek, 1907, *Kiotina dayaoshana* sp. nov., is described and illustrated based on males collected from Dayao Mountain, Guangxi Province, southern China. This represents the first formal report of this genus in Guangxi Province. The new species is characterized by standard pi-shaped epiproct sclerite, posteriorly projected hammer and presence of three specially shaped aedeagal lobes.

Key words: Plecoptera, Perlidae, *Kiotina dayaoshana*, description, new species.

Chen, Z. (2022). Description of a new species of *Kiotina* Klapálek, 1907(Plecoptera: Perlidae) from southern China. *Journal of the Entomological Research Society*, 24(1), 01-06.

Received: January 13, 2021 *Accepted: December 02, 2021*

INTRODUCTION

As the type genus of the tribe Kiotinini Uchida, 1990, *Kiotina* Klapálek, 1907 is a relatively small genus distributed in the southeastern Palearctic and northern Oriental regions. To date, 15 *Kiotina* species are known worldwide, including 11 species from China, three from Japan and one from North Korea (DeWalt, Maehr, Hopkins, Neu-Becker, & Stueber, 2021). Recent contributions to the taxonomy of *Kiotina* were made by Stark & Sivec (2008), Mo, Wang, Li, & Murányi (2019) and Su & Chen (2020). The taxonomic changes, current status and type information of Chinese *Kiotina* species have been reviewed by Su & Chen (2020). Current species delimitation in *Kiotina* is conducted mainly based on the shape of epiproct sclerite, spines on male tergum 10 and the shape of female subgenital plate (Stark & Sivec, 2008; Su & Chen, 2020).

In this study, a new species of *Kiotina* is identified and described based on material from Guangxi Province in southern China (Fig. 1). The male of the new species is described and compared with congeners. The distribution of Chinese *Kiotina* species are discussed.

MATERIAL AND METHODS

The specimens used in this study were collected by sweeping net and preserved in 75% ethanol. Male abdomens were removed and treated with 10% NaOH solution. Details of the morphology were studied with a SDPTOP SZM45 stereo microscope. Photos were taken with a Canon EOS 6D digital camera equipped with a Canon MP-E 65 mm 5X macro lens. All images were adjusted and assembled into plates with Adobe Photoshop CS6. Generic assignment of the new species follows Chen & Du (2018). The specimens are deposited in the Insect Collection of Jiangsu University of Science and Technology, Jiangsu Province, China (ICJUST).

RESULTS

***Kiotina dayaoshana* Chen, 2022 sp. nov.**

Distribution: The species is known only from Dayao Mountain of Guangxi Province, southern China (Fig. 1).

Material examined: Holotype: 1♂, China: Guangxi Province, Laibin City, Jinxiu County, Dayao Mountain (Fig. 2), 23.9701 N, 110.1175 E, 1100 m, 20.06.2020, Chun-Fu Feng. Paratype: 1♂, same locality and data as holotype.

Etymology: The species is named after its type locality in Dayao Mountain.

Description: Male body length (excluding antennae and cerci) 13.0-13.5 mm (n = 2), generally brown, with dark brown patterns. Head (Fig. 3) subquadrate, wider than long. Triocellate, anterior ocellus smaller than posterior ones; area between each posterior ocellus and compound eye with a pale oval spot. Ocellar area with a butterfly-shaped dark stigma extending backwards. M-line pale, anteriorly with a triangular dark stigma. Lateral areas of head pale and hairy. Antenna slender and dark brown, slightly longer than the abdomen.

Fig. 1. Distribution of *Kiotina* species in China. Type locality of *Kiotina dayaoshana* sp. nov. indicated by red, previous generic distribution indicated by blue.

Fig. 2. *Kiotina dayaoshana* sp. nov., habitat environment in Dayao mountain of Guangxi province, southern China. Photo by Mr. Qing-Yang Xu.

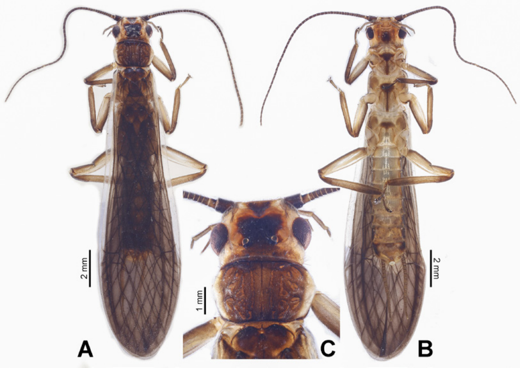
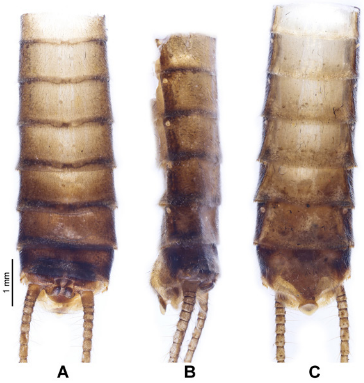


Fig. 3. *Kiotina dayaoshana* sp. nov., adult male; A. habitus, dorsal view; B. habitus, ventral view; C. head and pronotum, dorsal view.

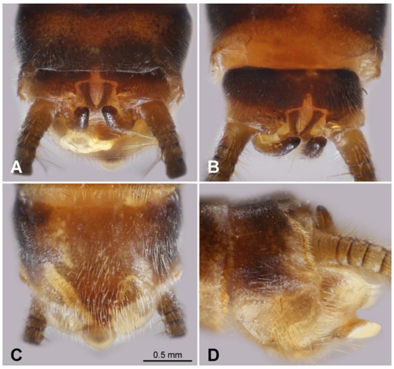
Pronotum (Fig. 3) oval, nearly as wide as head. Pronotal disc mostly dark brown with pale lateral margins, surface scattered with irregular rugosities. Meso- and metanota as wide as pronotum, mostly dark brown. Macropterous, wing membrane mostly dark brown except for the pale costal area, veins dark. Legs mostly dark brown, ventral aspects of femora pale.

Abdomen (Figs. 4-6) pale brown to dark brown, terminal segments darker than anterior ones. Tergum 10 with a bell-shaped, membranous median area which with a finger-shaped anterior projection. Epiproct sclerite resembling a reversed Pi-shape; arms straight and slightly thickened apically; base transversely straight, widened laterally with pointed apices. A pair of weakly sclerotized, small triangular spines present on each side of epiproct sclerite. A pair of blunt, larger projections on posterior margin of tergum 10 posterior to the two spines. Paraprocts long conical and upcurved, with blunt, sclerotized apices. Sternum 9 strongly sclerotized laterally, posteromedial margin extended backwards. Hammer large and glabrous, near transversely oval but somewhat projected at posterior margin. Aedeagus entirely membranous, wide from dorsal and ventral aspects whereas flat from lateral view. Dorsal aspect of aedeagus with an ear-shaped basal lobe, and a tongue-shaped apical lobe which curved ventrad and covered by dense triangular spines. Ventral aspect of aedeagus with a large heart-shaped apical lobe. Lateral aspect of aedeagus constricted at base, with a patch of pale scales at median ⅓ of the aedeagus.

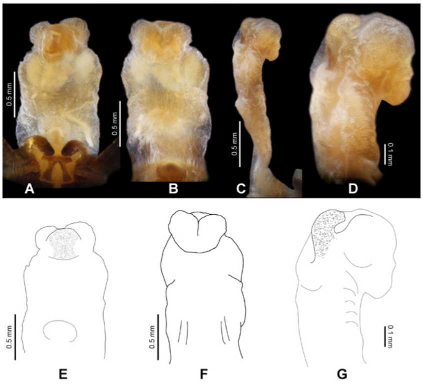
Diagnosis: Among the 12 species of *Kiotina* from mainland Asia, *K. albopila* (Wu, 1948) and *K. chiangi* (Banks, 1939) exhibits very tiny hammers that can be easily distinguished from the large hammer of the new species. The standard Pi-shaped epiproct sclerite of the new species furtherer separates it from *K. collaris* (Banks, 1937), *K. chekiangensis* (Wu, 1938), *K. decorata* (Zwick, 1973), *K. delicata* Stark & Sivec, 2008, *K. nigra* (Wu, 1938) and *K. quadrituberculata* Wu, 1948. The new species can be distinguished from *K. bifurcata* Stark & Sivec, 2008 by the absence of bifurcated lateral spines on male tergum 10, and from *K. resplendens* Banks, 1939 by the presence of finger-shaped paraprocts and posteriorly projected hammer. The new species is most similar to *K. bilobata* Mo, Wang, Li & Murányi, 2019 and *K. yexiaohani* Su & Chen, 2020. However, the new species exhibits dark body color, narrow finger-shaped notch on medial of male tergum 10, straight base of epiproct sclerite, dorsobasal aedeagal lobe, tongue-shaped dorsoapical aedeagal lobe and heart-shaped ventroapical aedeagal lobe. In *K. bilobata*, the body color is generally pale brown (which might be caused by the preservation in ethanol); the notch on medial area of male tergum 10 is wider and subtriangular; base of epiproct sclerite is curved; aedeagus has no dorsobasal lobe, tongue-shaped dorsoapical lobe or heart-shaped ventroapical lobe (Mo et al, 2019). When compared with *K. yexiaohani*, the new species can be easily separated by the different head pattern, much slender epiproct arms which with a much deeper median notch, much smaller spines on each side of epiproct sclerite, posteriorly projected hammer, and by different shaped aedeagus (Su & Chen, 2020).



Figs. 4. *Kiotina dayaoshana* sp. nov., adult male; A. abdomen, dorsal view; B. abdomen, lateral view; C. abdomen, ventral view.



Figs. 5. *Kiotina dayaoshana* sp. nov., adult male; A. terminalia, dorsal view; B. terminalia, dorsolateral view; C. terminalia, ventral view; D. terminalia, lateral view.



Figs. 6. *Kiotina dayaoshana* sp. nov., adult male; A. aedeagus, dorsal view; B. aedeagus, ventral view; C. aedeagus, lateral view; D. apex of aedeagus, lateral view; E. drawing of aedeagus, dorsal view; F. drawing of aedeagus, ventral view; G. drawing of apex of aedeaus, lateral view.

CONCLUSIONS AND DISCUSSION

The new species, *Kiotina dayaoshana* sp. nov. represents the first formal report of *Kiotina* in Guangxi Province, extending westwards the generic distribution of *Kiotina* in China (Fig. 1). All currently recognized *Kiotina* species in China are restricted in the southeastern provinces, including Zhejiang, Fujian, Guangdong, Taiwan, Jiangxi and Guangxi provinces (Su & Chen, 2020). Further investigations of these areas are expected to reveal more undescribed species of *Kiotina*.

ACKNOWLEDGEMENTS

The author is grateful to the editor and the reviewers for valuable reviews and manuscript improvements. This research is supported by the Natural Science Foundation of Jiangsu Province (No. BK20201009) and the Start-up Funding of Jiangsu University of Science and Technology (No. 1182931901).

REFERENCES

Banks, N. (1937). Neuropteroid insects from Formosa. *Philippine Journal of Science*, 62(3), 255-291, pls. 1-3.

Banks, N. (1939). New genera and species of neuropteroid Insects. *Bulletin of the Museum of Comparative Zoology at Harvard College*, 85(7), 439-504, pls. 1-9.

Chen, Z.T. & Du, Y.Z. (2018). A checklist and adult key to the Chinese stonefly (Plecoptera) genera. *Zootaxa*, 4375(1), 59-74.

DeWalt, R.E., Maehr, M.D., Hopkins, H., Neu-Becker, U., & Stueber, G. (2021, 03, January). *Plecoptera Species File Online*. Retrieved from http://Plecoptera.SpeciesFile.org.

Klapálek, F. (1907). Japonské druhy podčeledi Perlinae. *Rozpravy České Akademie*, 16(31), 1-28.

Mo, R.R, Wang, G.Q., Li, W.H., & Murányi, D. (2019). A remarkable new species of *Kiotina* Klapálek, 1907 (Plecoptera: Perlidae) from China. *Zootaxa*, 4623(3), 583-588.

Stark, B.P. & Sivec, I. (2008). Systematic notes on *Kiotina* Klapálek and *Hemacroneuria* Enderlein (Plecoptera: Perlidae), with description of four new species. *Illiesia*, 4(17), 161-175.

Su, T. & Chen, Z.T. (2020). A synopsis of *Kiotina* Klapálek, 1907 (Plecoptera: Perlidae) from China, with description of a new species from Zhejiang Province. *Zootaxa*, 4786(4), 583-591.

Uchida, S. (1990). *A revision of the Japanese Perlidae (Insecta: Plecoptera), with special reference to their phylogeny*. Tokyo Metropolitan University, Tokyo, Japan.

Wu, C.F. (1938). *Plecopterorum sinensium: A monograph of stoneflies of China (Order Plecoptera)*. Yenching University, Beijing, China.

Wu, C.F. (1948). Fifth supplement to the stoneflies of China (Order Plecoptera). *Peking Natural History Bulletin*, 17(2), 145-151.

Zwick, P. (1973). Plecoptera from Korea. *Annales Historico-Naturales Musei Nationalis Hungarici*, 65, 157-169.

J. Entomol. Res. Soc., 24(1): 07-21, 2022
Doi:0.51963/jers.v24i1.2011

Research Article
Online ISSN:2651-3579

Using Maximum Entropy Algorithm to Analyze Current and Future Distribution of the Asian hornet, *Vespa velutina*, in Europe and North Africa Under Climate Change Conditions

Hossam F. ABOU-SHAARA[1]* Areej A. AL-KHALAF[2]

[1]Department of Plant Protection, Faculty of Agriculture, Damanhour University, Damanhour, 22516, EGYPT
[2]Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, B.O. Box 84428 Riyadh 11671, SAUDİ ARABIA
e-mails: [1]hossam.farag@agr.dmu.edu.eg, [2]aaalkhalaf@pnu.edu.sa
ORCID ID: [1]0000-0001-7208-6526, [2]0000-0001-9278-0822

ABSTRACT

The Asian hornet, *Vespa velutina*, has invaded Europe during the last few years. This hornet is a dangerous pest to honey bee colonies and can cause significant economic damages. In this study, current and future distributions of this pest in Europe and North Africa were analyzed using maximum entropy algorithm. Different environmental factors were used in the Maxent model to predict the suitability of the study area for this pest. Two future models with two Shared Socio-economic Pathways (126 and 585) were used to estimate the future distribution of *V. velutina* in 2050. The Maxent model for *V. velutina* showed high performance based on the analysis of omission/commission rates and the area under curve. Jackknife test showed the high importance of temperature variables in *V. velutina* distribution. The model maps indicated the potential invasion of this pest to other areas in Europe and North Africa including deserts in Libya and Egypt. Negative consequences of such invasion on beekeeping and environmental balance are expected.

Key words: Honey bees, GIS, Maxent, pest, velutina.

INTRODUCTION

Abou-Shaara, H.F. & Al-Khalaf, A. A. (2022). Using maximum entropy algorithm to analyze current and future distribution of the Asian hornet, *Vespa velutina*, in Europe and North Africa under climate change conditions. *Journal of the Entomological Research Society,* 24(1), 07-21

Received: February 05, 2021 *Accepted: February 18, 2022*

Climate is changed rapidly and future climate conditions may negatively impact on honey bees, *Apis mellifera* L., and can be considered as a major future threat (Yörük & Sahinler, 2013). Indeed, the increase in temperature is the common phenomena of climate change. Such change in temperature can adversely affect activities of honey bees and beekeeping in side (Le Conte & Navajas, 2008; Abou-Shaara, 2016; Abou-Shaara, Owayss, Ibrahim, & Basuny, 2017) and the distribution of bee diseases and pests in the other side (Le Conte & Navajas, 2008) in addition to plant pollination and activity of pollinators (Hegland, Nielsen, Lazaro, Bjerknes, & Totland, 2009; Scaven & Rafferty, 2013; Rader et al, 2013). Bearing in mind that honey bees are crucial pollinators for many crops worldwide (Allen-Wardell et al, 1998; Abou-Shaara, 2014). In fact, honey bees have numerous numbers of pests which are distributed geographically (Le Conte & Navajas, 2008; Abou-Shaara & Staron, 2019). Some of them has recently succeeded to invade new regions other than their original locations including the Asian hornets, *Vespa velutina* (Lepeletier, 1836) (Hymenoptera: Vespidae) which were recorded in some Western European countries (López, González, & Goldarazena, 2011; Grosso-Silva & Maia, 2012; Monceau et al, 2014) and become established.

The predatory hornets from genus *Vespa* (Fam. Vespidae) include about 22 species (Archer, 2012). The common hornet in North Africa and the Mediterranean region is the oriental hornets, *Vespa orientalis* (Linnaeus, 1771) (Archer, 1998). This hornet was recorded accidentally in Madagascar (Carpenter and Kojima, 1997) and Mexico (Dvořák, 2006) without wide establishment. It causes damages to bee colonies which require developing methods for its control (Shaaban et al, 2015). Its activity is correlated with temperature and relative humidity (Taha, 2014) and generally extended from spring until autumn (Khodairy & Awad, 2013; Islam, Iftikhar, & Mahmood, 2015). Recently, the Asian hornet, *V. velutina* which occurs from Afghanistan to eastern China, and Indonesia (Carpenter & Kojima, 1997) has invaded France (Haxaire, Bouguet, & Tamisier, 2006), Spain (López et al, 2011), Portugal (Grosso-Silva & Maia, 2012), Italy (Demichelis et al, 2014), and Belgium (Monceau et al, 2013). This hornet is a predator to honey bees (Abrol, 1994; Abrol, 2006; Tan et al, 2007), and could cause damages to bee colonies in a similar way to the Oriental hornets. Also, the life cycle of this hornet is similar to the Oriental hornets including the presence of overwintering periods for queens which end up in early spring (Monceau et al, 2012). The presence of these two species at the same geographical range can be considered as a high risk to bee colonies and beekeeping as well as to the biodiversity in the invaded regions.

The social nature of these hornets as well as the dispersal range of queens in search for place for hibernation which is expected to be wide (Monceau et al, 2013) support their ability to invade additional countries in Europe and North Africa. Moreover, the short distances between Europe and North Africa in side and climate change in the other side greatly support the expansion of this hornet. This point has not been deeply studied. In fact, beekeeping is considered among the essential agricultural activities in North Africa especially Egypt due to the vital role of bees in plant pollination (Abou-Shaara, 2015; Al Naggar, Codling, Giesy, & Safer, 2018). Also, some Arabian countries import bee packages from Egypt especially Gulf countries (Al-Ghamdi &

Nuru, 2013; Al-Ghamdi, Alsharhi, & Abou-Shaara, 2016). The Oriental hornets are the main Vespidae predator to honey bees in North Africa which requires efforts from beekeepers to control them (Archer, 2012; Khodairy & Awad, 2013; Taha, 2014; Abou-Shaara, 2017). The potential invasion of the Asian hornets to North Africa can hinder beekeeping development and cause severe economic losses.

Predicting the geographical distribution of species is commonly achieved using climatic suitability models (Guisan & Thuiller, 2005). Different tools can be employed to predict species distribution in the future, and MaxEnt is one of these tools (Wei, Wang, Hou, Wang, & Wu, 2018; Jamal et al, 2021). The analysis depends on occurrence data of the species and assumed environmental variables (Hosni, Nasser, Al-Ashaal, Rady, & Kenawy et al, 2020; Jamal et al, 2021). Most models depend basically on analyzing environmental variables especially those aims to assess effects of climate change. Other variables like land cover and vegetation index could improve the models but they are not available for future conditions. This prevents their use in studying the influences of climate change on species distribution (Hosni et al, 2020). Therefore, in this study environmental variables were utilized to understand current and future distribution of the Asian hornets in Europe and North Africa, with discussing the consequences of the potential invasion of these hornets on beekeeping and environment.

MATERIALS AND METHODS

Occurrence locations

Some Asian countries are the original geographical locations of the Asian hornet, *Vespa velutina* (abbreviated as AH). However, this hornet species has invaded some countries in West Europe and showed high adaptability to its new environment. To understand the current and future distribution of AH in Europe and North Africa, only occurrence records from Europe (the new environment) were used in this study. Also, the geographical area of the study was limited to cover some European countries beside North Africa. The available records of the AH from the Global Biological Information Facility (GBIF.org, 2020) were mainly used. So, 295 confirmed locations were included in this study after excluding locations without coordinates or with uncertain distribution or with repeated coordinates.

Climate data

Five climate variables based on temperature and relative humidity with a spatial resolution of about 5 km^2 available from worldclim.org (Fick & Hijmans, 2017) were used in the analysis. These variables were: annual mean temperature (bio1), mean diurnal range (bio2), temperature seasonality (bio4), annual precipitation (bio12), and precipitation seasonality (bio15). These specific datasets were selected based on some initial analyses to select the most important variables (Hosni et al, 2020; Abou-Shaara et al, 2021).

Bioclimatic data available from WorldClim v2.1 released in January 2020 for the period from 1970 to 2000 were used to analyze the current global climate. The downscaled future climate data from the Coupled Model Intercomparison Project Phase 6 (CMIP6) (Eyring et al, 2016) from two climate models: the Meteorological Research Institute (MRI-ESM2-0; Institution ID: MRI) and the Beijing Climate Center Climate System Model (BCC-CSM2-MR; Institution ID: BCC) from two Shared Socio-economic Pathways (SSP126 and SSP585) were used to analyze future climate for 2050 (average from 2041-2060). The Shared Socio-economic Pathways (SSPs) are used by the Intergovernmental Panel on Climate Change (IPCC) in the IPCC AR6 instead of Representative Concentration Pathways (RCPs) in the IPCC AR5.

Modeling of current and future geographic distribution

The maximum entropy modeling utilizing Maxent v 3.4.1 (Phillips et al, 2020) was used to estimate current and future distribution of AH in Europe and North Africa. The model used 222 presence records for training and 73 for testing (25% random test points). Algorithm terminated after 500 iterations (17 seconds), 10218 points used to determine the Maxent distribution, and the regularization values: linear/quadratic/product: 0.050, categorical: 0.250, threshold: 1.000, hinge: 0.500.

For future conditions, the average from the two climate models MRI-ESM2-0 and BCC-CSM2-MR was presented using ssp126 (the low limit), ssp585 (the high limit), and the overall average for the two SSPs. This can present precise prediction about future distribution of AH. The output format was cumulative. The model images for current and future conditions were reclassified using ArcGIS 10.5 into four suitability degrees: rare (0-0.01), moderate (0.01-1), high (1-20), and very high (20-100) in line with Maxent guideline (Phillips, 2017) and previous study (Jamal et al, 2021).

Model performance

The contribution percentages of the analyzed variables in the model were calculated beside the response curve of each bioclimatic variable. The model was evaluated based on analysis of omission/commission rates. The omission rate and predicted area as a function of the cumulative threshold was analyzed. The receiver operating characteristic (ROC) were utilized to identify the area under curve (AUC) as an indication of the model performance. Also, the jackknife test of variables was presented. These parameters were selected based on previous studies (Hosni et al, 2020; Abou-Shaara & Darwish, 2021; Jamal et al, 2021).

RESULTS

Contribution percentages

Estimates of relative contributions of the five environmental variables to the Maxent model were 41.5%, 27.9%, 20.4%, 9%, and 1.2% to bio 4, bio 12, bio 1, bio 15, and bio 2, respectively. The highest contribution was to temperature seasonality, annual precipitation, and annual mean temperature. The response curves of variables with the highest contribution (Fig. 1) suggest specific favorable ranges of temperature and precipitation of AH in its new environment in West Europe.

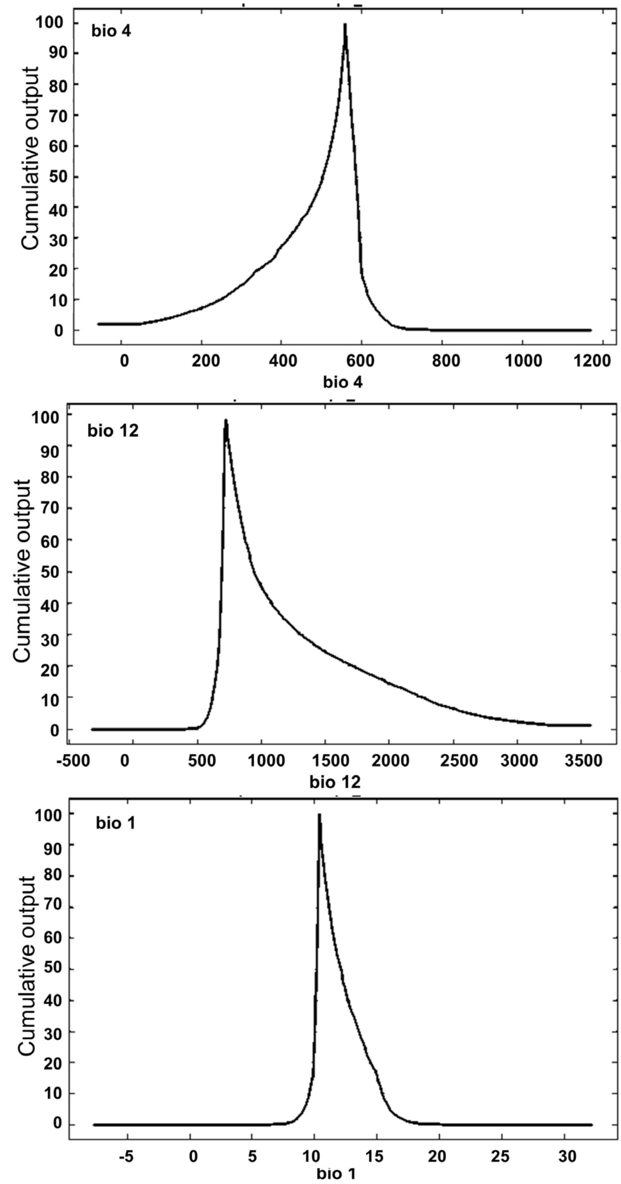


Fig. 1. Response curves of the most contributed variables in the model: temperature seasonality (bio4), annual precipitation (bio12), and annual mean temperature (bio1).

Current distribution

The current distribution (Fig. 2) reflects the real distribution of the AH in France, Spain, Belgium and Portugal as the new environment of this hornet in Europe. The South-Western parts of Europe were classified as very high suitable. Some other parts in Europe were classified as very high suitable including parts in England, Italy, and Turkey. Areas in some other European countries and in North Africa were classified as highly or moderately suitable. In general, the coastal regions in North Africa are moderately suitable for the AH. Also, some desert areas in Libya and Egypt as well as some parts close to North-East of Egypt were classified as moderately suitable. Regions with cold weather at North Europe and desert regions in North Africa were classified as rarely suitable for AH.

Future distribution

The model using the low limit of SSP126 (Fig. 3), the high limit of SSP 585 (Fig. 4), and the overall average (Fig.5) showed high similarities with the current distribution model with few exceptions as highlighted in Fig. 6. The three maps approximately showed the same future distribution except that the map based on the high limit of SSP differed slightly than the other two maps mainly in the suitability of some locations in Libya and Egypt to the AH. Approximately no changes will occur at West Europe than current distribution except that England will be more suitable for the AH.

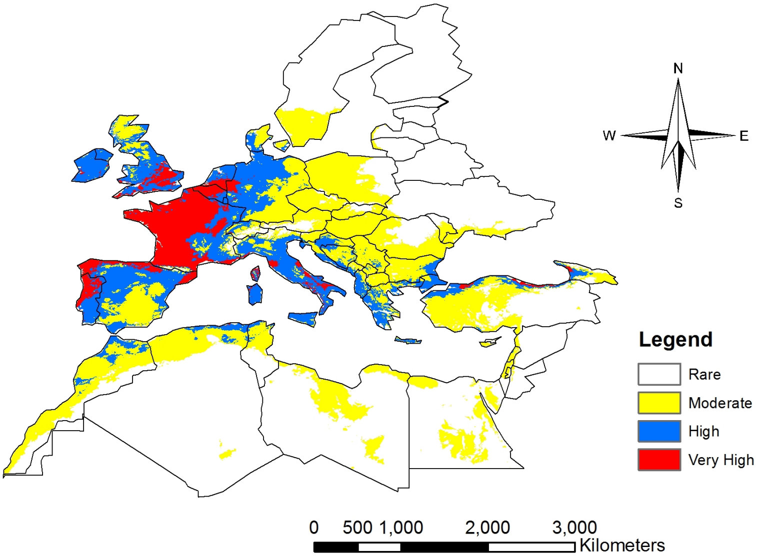


Fig. 2. Distribution of the Asian hornet under current climate conditions.

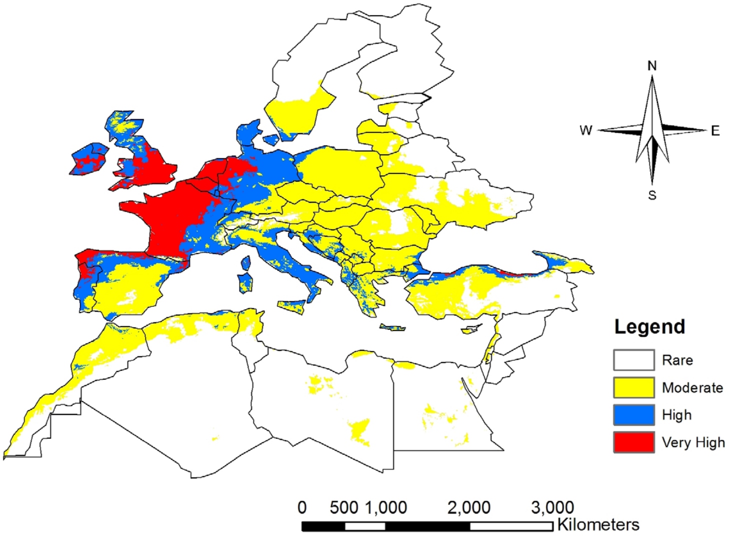


Fig. 3. Distribution of the Asian hornet under future climate conditions (SSP 126). This figure is the average of two climate models (MRI-ESM2-0 and BCC-CSM2-MR).

The locations with high suitability are not anticipated to be greatly changed than current (Fig. 6). The locations with moderate suitability in Europe will be approximately the same in the future. The coastal regions in North Africa and some locations in Libya and Egypt will be moderately suitable for the AH according to the maps based on the low limit of SSP and the overall average.

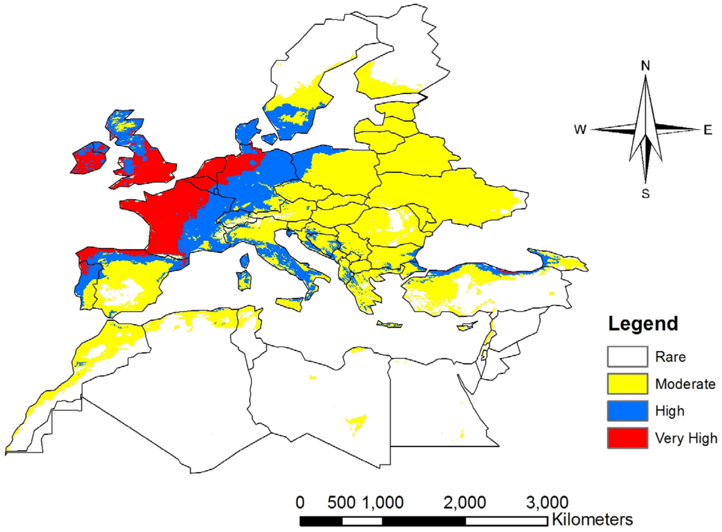


Fig. 4. Distribution of the Asian hornet under future climate conditions (SSP 585). This figure is the average of two climate models (MRI-ESM2-0 and BCC-CSM2-MR).

Model performance

The omission rate is calculated both on the training presence records and on the test records (Fig. 7). This rate is very close to the predicted omission. The receiver operating characteristic (ROC) curve for the training and the test data is shown in Fig. 8. The area under the curve (AUC) is 0.965 and 0.961± 0.006 for training and test data, respectively. Jackknife test using area under the curvy (AUC) on test data (Fig. 9) shows the high AUC of the variables over 0.7. bio 1 and 4 had the highest AUC, followed by bio 15, then bio 12, and finally bio 2.

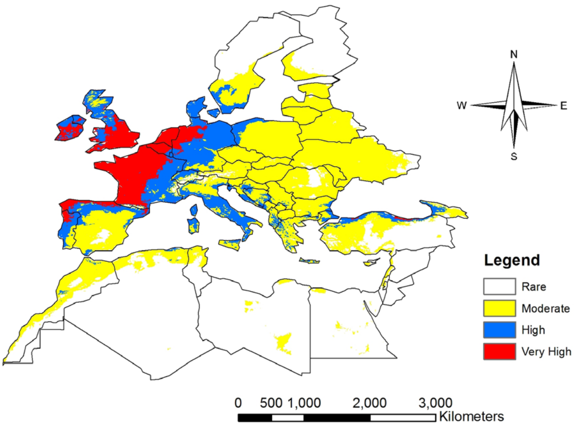
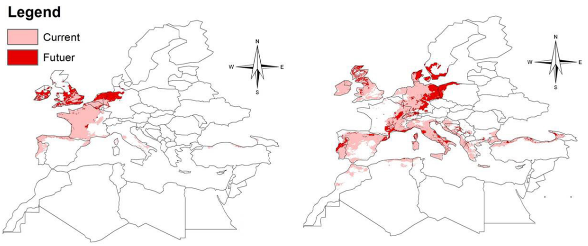
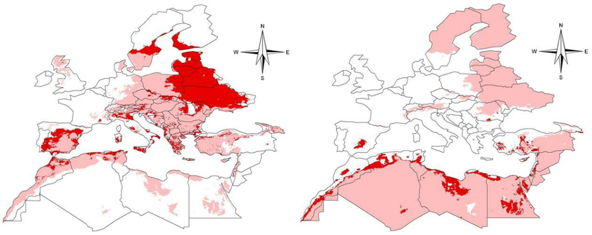


Fig. 5. Distribution of the Asian hornet under future climate conditions (SSP 126 and SSP 585). This figure is the average of two climate models (MRI-ESM2-0 and BCC-CSM2-MR).



(A) Very High (B) High



(C) Moderate (D) Rare

Fig. 6. Differences between current and future distribution (overall average) in each suitability class.

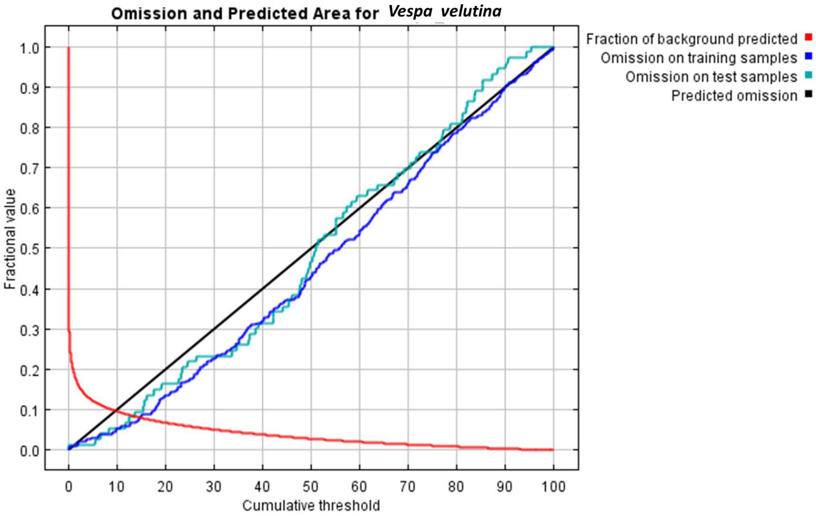


Fig. 7. The omission rate and predicted area as a function of the cumulative threshold.

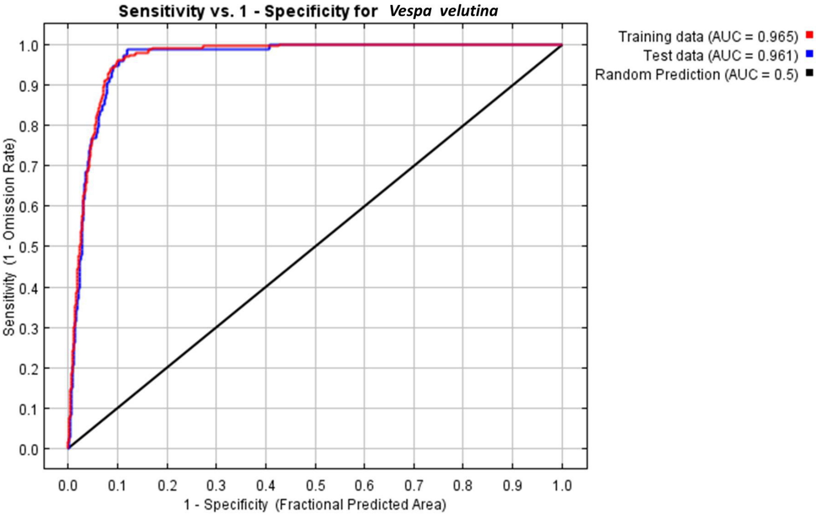


Fig. 8. The receiver operating characteristic (ROC) curve for the training and test data.

DISCUSSION

Contribution percentages

The variables with the highest contribution percentages were temperature seasonality, annual precipitation, and annual mean temperature. In fact, activities of Vespidae insects are impacted by temperature (Matsuura & Yamane, 1990; Taha, 2014).

Current distribution

Thus, two variables based on temperature (i.e. temperature seasonality and annual mean temperature) possessed 61.9% of the total. It seems that the AH becomes adapted to low/moderate temperature as showed from the response curves of highly contributed variables, which can be understood by its establishment in west Europe.

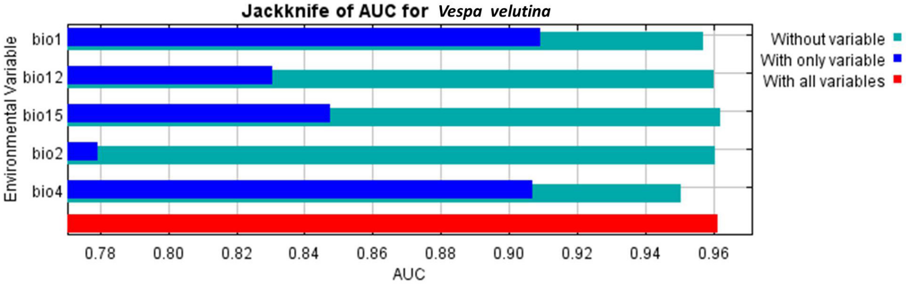


Fig. 9. Jackknife test using area under the curvy (AUC) on test data. bio1: annual mean temperature, bio2: mean diurnal range, bio4: temperature seasonality, bio12: annual precipitation, and bio15: precipitation seasonality.

The AH arrived in 2004 to Europe via South-Western France and then colonized in other countries including Spain, Portugal, and Belgium with good establishment (Haxaire et al, 2006; López et al, 2011; Grosso-Silva & Maia, 2012; Monceau et al, 2014). Notably, the current distribution obtained from the model showed high similarity with the real situation with some few exceptions. Thus, these countries were very high suitable for the AH. Accordingly, a previous study predicted the invasion success of AH in Western Europe especially in South-Western France based on data from its native range in Asia (Villemant et al, 2011). Most recently AH has been recorded in Hamburg, northern Germany (Husemann, Sterr, Maack, & Abraham, 2020). This supports the present study model as Germany among the highly suitable areas for AH. The exceptions reported in this study are the very high suitability of parts in England, Italy, and Turkey to AH. Perhaps the AH will colonize at these countries later. The geographical nature of each country may contribute in affecting the spread of AH such as the insular nature of England. In addition to this, some areas in other European countries and in North Africa were considered as highly or moderately suitable. This means that the AH can invade this countries especially environmental conditions at them are suitable for its occurrence. Moreover, the close distance between Morocco and Europe can facility the introduction of AH to North Africa.

In general, the coastal regions in North Africa are moderately suitable for the AH. This point is supported by a previous study as the occurrence of AH in Europe was characterized by specific environmental variable (high level of precipitation during the driest month of the year) which suggested the expansion of AH in the potentially invaded countries through coastal margins (Villemant et al, 2011). The invasion of AH to Africa can happen through Morocco and then through the coastal regions to invade other countries including Egypt. Also, AH can invade Egypt through the North-East part (Sinai) as some parts close to Sinai were classified as moderately suitable for the AH. Regions at North Europe with cold weather as well as most desert regions in North Africa were classified as rarely suitable for AH. It seems that the very low or high temperature at these regions caused such unsuitability for the occurrence of the AH.

Future distribution

Approximately, this is the first study to use the Shared Socio-economic Pathways (SSP) of two climatic models to predict future distribution of AH in Europe and North Africa. The old method for forecasting future conditions using Representative Concentration Pathways (RCPs) is currently outdated. The model showed high similarities with the current distribution model especially in West Europe except that England will be more suitable for the AH. No noticeable variations observed in regard to locations with high or moderate suitability for AH. Perhaps, future conditions will be in favor of AH colonization and establishment. On the contrary with the prediction that desert regions will become even drier due to climate change, which can negatively affect honey bees (Le Conte & Navajas, 2008). This study showed the potential distribution of the AH at coastal regions and some other locations in North Africa including deserts in Libya and Egypt.

Under future conditions, bee colonies were predicted to suffer from heat stress especially during summer (Abou-Shaara, 2016). The invasion of the AH to new regions will be another stress on bee colonies in addition to climate change. Especially, the AH has been reported to attack bee colonies fiercely (Shah & Shah, 1991; Tan et al, 2007; Kim, Choi, & Moon, 2006; Jung, Kim, Lee, & Baek 2008). Furthermore, honey bees at the invaded regions (e.g. in France) showed less ability to withstand the attack of AH (Rortais et al, 2010). This indicates that the damages from AH to apiaries will be high although honey bees showed some defensive behaviours against AH in Europe (Monceau et al, 2018). Another problem that the AH can compete the native hornet (*V. orientalis*) in attacking bee colonies which can cause high economic losses to beekeepers due to the loss of their colonies. In a previous study on AH and the European hornet, *Vespa crabro*, a high overlap in trophic preference was found with preference for honey bees than other insects or diets (Cini et al, 2018). Moreover, the AH can disturb the natural balance of living organisms in the invaded areas especially the prey spectrum of AH contains several insect species including some pollinators (Villemant et al, 2011).

Another key problem that the AH may transfer new diseases to bees and other Hymenoptera in the invaded areas as well as it can share some diseases with them. Especially AH and other Vespidae hornets generally are close in their genetic characteristics (Perrard et al., 2013; Takahashi, Okuyama, Minoshima, & Takahashi, 2018; Abou-Shaara & Elbanoby, 2020), and some of them can transport diseases to honey bees. For example, the oriental hornet (*Vespa orientalis*) can transfer the American Foulbrood disease to bees (Elhoseny, 2016). Moreover, Moku Virus and Israeli acute paralysis virus were detected in AH (Yañez, Zheng, Hu, Neumann, & Dietemann, 2012; Garigliany et al, 2017). Fortunately, some methods have been suggested for the control of AH (Monceau et al, 2014) include the use of traps (Demichelis et al, 2014), utilizing biocontrol agents (Darrouzet, Gévar, & Dupont, 2015), and the selection of bees with defensive behaviors (Monceau et al, 2018). However, more trends for the effective control of the AH are still required.

Model performance

The model used in the present study showed high performance as the omission rate was very close to the predicted omission. This is supported by previous studies (Abou-Shaara et al, 2021; Abou-Shaara & Darwish 2021). Also, the area under the curve (AUC) for training and test data was 0.965 and 0.961, respectively. Moreover, the Jackknife test showed the high AUC of the variables over 0.7. These values are close to 1, indicating the perfect discrimination of the used model especially that value more than 0.75 denotes to a very good fit of the model (Mulieri & Patitucci 2019; Hosni et al, 2020; Jamal et al, 2021).

CONCLUSION

The generated maps from this study are of beekeeping importance as they estimate the possible distribution of the predator hornet (*Vespa velutina*) to honey bees in the study regions (Europe and North Africa). Also, the model maps illustrated the effects of climate change on AH distribution based on the Shared Socio-economic Pathways. According to this study, some parts in Europe and North Africa including deserts in Libya and Egypt showed moderate to high suitability for the distribution of AH under current and future climate conditions. This suggests that these regions could be invaded by AH, causing damages to beekeeping sector and harming the biodiversity. So, the study models act as an alert for these countries to avoid the possible invasion of AH. Efforts to follow the expansion of the AH in Europe and to prevent it from invading other countries should be planned by the responsible authorities. In fact, the environmental conditions in North Africa are somewhat similar to those in the Levant and the Gulf countries. So, further study to follow the potential expansion of AH to such areas is required.

ACKNOWLEDGMENT

We acknowledge Princess Nourah bint Abdulrahman University Researchers Supporting Project number PNURSP2022R37, Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia for supporting this work. Also, thanks are given to the World Climate Research Programme and the climate modeling groups for producing and making available their model output, and funding agencies who support CMIP6.

REFERENCES

Abou-Shaara, H.F. (2014). The foraging behaviour of honey bees, *Apis mellifera*: a review. *Veterinarni Medicina*, 59(1), 1-10.

Abou-Shaara, H.F. (2015). Potential honey bee plants of Egypt. *Agronomical Research in Moldavia*, 48(2), 99-108.

Abou-Shaara, H.F. (2016). Expectations about the potential impacts of climate change on honey bee colonies in Egypt. *Journal of Apiculture*, 31(2), 157-164.

Abou-Shaara, H.F. (2017). Morphological characterization and wing description of *Vespa orientalis orientalis* queens. *Biotechnology in Animal Husbandry,* 33(2),251-259.

Abou-Shaara, H.F., Owayss, A.A., Ibrahim, Y.Y. & Basuny, N.K. (2017). A review of impacts of temperature and relative humidity on various activities of honey bees. *Insectes Sociaux*, 64, 455-463.

Abou-Shaara, H.F. & Staron, M. (2019). Present and future perspectives of using biological control agents against pests of honey bees. *Egyptian Journal of Biological Pest Control*, 29(1), 1-7.

Abou-Shaara, H.F. & Elbanoby, M.I. (2020). A bioinformatics study to detect the genetic characteristics of Vespa Hornets (Hymenoptera: Vespidae). *Journal of Entomological Research Society*, 22(3), 227-237.

Abou-Shaara, H.F. & Darwish, A.A.E. (2021). Expected prevalence of the facultative parasitoid *Megaselia scalaris* of honey bees in Africa and the Mediterranean region under climate change conditions. *International Journal of Tropical Insect Science*, 41, 3137-3145.

Abou-Shaara, H.F., Alashaal, S.A., Hosni, E.M., Nasser, M.G., Ansari, M.J., Alharbi, S.A. (2021). Modeling the invasion of the large hive beetle, *Oplostomus fuligineus*, into north Africa and South Europe under a changing climate. *Insects* 12(4), 1-12.

Abrol, D.P. (1994). Ecology, behaviour and management of social wasp, *Vespa velutina* Smith (Hymenoptera: Vespidae), attacking honeybee colonies. *Korean Journal of Apiculture*, 9, 5-10.

Abrol, D.P. (2006). Defensive behaviour of *Apis cerana* F. against predatory wasps. *Journal of Apicultural Science,* 50, 39-46.

Al Naggar, Y., Codling, G., Giesy, J.P., & Safer, A. (2018). Beekeeping and the need for pollination from an agricultural perspective in Egypt. *Bee World,* 95(4), 107-112.

Al-Ghamdi, A.A., Alsharhi, M.M., & Abou-Shaara, H.F. (2016). Current status of beekeeping in the Arabian countries and urgent needs for its development inferred from a soci-economic analysis. *Asian Journal of Agricultural Research,* 10, 87-98.

Al-Ghamdi, A. & Nuru, A. (2013). Beekeeping in the Kingdom of Saudi Arabia opportunities and challenges. *Bee World,* 90(3), 54-57.

Allen-Wardell, G., Bernhardt, P., Bitner, R., Burquez, A., Buchmann, S., Cane, J., Cox, P.A., Dalton, V., Feinsinger, P., Ingram, M., Inouye, D., Jones, C.E., Kennedy, K., Kevan, P., Koopowitz, H., Medellin, R., Medellin-Morales, S., Nabhan, G.P., Pavlik, B., Tepedino, V., Torchio, P., & Walker, S. (1998). The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conservation Biology,* 12(1), 8-17.

Archer, M.E. (1998). Taxonomy, distribution and nesting biology of *Vespa orientalis* L. (Hym., Vespidae). *Entomologist's Monthly Magazine,* 134, 45-51.

Archer, M. E. (2012). *Vespine wasps of the world: behavior, ecology & taxonomy of the Vespinae*. Manchester: Siri Scientific Press.

Wei, B., Wang, R., Hou, K., Wang, X., & Wu, W. (2018). Predicting the current and future cultivation regions of *Carthamus tinctorius* L. using MaxEnt model under climate change in China. *Global Ecology and Conservation,* 16, 1-11.

Carpenter, J.M. & Kojima, J. (1997). Checklist of the species in the subfamily Vespinae (Insecta: Hymenoptera: Vespidae). *Natural History Bulletin of Ibaraki University*, 1, 51-92.

Cini, A., Cappa, F., Petrocelli, I., Pepiciello, I., Bortolotti, L., & Cervo, R. (2018). Competition between the native and the introduced hornets *Vespa crabro* and *Vespa velutina*: a comparison of potentially relevant life-history traits. *Ecological Entomology,* 43(3), 351-362.

Darrouzet, E., Gévar, J., & Dupont, S. (2015). A scientific note about a parasitoid that can parasitize the yellow-legged hornet, *Vespa velutina nigrithorax*, in Europe. *Apidologie,* 46(1), 130-132.

Demichelis, S., Manino, A., Minuto, G., Mariotti, M., & Porporato, M. (2014). Social wasp trapping in north west Italy: comparison of different bait-traps and first detection of *Vespa velutina*. *Bulletin of Insectology,* 67(2), 307-317.

Dvořák, L. (2006). Oriental hornet *Vespa orientalis* Linnaeus, 1771 found in Mexico (Hymenoptera, Vespidae, Vespinae). *Entomological Problems,* 36, 80.

Elhoseny, E.N. (2016). Oriental hornet (*Vespa orientalis*) as AFB disease vector to honeybee (*Apis mellifera* L.) colonies. *Sciences,* 6(04), 934-940.

Eyring, V., Bony, S., Meehl, G. A., Senior, C.A., Stevens, B., Stouffer, R.J., & Taylor, K.E. (2016). Overview of the coupled model intercomparison project phase 6 (CMIP6) experimental design and organization, *Geoscientific Model Development,* 9, 1937-1958.

Fick, S.E. & Hijmans, R.J. (2017). WorldClim 2: new 1km spatial resolution climate surfaces for global land areas. *International Journal of Climatology,* 37 (12), 4302-4315.

Garigliany, M., Taminiau, B., El Agrebi, N., Cadar, D., Gilliaux, G., Hue, M., Desmecht, D., Daube, G., Linden, A., Farnir, F., De Proft, M., & Saegerman C. (2017). Moku Virus in invasive Asian hornets, Belgium, 2016. *Emerging Infectious Diseases Journal*, 23, 2109-2112.

GBIF.org. (2020, May 5). *Global biodiversity information facility*. Retrieved from https://doi.org/10.15468/dl.tkn6xe

Grosso-Silva, J.M. & Maia, M. (2012). *Vespa velutina* Lepeletier, 1836 (Hymenoptera, Vespidae), new species for Portugal. *Arquivos Entomolóxicos,* 6, 53-54.

Guisan, A. & Thuiller, W. (2005). Predicting species distribution: offering more than simple habitat models. *Ecology letters*, 8, 993-1009.

Haxaire, J., Bouguet, J.P. & Tamisier, J.P. (2006). *Vespa velutina* Lepeletier, 1836, uneredoutable nouveauté pour la faune de France (Hym., Vespidae). *Bulletin de la Société Entomologique de France*, 111(2), 194.

Hegland, S.J., Nielsen, A., Lazaro, A., Bjerknes, A.L., & Totland, O. (2009). How does climate warming affect plant-pollinator interactions?. *Ecology letters*, 12, 184-195.

Hosni, E.M., Nasser, M.G., Al-Ashaal, S.A., Rady, M.H., & Kenawy, M.A. (2020). Modeling current and future global distribution of Chrysomya bezziana under changing climate. *Scientific Reports*, 10, 1-10.

Husemann, M., Sterr, A., Maack, S., & Abraham, R. (2020). The northernmost record of the Asian hornet *Vespa velutina nigrithorax* (Hymenoptera, Vespidae). *Evolutionary Systematics*, 4, 1-4.

Islam, N., Iftikhar, F., & Mahmood, R. (2015). Seasonal variations in hornet's spp. and efficiency of different traps as a tool for control. *American Journal of Agricultural Science*, 2, 223-230.

Jamal, Z.A., Abou-Shaara, H.F., Qamer, S., Alotaibi, M.A., Khan, K.A.; Khan, M.F., Bashir, M.A., Hannan, A., AL-Kahtani, S.N., Taha, E.A., Anjum, S.I., Attaullah M., Raza, G. & Ansari, M.J. (2021). Future expansion of small hive beetles, *Aethina tumida*, towards North Africa and South Europe based on temperature factors using maximum entropy algorithm. *Journal of King Saud University-Science*, 33(1), 101242.

Jung, C., Kim, D.W., Lee, H.S., & Baek, H. (2008). Some biological characteristics of a new honeybee pest, *Vespa velutina nigrithorax* Buysson 1905 (Hymenoptera: Vespidae). *Korean Journal of Apiculture,* 24, 61-65.

Khodairy M.M & Awad A.A. (2013). A study on the sensory structure, in relation to some behavioral ecology of the oriental hornet (*Vespa orientalis* L.)) (Hymenoptera: Vespidae). *Life Science Journal,* 10, 1207-1216.

Kim, J.K., Choi, M., & Moon, T.Y. (2006). Occurrence of *Vespa velutina* Lepeletier from Korea, and a revised key for Korean *Vespa* species (Hymenoptera: Vespidae). *Entomological Research*, 36, 112-115.

Le Conte, Y. & Navajas M. (2008). Climate change: impact on honey bee populations and diseases. *Revue Scientifique et Technique,* 27(2), 499-510.

López, S., González, M., & Goldarazena, A. (2011). *Vespa velutina* Lepeletier, 1836 (Hymenoptera: Vespidae): first records in Iberian Peninsula. *EPPO Bulletin,* 41, 439-441.

Matsuura, M. & Yamane, S. (1990). *Biology of vespine wasps*. Springer, Verlag, Berlin.

Monceau, K., Arca, M., Leprêtre, L., Bonnard, O., Arnold, G., & Thiéry, D. (2018). How *Apis mellifera* behaves with its invasive hornet predator *Vespa velutina*?, *Journal of Insect Behavior,* 31(1), 1-11.

Monceau, K., Bonnard, O., & Thiery, D. (2012). Chasing the queens of the alien predator of honeybee: a water drop in the invasiveness ocean. *Open Journal of Ecology,* 2,183-191.

Monceau, K., Maher, N., Bonnard, O., & Thiéry, D. (2013). Predation dynamics study of the recently introduced honeybee killer Vespa velutina: learning from the enemy. *Apidologie*, 44, 209-221.

Monceau, K., Bonnard, O., & Thiéry, D. (2014). *Vespa velutina*: a new invasive predator of honeybees in Europe. *Journal of Pest Science,* 87(1), 1-16.

Mulieri, P.R. & Patitucci, L.D. (2019). Using ecological niche models to describe the geographical distribution of the myiasis-causing *Cochliomyia hominivorax* (Diptera: Calliphoridae) in southern South America. *Parasitology Research*, 118, 1077-1086.

Perrard, A., Pickett, K.M., Villemant, C., Kojima, J.I., & Carpenter, J. (2013). Phylogeny of hornets: a total evidence approach (Hymenoptera, Vespidae, Vespinae, Vespa). *Journal of Hymenoptera Research,* 32,1-15.

Phillips, S.J. (2017) (2020, May 05). *A brief tutorial on maxent*. Retrieved from http://biodiversityinformatics.amnh.org/open_source/maxent/.

Phillips, S.J., Dudík M., & Schapire, R.E. (2020, March 20). Maxent software for modeling species niches and distributions (Version 3.4.1). Retrieved from http://biodiversityinformatics.amnh.org/open_source/maxent/.

Rader, R., Reilly, J., Bartomeus, I., & Winfree, R. (2013). Native bees buffer the negative impact of climate warming on honey bee pollination of watermelon crops. *Global Change Biology,* 19(10), 3103-3110.

Rortais, A., Villemant, C., Gargominy, O., Rome, Q., Haxaire, J., Papachristoforou, A., & Arnold, G. (2010). A new enemy of honeybees in Europe: The Asian hornet *Vespa velutina*. In Settele, J., (Ed.). *Atlas of Biodiversity Risks-from Europe to the Globe, From Stories to Maps* (182-187). Pensoft, Sofia, Moscow.

Scaven, V.L. & Rafferty N.E. (2013). Physiological effects of climate warming on flowering plants and insect pollinators and potential consequences for their interactions. *Current Zoology,* 59(3), 418-426.

Shaaban, Y., El-Ashkar, A., El-Ghobashy, H., & Ghania, A. (2015). A new trap for controlling the oriental hornet (*Vespa orientalis* L.) under Egyptian apiaries conditions. *Journal of Soil Sciences and Agricultural Engineering,* 6(12), 1515-1526.

Shah, F. & Shah, T. (1991). *Vespa velutina*, a serious pest of honey bees in Kashmir. *Bee World,* 72, 161-164.

Taha A.A. (2014). Effect of some climatic factors on the seasonal activity of oriental wasp, *Vespa orientalis* L. attackting honeybee colonies in *Dakahlia governorate*, Egypt. *Egyptian Journal of Agricultural Research,* 92, 43-51.

Takahashi, R., Okuyama, H., Minoshima, Y.N., & Takahashi, J.I. (2018). Complete mitochondrial DNA sequence of the alien hornet *Vespa velutina* (Insecta: Hymenoptera) invading Kyushu Island, Japan. *Mitochondrial DNA Part B* 3, 179-181.

Tan, K., Radloff, S.E., LI, J.J., Hepburn, H.R., Yang, M.X., Zhang, L.J., & Neumann, P. (2007). Bee-hawking by the wasp, *Vespa velutina*, on the honeybees *Apis cerana* and *A. mellifera*. *Naturwissenschaften,* 94, 469-472.

Villemant, C., Barbet-Massin, M., Perrard, A., Muller, F., Gargominy, O., Jiguet, F., & Rome, Q. (2011). Predicting the invasion risk by the alien bee-hawking Yellow-legged hornet *Vespa velutina nigrithorax* across Europe and other continents with niche models. *Biological Conservation,* 144(9), 2142-2150.

Yañez, O., Zheng, H.Q., Hu, F.L., Neumann, P., & Dietemann, V. (2012). A scientific note on Israeli acute paralysis virus infection of Eastern honeybee *Apis cerana* and vespine predator *Vespa velutina*. *Apidologie,* 43, 587-589.

Yörük, A. & Sahinler, N. (2013). Potential effects of global warming on the honey bee. *Uludag Bee Journal,* 13(2), 79-87.

J. Entomol. Res. Soc., 24(1): 23-31, 2022
Doi:0.51963/jers.v24i1.2019

Research Article
Online ISSN:2651-3579

Overwintering of Coccinellids (Coleoptera: Coccinellidae) in the Center of Iran

Mehdi ZARE KHORMIZI[1]* Jahanshir SHAKARAMI[2]
Oldřich NEDVĚD[3,4] Katayoun PAHLAVAN YALI[5]
Minoo HEIDARI LATIBARI[4,6] Mostafa GHAFOURI MOGHADDAM[7]

[1]Yazd Provincial Office of Department of Environment, Yazd, IRAN.
[2]Department of Plant Protection, Faculty of Agriculture, Lorestan University, Khorramabad, IRAN
[3]Faculty of Science, University of South Bohemia, České Budějovice, CZECH REPUBLIC
[4]Czech Academy of Sciences, Biology Centre, Institute of Entomology, České Budějovice, CZECH REPUBLIC
[5]Department of Plant protection, College of Agriculture, University of Tehran, Tehran, IRAN.
[6]Department of Plant protection, College of Agriculture, Ferdowsi University of Mashhad, Mashhad, IRAN
[7]Department of Plant Protection, College of Agriculture, University of Zabol, Zabol, P.O. Box: 98615-538, IRAN
e-mails: [1]*zare7002@gmail.com, [2]shakarami.j@gmail.com, [3,4]nedved@prf.jcu.cz, [5]k.pahlavanyali@ut.ac.ir, [4,6]minoo.heidarilatibari@mail.um.ac.ir, [4,6]heidam00@prf.jcu.cz, [7]ghafourim@uoz.ac.ir
ORCID IDs:[1]0000-0002-1540-1144, [2]0000-0003-3948-4113, [3,4]0000-0001-9932-3456, [5]0000-0003-2958-2975, [4,6]0000-0003-4158-8034, [7]0000-0002-1942-9689

ABSTRACT

Overwintering adult coccinellids were collected underneath the bark of tree trunks of apricot, pomegranate, almond and mulberry in the Mehriz region of Yazd province, Iran, in 2009 to 2012 at elevations 1420-1520 meters. We identified and illustrated the following six species: *Adalia bipunctata* (Linnaeus, 1758): 61 individuals, *Exochomus quadripustulatus* (Linnaeus, 1758): 7 individuals, *Exochomus undulatus* Weise, 1878: 26 individuals, *Hippodamia variegata* (Goeze, 1777): 1 individual, *Oenopia conglobata* (Linnaeus, 1758): 95 individuals, *Scymnus subvillosus* (Goeze, 1777): 3 individuals. Coccinellids occasionally overwintered in multispecies groups. Pomegranate was host of most overwintering coccinellids.

Key words: Coccinellidae, aggregation, diapause, overwintering, distribution, Iran.

Zare Khormizi, M., Shakarami, J., Nedvěd, O., Pahlavan Yali, K., Heidari Latibari, M., & Ghafouri Moghaddam, M. (2022). Overwintering of coccinellids (Coleoptera: Coccinellidae) in the center of Iran. *Journal of the Entomological Research Society,* 24(1), 23-31.
Received: February 18, 2021 *Accepted: February 18, 2022*

INTRODUCTION

Hibernacula of coccinellid beetles in temperate climate may be scattered in agricultural areas, along forest margins, gardens, dry vegetation and even on the walls of buildings. Certain hypsotactic species fly to prominent hilltops, often several kilometers away from where they reproduced. At these sites, diapausing adults stay from mid-summer (July-August) until the following spring (April-May), sheltering in various structures and largely refraining from feeding (Hagen, 1962; Hodek, 1996). Thus, they spend a considerable time in (aestivo-) hibernation and have physiological and behavioural adaptations that minimize mortality during this period. Overwintering at high elevations may be adaptive but sometimes turns into ecological trap (Güven, Gölluoğlu, & Ceryngier, 2015).

Hibernating adults may aggregate in clumps, which usually consist of a few but sometimes tens of thousands of individuals. Ecological theory predicts that clumping may decrease the risk of predation and/or parasitism (Sillen-Tullberg and Leimar, 1988; Turchin and Kareiva, 1989; Mooring & Hartl, 1992). However, the high population density may also facilitate the spread of disease (Bellows & Hassell, 1999). Aggregation was studied in three species that differ in their overwintering habits, *Coccinella septempunctata* L., *Hippodamia undecimnotata* (Schneider) and *Hippodamia variegata* (Goeze) by Honek, Martinkova, & Pekar (2007). The hibernation behaviour of *C. septempunctata* is plastic (Hodek, 1960). Adults of this species move between habitats (Ricci, Pontil & Pires, 2005) before flying to hibernacula (Honek, 1990; Nedved, Ceryngier, Hodkova, & Hodek, 2001). Many individuals of *C. septempunctata* and other species hibernate at lowland sites with warm microclimates (southern slopes, woodland margins and roadsides), where they overwinter under dead leaves or other dry vegetation, singly or in small groups (Honek, 1989). Adults of *C. septempunctata* and *H. variegata* that fly to hilltop hibernacula overwinter in grass tussocks or under stones, singly or in groups that may consist of several tens of individuals (Honek, 1989; Ceryngier, 2000; Güven et al, 2015). Adults of *H. undecimnotata* and *Tytthaspis sedecimpunctata* form large aggregations (thousands or more individuals) in crevices on hill tops (Hodek 1960, Nedvěd, 2006). According to Novak & Grenarova (1967), much higher resistance to low temperature as well as to low moisture was found in *A. bipunctata*, which hibernates in bark crevices, than in *C. septempunctata*, *Coccinella quinquepunctata* and *Exochomus quadripustulatus*, which hibernate in litter. Individuals exposed to cool and dry climatic conditions develop stronger cold hardiness (Nedvěd, 1993; 1995). However, coccinellids can move between exposed sites on bark to shelters even during winter, causing apparent changes of their abundance (Holecová et al, 2018). The abundance of overwintering adults greatly varies among years (Honek & Martinkova, 2005).

Location and timing of hibernation affect the survival of coccinellids and their presence on specific plants early in the next season where they are needed for suppression of pests. The present study was undertaken as part of an inventory of overwintering natural enemies in agricultural Mehriz region, Iran.

MATERIAL AND METHODS

Three locations in Mehriz region of Yazd province, Iran, were sampled: Mehriz (Baghdad Abad 31°34´25˝N, 54°26´13˝E, 1502 m, and Stehrij nearby), Khormiz (31°32´30˝N, 54°26´15˝E, 1520 m) and Saryazd (31°36´23˝N, 54°55´07˝E, 1420 m) (Fig. 1). Sampling in each station was done on ten old trees of apricot, pomegranate, almond, and mulberry, which were selected randomly, repeatedly from September 2009 to March 2010 and from December 2011 to March 2012. Minimum temperatures in January in the region were 6.4°C. The height where the individuals were sampled was measured using Measuring tape (2010-2012). Adult specimens were collected from under the bark of selected trees, using a screwdriver to pry the bark loose. All collected specimens were killed in a cyanide killing jar and pinned. each specimen was tagged with the information about the host plant, locality, and date of collection. Genitalia of both sexes were dissected by softening the specimens in hot water, removing the abdomen, placing it in a dilute solution of potassium hydroxide until the fat and muscle were dissolved, then rinsing the abdomen and genitalia in clean water, aimed to prepare microscopic slides for more examination. Morphological identification was done based on Nedved (2015).

Fig. 1. Sampling localities of the coccinellids in the Yazd province (Iran).

RESULTS

Six species of coccinellids were collected overwintering in early 2010 (Table 1): *Adalia bipunctata* (Linnaeus, 1758): 27 individuals, *Exochomus quadripustulatus* (Linnaeus, 1758): 5 individuals, *Exochomus undulatus* (Weise, 1878):2 individuals, *Hippodamia variegata* (Goeze, 1777): 1 individual, *Oenopia conglobata* (Linnaeus, 1758): 42 individuals, *Scymnus subvillosus* (Goeze, 1777): 1 individual. Dorsal colour pattern was highly variable in *A. bipunctata*, with two morphs and four aberrations found in this survey (Fig. 2). No coccinellids were found from September to December 2009 and in March 2010. The highest number of species and individuals were obtained from pomegranate.

In 2011-2012, the same species, except *H. variegata*, and in similar numbers were sampled (Table 1), here provided with the mean height of sampling: *A. bipunctata*: 34 individuals, 1.2 m; *E. quadripustulatus*: 2 individuals, 1.7 m; *E. undulatus*: 17 individuals, 1.6 m; *O. conglobata*: 53 individuals, 1.0 m; *Scymnus subvillosus* (Goeze, 1777): 3 individuals, 1.5 m. Coccinellids were mostly found individually. The exception was a group of 12 *O. conglobata* with 9 *A. bipunctata* and two *E. undulatus* on mulberry.

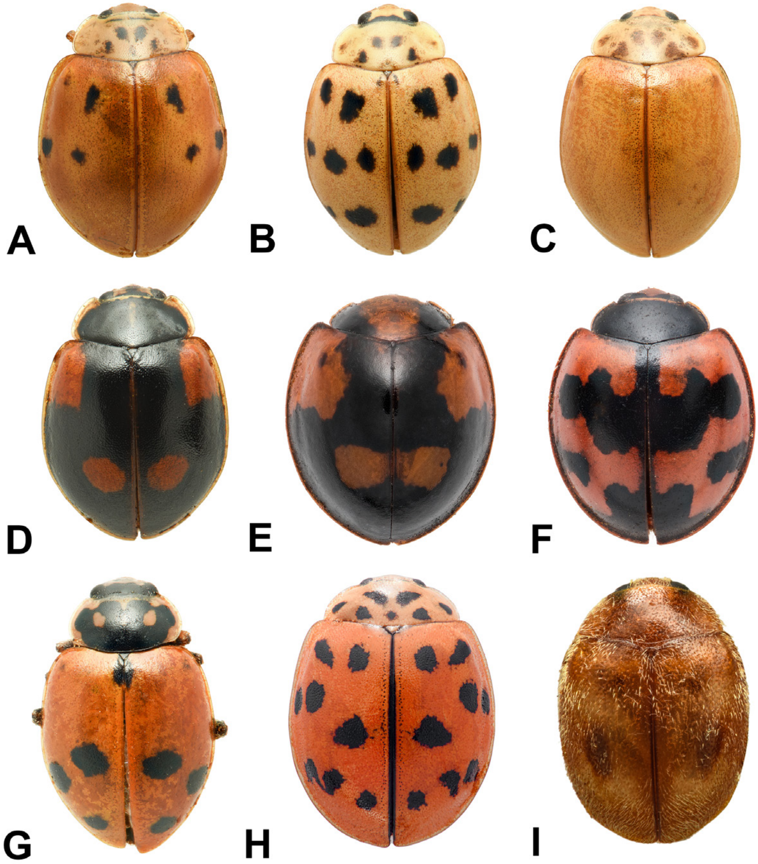


Fig. 2. Coccinellid species collected from the Yazd province during overwintering, dorsal view. A, B, C, D. Diverse colour morphs of *Adalia bipunctata* (Linnaeus, 1758); E. *Exochomus quadripustulatus* (Linnaeus, 1758); F. *Exochomus undulatus* Weise, 1878; G. *Hippodamia variegata* (Goeze, 1777); H. *Oenopia conglobata* (Linnaeus, 1758); I. *Scymnus subvillosus* (Goeze, 1777).

Table 1. Sampling locations and dates of ladybird species collected in the Yazd province along with host plant information during 2010–2012.

Location	Date	Host plant	Tree height (m)	Number
***Adalia bipunctata* (Linnaeus, 1758)**				
Khormiz	06.01.2010	Mulberry	1	1
Saryazd	26.01.2010	Pomegranate	1.64	3
Stehrij	19.02.2010	Almond	2.60	1
Khormiz	22.12.2011	Almond	3.00	1
Khormiz	02.01.2012	Pomegranate	1.00	1
Saryazd	25.01.2012	Pomegranate	1.68	2
Baghdad Abad	04.02.2012	Pomegranate	1.52	2
Stehrij	09.02.2012	Pomegranate	1.22	1
Baghdad Abad	11.02.2012	Mulberry	0.89	9
Stehrij	12.02.2012	Pomegranate	1.60	1
Stehrij	13.02.2012	Pomegranate	1.37	2
Baghdad Abad	15.02.2012	Pomegranate	1.09	2
Baghdad Abad	18.02.2012	Pomegranate	0.58	1
Baghdad Abad	23.02.2012	Pomegranate	0.48	1
Stehrij	26.02.2012	Pomegranate	1.66	1
Baghdad Abad	26.02.2012	Pomegranate	1.56	1
Baghdad Abad	29.02.2012	Pomegranate	1.38	1
Baghdad Abad	03.03.2012	Pomegranate	0.93	6
Baghdad Abad	03.03.2012	Pomegranate	1.56	2
***Exochomus quadripustulatus* (Linnaeus, 1758)**				
Saryazd	28.01.2010	Pomegranate	1.46	3
Stehrij	10.02.2010	Pomegranate	1.32	2
Saryazd	25.01.2012	Pomegranate	1.68	2
***Exochomus undulatus* (Weise, 1878)**				
Khormiz	29.01.2010	Apricot	1	1
Saryazd	02.02.2010	Almond	2.50	1
Khormiz	23.12.2011	Pomegranate	2.00	1
Khormiz	29.12.2011	Pomegranate	3.00	1
Khormiz	11.01.2012	Pomegranate	2.00	1
Khormiz	14.01.2012	Pomegranate	2.00	1
Khormiz	16.01.2012	Apricot	2.00	1
Khormiz	21.01.2012	Pomegranate	2.50	1
Saryazd	23.01.2012	Pomegranate	2.00	1
Saryazd	28.01.2012	Pomegranate	1.46	1
Saryazd	31.01.2012	Pomegranate	1.22	1
Saryazd	03.02.2012	Pomegranate	1.16	1
Saryazd	05.02.2012	Pomegranate	0.46	1
Baghdad Abad	11.02.2012	Mulberry	0.89	2
Baghdad Abad	14.02.2012	Pomegranate	1.65	1
Baghdad Abad	03.03.2012	Pomegranate	0.93	2
Baghdad Abad	03.03.2012	Pomegranate	1.56	1

Table 1. Continued.

Location	**Date**	**Host plant**	**Tree height (m)**	**Number**
Hippodamia variegata **(Goeze, 1777)**				
Stehrij	08.02.2010	Pomegranate	1.24	1
Oenopia conglobata **(Linnaeus, 1758)**				
Khormiz	13.01.2010	Apricot	1	3
Saryazd	28.01.2010	Almond	2.50	2
Mehriz	08.02.2010	Pomegranate	1.20	2
Khormiz	26.12.2011	Pomegranate	2.50	1
Khormiz	18.01.2012	Apricot	1.00	1
Baghdad Abad	04.02.2012	Pomegranate	1.52	1
Stehrij	07.02.2012	Pomegranate	1.65	1
Baghdad Abad	07.02.2012	Pomegranate	1.54	4
Baghdad Abad	11.02.2012	Mulberry	0.89	12
Baghdad Abad	14.02.2012	Pomegranate	1.65	1
Baghdad Abad	14.02.2012	Apricot	0.69	1
Baghdad Abad	15.02.2012	Pomegranate	0.89	1
Baghdad Abad	18.02.2012	Pomegranate	0.58	2
Baghdad Abad	18.02.2012	Pomegranate	1.56	1
Baghdad Abad	20.02.2012	Pomegranate	1.19	3
Baghdad Abad	20.02.2012	Pomegranate	1.33	1
Baghdad Abad	23.02.2012	Mulberry	0.48	1
Baghdad Abad	23.02.2012	Pomegranate	1.26	1
Baghdad Abad	26.02.2012	Pomegranate	1.56	4
Baghdad Abad	26.02.2012	Pomegranate	1.08	2
Baghdad Abad	29.02.2012	Pomegranate	1.38	2
Baghdad Abad	29.02.2012	Pomegranate	0.61	2
Stehrij	29.02.2012	Pomegranate	1.09	1
Baghdad Abad	03.03.2012	Pomegranate	0.48	1
Stehrij	03.03.2012	Apricot	0.52	2
Baghdad Abad	05.03.2012	Pomegranate	0.89	1
Stehrij	05.03.2012	Pomegranate	0.38	1
Baghdad Abad	07.03.2012	Pomegranate	0.49	2
Stehrij	07.03.2012	Pomegranate	0.41	1
Baghdad Abad	09.03.2012	Pomegranate	0.89	1
Stehrij	09.03.2012	Pomegranate	0.33	1
Scymnus subvillosus **(Goeze, 1777)**				
Khormiz	13.01.2010	Pomegranate	1.30	1
Khormiz	05.01.2012	Pomegranate	1.00	1
Khormiz	08.01.2012	Pomegranate	2.00	1

DISCUSSION

Most coccinellid species in temperate region overwinter as adults, although *Scymnus abietis* (Paykull, 1798) also as larvae (Nedvěd & Honěk, 2012) and *S. impexus* Mulsant, 1850 as eggs (Delucchi, 1954). Different microhabitats are used for hibernation by the Coccinellidae (Hodek, 1960; 1973). They choose sites which offer shelter from cold and moisture. We observed six species of coccinellids typical for fruit trees overwintering directly in the orchards under the bark of tree species that were probably also host plants for these species during the breeding period. Only *H. variegata*, common in most of Iran, but found in a single individual in our study, prefers herbs such as alfalfa and overwintering sites among dry herbs. Anyway, various insects can fly long distances to find large trees with space under the bark for overwintering (Spitzer et al, 2010).

Pomegranate was far the richest tree for overwintering coccinellids, either measured as number of species, number of aggregations or individuals. *Adalia bipunctata* and *O. conglobata* were common in other soft fruit and stone fruit orchards in Iran, forming Müllerian mimicry ring by having similar elytral colour pattern (Nedvěd, Biranvand, Shakarami, & Şenal, 2020; Nedvěd, 2021).

Forming overwintering aggregations is common for many species, especially Hippodamia undecimnotata may establish very conspicuous overwintering clumps consisting of several thousands of individuals. *H. variegata* overwinters in a variety of lowland and hilltop hibernacula, singly or in small groups, frequently together with other coccinellid species (Hodek, 1960) but not under tree bark. A single larger aggregation found in the present study consisted of three species: *O. conglobata*, *A. bipunctata* and *E. undulatus*.

More *E. undulatus* were found under the bark in January, while the other two common species, *A. bipunctata* and *O. conglobata* were regularly sampled also in February and March. This finding seems to confirm the early spring activity of Exochomus species, documented in *E. quadripustulatus* in Central Europe. This species was observed to look for food and mates already in late February (Nedvěd, 1995). Contrary to our present study using samples under bark, coccinellids sampled on branches of pine *Pinus sylvestris* in Slovakia were common in November but almost absent in February (Holecová et al, 2018).

We confirmed presence of several predatory ladybird species in orchards in winter where they can become valuable natural enemies of pests during the vegetation season (Heidari Latibari, Zare Khormizi, Sahamian, Dehghan Dehnavi & Moravvej, 2018). Human activities greatly affect the efficiency of natural enemies. Conservation and protection of natural enemies, as a form of biological control, including their overwintering habitats can improve biological control efforts.

ACKNOWLEDGEMENTS

We are grateful to all the people who helped us from different aspects since our study would have been impossible without their generous help and efforts. The manuscript benefited from comments by Guy A. Hanley (Northern Plains Entomology). We also thank the anonymous reviewers for critically reviewing the manuscript.

REFERENCES

Bellows, T.S. & Hassell, M.P. (1999). Theories and mechanisms of natural population regulation. In T.S. Bellows & T.V. Fisher (Eds.). *Handbook of biological control* (pp. 17-44). Academic Press, San Diego.

Ceryngier, P. (2000). Overwintering of *Coccinella septempunctata* (Coleoptera: Coccinellidae) at different altitudes in the Karkonosze Mts, SW Poland. *European Journal of Entomology*, 97, 323-328.

Delucchi, V. (1954). *Pullus impexus* (Muls.) (Coleoptera, Coccinellidae), a predator of *Adelges piceae* (Ratz.) (Hemiptera, Adelgidae), with notes on its parasites. *Bulletin of Entomological Research*, 45(2), 243-278.

Güven, O., Göllüoğlu, H., Ceryngier, P. (2015). Aestivo-hibernation of *Coccinella septempunctata* (Coleoptera: Coccinellidae) in a mountainous area in southern Turkey: Is dormancy at high altitudes adaptive? *European Journal of Entomology*, 112, 41-48.

Hagen, K.S. (1962). Biology and ecology of predaceous Coccinellidae. *Annual Review of Entomology*, 7, 289-326.

Heidari Latibari, M., Zare Khormizi, M., Sahamian, E., Dehghan Dehnavi, L., & Moravvej, G. H. (2018). Faunistic survey of Aphidoidea (Hemiptera) and associated predatory ladybirds in orchards, Yazd Province, Iran. *EPPO Bulletin*, 48(1), 160-163.

Hodek, I. (1960). Hibernation-bionomics in Coccinellidae. *Čas. čs. Spol. Entomol.*, 57, 1-20.

Hodek, I. (1973). *Biology of Coccinellidae*. Academy of Science Prague, Dr W. Junk, Publishers, The Hague and Academia, Czechoslovak, Prague.

Hodek, I. (1996). Dormancy. In I. Hodek & A. Honek (Eds.). *Ecology of Coccinellidae* (pp. 239-318), Kluwer Academic Publishers, Dordrecht.

Holecová, M., Zach, P., Hollá, K., Šebestová, M., Klesniaková, M., Šestáková, A., Honěk, A., Nedvěd, O., Parák, M., Martinková, Z., Holec, J., Viglášová, S., Brown, P.M.J., Roy, H.E., & Kulfan, J. (2018). Overwintering of ladybirds (Coleoptera: Coccinellidae) on Scots pine in Central Europe. *European Journal of Entomology*, 115, 658-667.

Honek, A. & Martinkova, Z. (2005). Long term changes in abundance of *Coccinella septempunctata* (Coleoptera: Coccinellidae) in the Czech Republic. *European Journal of Entomology*, 10, 443-448.

Honek, A, Martinkova, Z., & Pekar, S. (2007). Aggregation characteristics of three species of Coccinellidae (Coleoptera) at hibernation sites. *European Journal of Entomology*, 104 (1), 51-56.

Honek, A. (1989). Overwintering and annual changes of abundance of *Coccinella septempunctata* in Czechoslovakia (Coleoptera, Coccinellidae). *Acta Entomologica Bohemoslovaca*, 86, 179-192.

Honek, A. (1990). Seasonal changes in flight activity of Coccinella septempunctata L. (Coleoptera, Coccinellidae). *Acta Entomologica Bohemoslovaca*, 87, 336-341.

Mooring, M.S & Hartl, B.L. (1992). Animal grouping for protection from parasites - selfish herd and encounter-dilution effects. *Behaviour,* 123, 173-193.

Nedvěd, O. & Honěk, A. (2012). Life History and Development. In Hodek, I., van Emde,n H., & Honěk, A. (Eds). *Ecology and behaviour of the ladybird beetles (Coccinellidae)* (pp. 54-109). Wiley-Blackwell.

Nedvěd, O. (2015). Brouci čeledi slunéčkovití (Coccinellidae) střední Evropy = Ladybird beetles (Coccinellidae) of Central Europe. Ladybird beetles (Coccinellidae) of Central Europe. Prague: Academia

Nedvěd, O. (1993). Comparison of cold hardiness in two ladybird beetles (Coleoptera: Coccinellidae) with contrasting hibernation behaviour. *European Journal of Entomology*, 90, 465-470.

Nedvěd, O. (1995). Cold reacclimation in postdormant *Exochomus quadripustulatus* (Coleoptera: Coccinellidae). *Cryo-Letters*, 16, 47-50.

Nedvěd, O. (2006). Ephemeral overwintering aggregations of ladybirds in South Bohemia. *Silva Gabreta,* 12(3), 151-155.

Nedvěd O. (2021). Bidirectional Müllerian Mimicry between *Adalia bipunctata* (Linnaeus) and O*enopia conglobata* (Linnaeus) (Coleoptera: Coccinellidae). *Outreach Science*, 120, 42-45.

Nedvěd, O., Biranvand, A., Shakarami, J., & Şenal, D. (2020). Potential Müllerian mimicry between A*dalia bipunctata* (L.) and *Oenopia conglobata* (L.) (Coleoptera: Coccinellidae) in Iran. *The Coleopterists Bulletin*, 74, 161-167.

Nedved, O., Ceryngier, P., Hodkova, M., & Hodek, I. 2001. Flight potential and oxygen uptake during early dormancy in *Coccinella septempunctata*. *Entomologia Experimentalis et Applicata*, 99, 371-380.

Novak, B. & Grenarova, A. (1967). Coccinelliden and der grenze des feld- und Waldbiotops Hibernationsversuchemit den Imagines fuhrender Arten. In: *Konferenz uber die schadlinge der Hackfruchte III* (pp. 49-59), Praha.

Ricci, C., Pontil, L., & Pires, A. (2005). Migratory flight and pre diapause feeding of Coccinella septempunctata (Coleoptera) adults in agricultural and mountain ecosystems of Central Italy. *European Journal of Entomology*, 102, 531-538.

Sillen-Tullberg, B., & Leimar**,** O. (1988). The evolution of gregariousness in distasteful insects as a Défense against predators. *The American Naturalist*, 132, 723-734.

Spitzer, L., Konvička, O., Tropek, R., Roháčová, M., Tuf, I.H., & Nedvěd, O. (2010). Overwintering communities of arthropods on white fir (*Abies alba*) in the Moravian Wallachia region (West Carpathians, Czech Republic). *Časopis Slezského Zemského Muzea*, 59, 217-232.

Turchin, P. & Kareiva, P. (1989). Aggregation in *Aphis varians*: An effective strategy for reducing predation risk. *Ecology*, 70, 1008-1016.

J. Entomol. Res. Soc., 24(1): 33-45, 2022
Doi: 10.51963/jers.v24i1.2102

Research Article
Online ISSN:2651-3579

The First Report of Life Cycle for *Ornithoptera croesus* (Wallace, 1859) Endemic Butterfly Found Bacan Islands

Abdu MAS'UD[1]* Sundari SUNDARI[2] Mohamad AMIN[3] Alisi ALISI[4]

[1,2]Faculty of Teacher Training & Education, University of Khairun, Ternate, INDONESIA
[3]Department of Biology, Faculty of Mathematics and Natural Sciences, State University of Malang, Malang, INDONESIA
[4]Conservationists, Endemic Butterfly Island Bacan North Maluku, Ternate, INDONESIA
e-mail: *abdumasud@unkhair.ac.id, sundari@unkhair.ac.id, mohamad.amin.fmipa@um.ac.id, alisibacanisland@gmail.com
ORCID IDs: [1]0000-0002-5813-7187, [2]0000-0001-9440-3350, [3]0000-0002-7900-4017, [4]0000-0002-4028-2327

ABSTRACT

This study reports for the first time the survival and length of time the perfect metamorphosis of *Ornithoptera croesus* endemic butterflies from Bacan Island. The purpose of this study is to describe the metamorphosis phase of *O. croesus* for the length of time needed for metamorphosis and *O. croesus* survival ability in Mussaenda (*Mussaenda pubescens*) and Asoka (*Saraca asoca*) flowers as a database of endemic butterfly conservation policy in Bacan Island. This research was conducted from July to October 2020. The eggs were collected from mussaenda and asoka plants in the Mount Sibela nature reserve on Bacan Island, and then reared in captivity with a cage size of 50x50x50 cm. The results showed that the survival percentage of *O. croesus* was in the low category with a value of 55%. Furthermore, it is found out that the time required for the metamorphosis of *O. croesus* is 93-100 days, including the longer category compared to other *Papilio*. The results of this study recommend that *O. croesus* needs attention in the conservation of local resources, considering the low life span and long metamorphosis are feared to be extinct.

Key words: Life cycle, butterfly, hostplant, *Ornithoptera croesus.*

Mas'ud, A., Sundari, S., Amin, M., & Alisi, A. (2022). The first report of life cycle for *Ornithoptera croesus* (Wallace, 1859) endemic butterfly found bacan islands. *Journal of the Entomological Research Society,* 24(1), 33-45.

Received: July 15, 2021 *Accepted: February 14, 2022*

INTRODUCTION

Ornithoptera croesus butterflies in their development experience a life cycle better known as metamorphosis. Metamorphosis is a series of changes in shape and size from eggs to imago. Butterflies undergo a perfect metamorphosis which includes egg stages, larvae, cocoons and imago (Snodgrass, 1961; Jumar, 2000; Helmiyetti et al, 2013). *O. croesus* butterflies choose specific food plants such as the mussaenda and asoka plants to lay their eggs (Mas’ud et al, 2019). The larvae of the Papilionidae butterfly are plant eaters, while the adult butterfly feed is flower nectar (Jordano & Gomariz, 1994; Borror, Triplehorn, & Johnson, 1996; Zulnawati, Dahelmi, & Rahayu, 2018; Mas’ ud, Corebima, Haerullah, Hasan, & Alisi, 2019). The relationship of *O. croesus* with its host plants shows a pattern of relationship, especially in the larval phase. In the larval phase the *O. croesus* butterfly requires feed from a specific host plant.

There are six host plant families of Papilionidae species, namely Aristolochiaceae, Rutaceae, Lauraceae, Annonaceae, Magnoliaceae, and Piperaceae (Soekardi, 2012). It is stated that the life cycle of same species butterflies maintained in different host plants would have differences in larval phase length and imago size (Suwarno et al, 2007; Lytan & Firake, 2012). Furthermore, Suwarno et al, (2007) reported that *Papilio polytes* butterflies reared on the *Citrus reticulata* host plants had shorter larval phases. Whereas *Papilio polytes* that are maintained on the *Citrus hystrix* host plant have a larger imago size.

The research findings of Suwarno et al, (2007) can be explained that the availability of host plants influences the life cycle length of the butterfly. Some possibilities that can arise in butterflies due to the various qualities of the host plant include, a species can experience a long life cycle and become a normal-sized adult individual or experience a short larval stage and then become a small-sized or disabled adult. For growth, larvae need water and nitrogen obtained from the host plants that they eat (Lincoln, Couvet, & Sionit, 1986; Fajer, 1989; Baylis & Pierce, 1991; Helmiyetti et al, 2010).

Mussaenda and asoka plants are food plants of the *O. croesus* butterfly located in the Sibela mountain nature reserve of Bacan Island (Mas’ud, 2018). At the height of 20 meters above sea level, there is a butterfly breeding area owned by a researcher and conservationist of the Bacan island butterfly, which has been breeding several taxa of endemic local butterflies, Bacan island, including *O. croesus, Papilio ulysses telegonus, Papilio deiphobus, Papilio lorquinianus gelia, Papilio fuscus lapathus, Troides hypolitus, Troides criton, Graphium milon*, and *Hebomoia glaucippe sulphurea.* Information about the life cycle from eggs to imago of *O. croesus* butterflies that live on the mussaenda and asoka host plants is still limited and has not been carried out, so research needs to be carried out with the aim to collect more complete data on the life cycle of butterflies. The *O. croesus* butterfly is kept in captivity by providing mussaenda and asoka host plants. It is hoped that the results of this study can be used as a reference in endemic butterfly conservation policies on Bacan Island.

MATERIAL AND METHODS

Provision of eggs

This research has been carried out from July to October 2020 using the ex post facto method, to find the causes that allow for changes in the butterfly development phase starting from the egg phase to the imago phase. The tools used in this study include (1) measuring devices in the form of calipers, (2) psychrometers, (3) label paper, (4) petri dishes, (5) room thermometers, (6) stretching boards, (7) ovens, (8) digital scales and (9) digital camera. The research procedure is as follows.

O. croesus butterfly eggs collected from mussaenda and asoka plants in the Sibela mountain nature reserve area of Bacan island, and kept in the Bacan island butterfly breeding with a maintenance cage size of 50x50x50 cm are eggs that have just been laid by the female butterfly of *O. croesus*. The eggs are observed. Each collected egg is placed on a petri dish and labelled with the date and egg identification number. Then the eggs are kept in a maintenance cage so that their life cycle can be observed until they reach the adult stage. (egg stage, larvae, pre pupae, pupa to become imago).

Life cycle of *O. croesus*

The life cycle of a butterfly is calculated from egg to adult. During the observation, measurements were taken of the duration of the egg, larvae, pre-pupa and pupa stages. Measurements were made on the shape, color, diameter and height of the egg, body length and diameter of the head shell on the larvae, the length of the antennae the span of the wing and the length and width of the front and hind wings. The *O. croesus* butterfly cycle data and morphological characteristics of each *O. croesus* individual stage obtained were analyzed descriptively. Furthermore, the egg stage is observed until the eggs hatch into larvae. Instar larvae are counted since larvae hatch from eggs. The second instar larvae are counted after the larvae have changed their first skin until their second skin changes, and so on until the last instar larvae (fourth instar) are finished. During maintenance, feeding of larvae in the form of mussaenda and asoka leaves is carried out. Larvae are transferred from petri dishes to feed leaves placed in a glass from the larvae entering the second instar to the fourth instar larvae, each labelled with information about each larva. At the time of the study also carried out measurements of temperature using a thermometer in the maintenance room and the relative humidity of the air using a psychrometer.

RESULTS

The results of this study obtained information that 4 *O. croesus* butterflies were found in the host plants of mussaenda and asoka as many as 4 individual *O. croesus* butterflies with a life cycle as shown in Table 1. In this study, 8 eggs were successfully collected, but only 5 eggs out of 8 successfully metamorphosed into larvae.

Table 1.Developmental phases of 4 *O. croesus* individuals

Developmental phase	**time**
1) egg preparation period	7 days
2) egg development period	28 ays
3) The period of development of larvae / caterpillars	28- 30 days
4) Development of cocoons	28 ays
5) Egression time from pupa as a young *O. croesus*	1 minute, 34 seconds
6) Life time as an adult	30-35 days

Three eggs did not succeed in hatching eggs because parasites were exposed. 5 larvae that live, which managed to grow into imago only 4 larvae, 1 larvae died during instar 3. The description of the length of time required for each phase of *O. croesus* metamorphosis is: the mating phase (copulation) of the *O. croesus* butterfly for 9 hours. Furthermore, the egg preparation phase on the host plant lasts for 7 days. The time for egg development is 28 days and the larval development period is 28-30 days. Furthermore, the pupa development period was 28 days and the time of aggression from pupa to young *O. croesus* was 1 minute 34 seconds. The time required for the development of imago (young butterflies) into adults is 30-35 days. The total time required for the developmental phase of the life cycle of the *O. croesus* butterfly started from the egg preparation period, the egg development period, the larval development period, the pupal development period, the expulsion period into young *O. croesus*, and the butterfly development period. Imago (young butterflies) become adults for 93-100 days. The environmental conditions consist of a temperature of around 29.5^{0}C, relative humidity of about 79%, light intensity of 112.3, and rainfall of around 212.3.

Furthermore, the results of the study also obtained information about the description of the phases of the metamorphosis process in the egg preparation phase which begins with copulation in *O. croesus* butterflies between males and females lasting for 9 hours (Fig. 1).

Fig 1. Copulation (marriage) of *O. croesus* butterflies on mussaenda plants.

Eggs are the first stage in the life cycle of *O. croesus*. Eggs are full of nutritious liquid that caterpillars use for their growth. The egg has a special hard shell to protect the caterpillars that grow inside. Eggs are usually placed under the leaves so that predatory animals do not find it and it is difficult to eat it.

Fig 2. *O. croesus* eggs.

Egg stadia of *O. croesus* on mussaenda and ashoka host plants

After mating, *O. croesus* lays his eggs on the lower of the leaves of the mussaenda and asoka plants. The leaves in the vines (mussaenda and asoka) chosen for oviposition are usually light green leaves and not stiff for them to lay eggs in the tip area (30 cm from the tip) to full. Female butterflies generally do not lay eggs on the terminal part of the leaf, and usually one egg is placed on only one leaf (Fig. 2).

Stadia larva/caterpillar of *O. croesus* on mussaenda and asoka host plants

Instar larvae will hatch from eggs in summer by making holes. The initial hatching of the egg becomes a caterpillar with a caterpillar length of approximately 2 millimeters. The caterpillar is dark brown in color and has spines that vary from 60 to 66 spines. During the instar growth process, the caterpillar molts 4 times. Then after 7 days the caterpillar molts again and then eats the skin on 14 days later the caterpillar releases its skin and cocoon.

The first and second instars are black and soft and there are tapered spines in all segments but the 6th segment has a bright yellow tapered thorn with a black tip, and a black head. The third and fourth instars are also black or purplish brown with yellow spines except in the 6th segment. The third and fourth instars vary according to body color; starting from black, chocolate or cream with thorns in the 6th segment brown orange and black tipped. The back color of the larvae is very uniform and ends in blackish brown except on the 6th segment with pale yellow to orange spines. The head of the larvae is brown with bright yellow, the first part of the thoracic segment is dark in color with yellow osmeterium (Fig. 3). When an adult larva can reach 7 cm long.

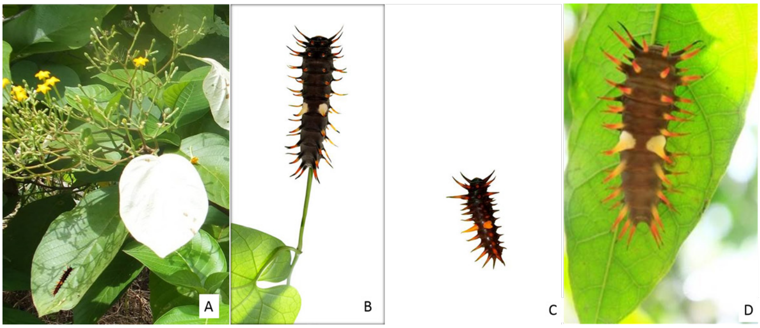


Fig 3. Larvae/caterpillar *O. croesus* (A. intar first, B. second instar, C. third instar, D. fourth instar).

At rest, the larvae under the leaves also do a complete ecdysis and they will always consume the skin removed. The body of the larvae of *Ornithoptera* sp. undergoes a change into a solid mass (concentrated mixture) of cells that will turn into butterflies within 14 days. Furthermore, the number of thorns and the pairs legs can be seen in Fig. 4.

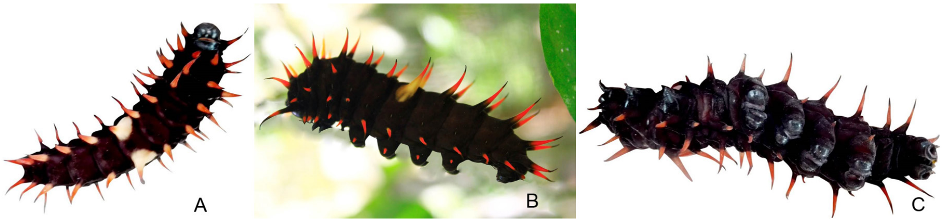


Fig 4. Larvae/caterpillar *O. croesus* (A = number of thorns; B = pairs legs; C = five pairs of legs in small meaty structure).

Based on Fig. 4 it can be explained as follows: (1) the number of upper spines is 24, (2) the number of right and left spines is 18 and 21, respectively. (a; true legs ie there are three pairs of true feet candidates, (b; mid abdominal prolegs that is there are four pairs of small structured meat found in the mid-abdomen to walk and hold and (c; anal proleg that is a pair of small structured flesh found in the the back end (the anus) functions to walk and hold, then on the last day, the larvae ascend to a safe place to spin and hang their heads down in a "J" shape for 12 hours. (Fig. 5)

The first, second and third instars experience nitrogen concentrations increase more quickly in the summer when the nutrient concentration in the leaves is also high, so that the size of the instar becomes larger compared to the size of the instar when eating less nutritious leaves.

Fig 5.*O. croesus* larvae spinning and hanging their heads down in the shape of the letter “J”.

Fig 6. *O. croesus* cocoon (*O. croesus* cocoon is usually seen attached to the foodplant).

Stadia pupa of *O. croesus* on mussaenda and asoka host plants

O. croesus butterflies that have been obtained as many as 4 individual *O. croesus*, 1 male butterfly and 3 female butterflies have developed into adulthood by undergoing a change in metamorphosis from larvae to cocoons for 14 days or two weeks. When the instar prepares itself to become a cocoon, the adult larva instar leaves the leaf of the host plant (Fig. 6), the adult larvae move to the bush and look for a new place for approximately 1-2 days, the adult larvae release a black liquid (meconium), then spin and attach the tail segment and proleg terminal section towards the bottom of the leaf, forming a silk thread that will support the weight of the pupa. Before removing the skin, the larvae of the butterfly extend, grow up and remain silent for 2 days.

The final ecdysis process takes place quickly because the larvae skin is rolled back and finally ejected again by a flick of the terminal segment, before being reinserted into the cremaster attached to a silk base on the leaf. Overall in the process of metamorphosis from larvae to cocoons takes about 3-4 days.

Butterflies young of *O. croesus* on mussaenda and ashoka host plants

Imago *O. croesus* has a long process of separating the skin from its shell into a young *O. croesus* butterfly with a time of 1 minute 34 seconds. While during the development of young *O. croesus* becomes an adult that is for 30-35 days. *O. croesus* young butterfly who broke away from his shell by crawling out. Next they look for food and drink in the nectar of the mussaenda flower, using special organs such as the mouth called "proboscis". Furthermore, the process of separating the imago skin from its shell into young *O. croesus* as shown in Fig. 7 is: a) After about 2 weeks in the form of pupae, the cocoon (chrysalis) begins to appear transparent. It is cocoon open when *O. croesus* is ready to come out, usually early in the morning, b); This stomach is filled with hemolymph, fluid like blood, and will be flowed into its wings in order to develop fully, c); *O. croesus's* stomach continues to flow hemolymph into wings, the proboscis begin to be removed and extended from its mouth. d); Wingtip is the last part to be filled with fluid. In addition to flowing hemolymph into its wings, *O. croesus* also releases orange-colored liquid which is a waste product /impurities resulting from the process of metamorphosis. e); in this phase the *O. croesus* wing has fully developed, it takes about 1 hour for the drying process before *O. croesus* is ready to fly, and f); *O. croesus* is ready to fly.

At the time of its initial growth, the body of *Ornithoptera* spp. was generally wet, wrinkled, its wings softer than silk and its body filled with a fat-like liquid. *Ornithoptera* spp. butterflies remove themselves from the cocoon by pushing the panel legs. The phases of the perfect metamorphosis process of *O. croesus* can be seen in Fig. 8.

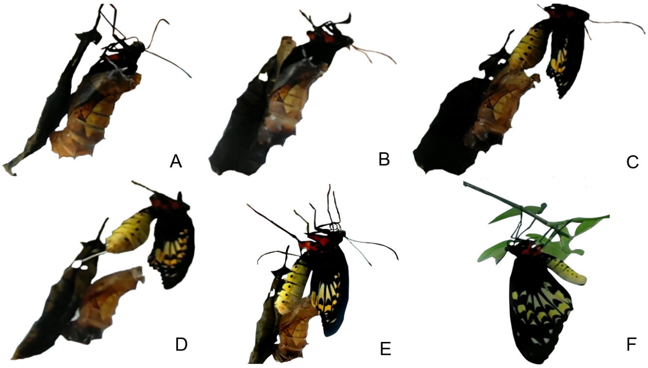


Fig 7.Imago *O. croesus* has a long process of separation of the skin of imago from its shell into a young *O. croesus*.

DISCUSSION

The results of this study indicate that the *O croesus* butterfly has a survival rate of 50%-62,5% on collection data. Based on these results, it can be said that O croesus has a lower survival rate than the *Papilio demoleus* butterfly 80% (Helmiyetti et al, 2010), the *Papilio polytes* butterfly 66.66% (Astuti, 2005). Butterflies have different survival abilities depending on environmental conditions and adaptability. In the butterflies *Graphium agamemnon*, *Papilio demoleus* and *Papilio polytes* have different life cycles at temperatures around 27º-29ºC in the host plants *Michellia champaka* (Magnoliaceae) and *Annona muricata* (Annonacea). In general, insects on host plants *Michellia champaka* (Magnoliaceae) and *Annona muricata* (Annonacea) have a minimum temperature range of 15ºC, an optimum temperature of 25ºC and a maximum temperature of 45º (Jumar, 2000).

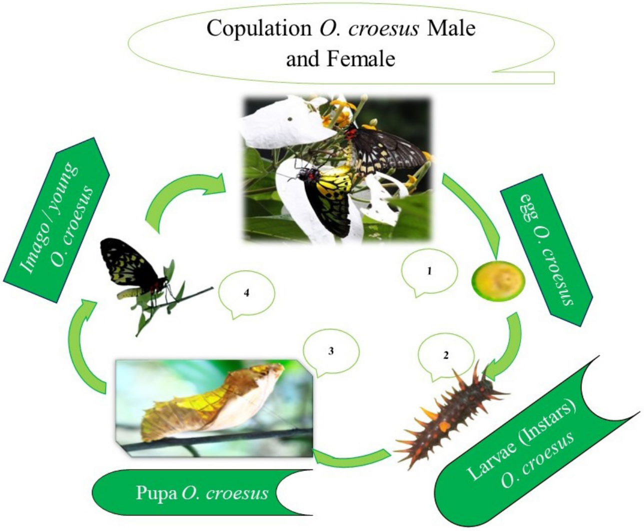


Fig 8.Development phase of the perfect metamorphosis process of *O. croesus*. (1. egg; 2. larvae; 3. pupa; 4. imago).

The results of research by Dahelmi, Salmah, & Yulnetti (2013) found that the life cycle of *Graphium agamemnon* lasts for 33-43 days, but in the host plant *Citrus aurantifolia* the life cycle of *Papilio demoleus* and *Papilio polytes* lasts for 31-42 days (Astuti, 2005). In this study, it is known that the life cycle of the butterfly *O. croesus* is 93 to 100 days. The day long description for metamorphosis starts from the egg preparation period for 9 hours, the egg development period for 7 days, the larval development period for 28-30 days, the development period for pupae for 28 days, the long expiration time being *young O. croesus* 1 minute 34 seconds and the development period imago (young butterfly) becomes an adult of 30-35 days. The life cycle of the butterflies *Papilio demoleus* and *Papilio polytes* which on the host *Citrus calamansi* is shorter because it has a higher temperature at the time of the study (Helmiyetti et al, (2013) and Astuti (2005). As also stated by Jumar (2000) generally if the temperature rises, the metabolic process will also be faster and the time to complete the insect development cycle is also faster. Faster metabolic rates cause a reduction in substances needed by insects. This makes the age of the insect will be shorter. In addition to the temperature factor, the type of host plant is also thought to influence the length of the butterfly's life cycle.

In line with research Helmiyetti et al (2010) reported that the life cycle of *Papilio demoleus* for 22-24 days and *Papilio polytes* for 22-26 days on the host plant *Citrus maxima.* The life cycle of *Papilio demoleus* became longer, namely 27-31 and the life cycle of *Papilio polytes* for 27-28 days on different host plants, namely *Citrus calamansi.* The results of this study indicate the duration of 93-100 days in *O. Croesus*. Based on the results of the study note that the water content of *Citrus calamansi* leaves is 66.58 ± 0.02%; in grapefruit (*Citrus maxima*) contains a water content of 71.56% (Helmiyetti et al, 2010). Water and protein content is very important for larval growth, especially in the early days. The quality of the host plant will affect the length of time needed for larvae to carry out further development and affect the size of the larvae (Jordano & Gomariz, 1994; Ojeda-Avila, Woods, & Raguso, 2003; Suwarno et al, 2007; Zulnawati et al, 2018). In this study the Asoka and Musaenda plants have a relatively little water content compared to citrus plants.

At the egg stage of *O. croesus*, lays its eggs under the leaves of Mussaenda and Asoka. In line with Sands & New (2013) states that female butterflies begin to collect their eggs under the leaves of plants that become food (foodplant). The egg stadia of *Papilio demoleus* and *Papilio polytes* on the host plant of *Citrus calamansi* was 3 days. This statement is in line with the research of Suwarno, Salmah, M.R., Hassan, A.A., & Norani (2007) who reported that the egg stadia in *Papilio polytes* on four types of host plants lasted for 3 days. However, it is relatively shorter when compared with Helmiyetti et al (2010) 2-4 days and Astuti (2005) which lasts 2-5 days. In *O. croesus* the egg stadia is 7 days old. Selvey (2008) measured the eggs of *Ornithoptera* spp in general as long as 2.3-2.5 mm in diameter and were initially greenish yellow, becoming pale yellow in a few days and brown yellow one or two days before hatching. Furthermore, Selvey (2008) states that after the eggs incubate, the larvae move to the host plant (foodplant) and look for soft leaves to eat small hairs (trichomes) at the bottom of the leaf as well as a place to stick.

In general, the time required for the larval stage of the *O. croesus* butterfly is 28-30 days. Furthermore, the duration of the 1st instar larvae to 4th instar larvae on Papilio demoleus lasted for 2-3 days. In instar larvae 5, the time needed is 5-6 days. This is also in line with the results of research Helmiyetti et al (2010, 2013); Dahelmi et al (2013) and Astuti (2005) who reported that the 5th instar larvae in this type of butterfly lasted for 4-5 days.

The concentration of nitrogen in the host plant can affect the number of instars in butterflies and the level of instar development (larvae), size and productivity of adult butterflies (Taylor, 1984). In the rainy season the instars experience a decrease in nitrogen concentration which is influenced by lower nutrient concentrations in the leaves (Taylor & Sands, 1986). Birdwing butterflies usually eat the leaves of vines (mussaenda), but there are certain species such as *O. richmondia, O. euphorion* and *O. priamus*, usually eating shoots, flowers, seed capsules and stems (Feeny, 1995). Birdwing butterflies generally prefer the leaves of vines because in addition to obtaining food they are also used as a place to lay eggs, breed and can be used as a place to live in limited conditions to protect them from predators and parasitoids (Feeny, 1995; Omura & Honda, 2005; Scott, 2006). Generally, birdwing butterfly species only develop on one or two leaves (Sands, 2008). Common & Waterhouse (1981) and Feeny (1995) stated that *Ornithoptera* spp. larvae generally have tubular organs, these organs are used when there is danger by producing volatile odors that are used to expel predators. It further state that the development phase of *Ornithoptera* spp. larvae generally digest food for 22-46 days until it enters the cocoon stage. The pupa stage of *O. croesus* was 28 days and the pupa stage of the *Papilio demoleus* butterfly was 9-11 days. This is different from the research of Helmiyetti et al (2010) and Astuti (2005) with a shorter pupa stage time and the results of Suwaro et al (2007) research with a longer pupa stage time than the *O. croesus* pupa stage. The difference in the duration of the pupal period is thought to be due to the different host plants used. When danger is present, all larval instars will bend the body backward and open two yellow osmeterium branches at the anterior end of the prothoracic segment to protect themselves (Common & Waterhouse, 1981; Feeny, 1995; Selvey, 2008; Sands & New, 2013). At the end of the pupa stage the event of the emergence of Ornithoptera spp is called “eclosion” (Common & Waterhouse, 1981; Feeny, 1995; Selvey, 2008; Sands & New, 2013).

In general, the results of this study indicate that the survival of *O. croesus* butterflies is in the low category when compared to other types of Papilio butterflies. Likewise, the length of the life cycle or the length of metamorphosis of *O. croesus* butterflies in the old category compared to other types of butterflies. Based on the results of this study, the *O. croesus* butterfly is a type of butterfly that needs special attention from conservationists considering its ability and low life cycle so that it is feared threatened with extinction.

CONCLUSIONS

The study reported that the survival rate of the *O. croesus* butterfly category was low at 55%. Larvae hatched from only 5 out of 8 eggs collected. 3 eggs did not succeed in hatching eggs because parasites were exposed. 5 larvae that live, which managed to grow into imago only 4 larvae, 1 larvae died during instar 3. Furthermore, it was also reported that the length of the life cycle of *O. croesus* in the long category is 93-100 days for the 9-hour egg preparation phase, the period egg development 7 days, larval development period 28-30 days, pupa development period 28 days, long expulsion to be young *O. croesus* 1 minute 37 seconds, and the development period of imago (young butterfly) becomes adult 30-35 days.

ACKNOWLEDGEMENTS

The researcher would like to thank Mukhlis, S.Pd, who helped with the collection of data in the field during the study.

REFERENCES

Astuti, H. (2005). *Life cycle of some Papilionidae butterflies found on UNIB campus*. Thesis of the Department of Biology, Faculty of Mathematics and Natural Sciences, Bengkulu University, Benkulu, Endonesia.

Baylis, M. & Pierce, N.E. (1991). The effect of host plant quality on the survival of larvae and oviposition by adults of an ant-tended lycaenid butterfly, *Jalmenus evagoras*. *Ecological Entomology*, 16(1), 1-9.

Borror, D.J., Triplehorn, C.A., & Johnson, N.F. (1996). *Introduction to insect study.* 6th ed, Gadjah Mada University Press, Yogyakarta, Indonesia.

Common, I.F.B. & Waterhouse, D.F. (1981). *Butterflies of Australia*. (2nd edn.) Angus & Robertson, Melbourne, Australia.

Dahelmi, D., Salmah, S., & Yulnetti, Y. (2013). Notes on the Premature Stage of the Butterfly *Graphium agamemnon* L. (Lepidoptera: Papilionidae). *Prosiding Semirata 2013*, 1(1).

Fajer, E.D. (1989). The effects of enriched CO_2 atmospheres on plant-insect herbivore interactions: growth responses of larvae of the specialist butterfly, *Junonia coenia* (Lepidoptera: Nymphalidae). *Oecologia*, 81(4), 514-520.

Feeny, P.P. (1995). Ecological opportunism and chemical constraints on the host associations of swallowtail butterflies. In Scriber, J. M., Tsubaki, Y., & Lederhouse, R. C. (Eds.), *Swallowtail Butterflies: Their Ecology and Evolutionary Biology* (pp. 9-16). Scientific Publ., Gainesville, FL.

Helmiyetti, H., Fadillah, F., & Manaf, S. (2013). Life cycle of several types of papilionidae butterflies on host plants of *Calamansi citrus* (*Citrofurtunella microcarpa*). *Konservasi Hayati*, 9(2), 7-17.

Jordano, D. & Gomariz, G. (1994). Variation in phenology and nutritional quality between host plants and its effect on larval performance in a specialist butterfly, *Zerynthia rumina*. *Entomologia experimentalis et applicata*, 71(3), 271-277.

Jumar. (2000). *Insect Entomology.* PT. Rineka Cipta, Jakarta

Helmiyetti, H., Dahelmi, D., & Diana, S.Y. (2010). Premature stage of several types of papilionidae butterflies on bali host plants (*Citrus maxima Merr*.). *Konservasi Hayati*, 6(2), 9-19.

Lincoln, D.E., Couvet, D., & Sionit, N. (1986). Response of an insect herbivore to host plants grown in carbon dioxide enriched atmospheres. *Oecologia*, 69(4), 556-560.

Lytan, D., & Firake, D.M. (2012). Effects of different host Plants and rearing atmosphere on life cycle of large white cabbage butterfly, *Pieris brassicae* (Linnaeus). *Archives of Phytopathology and Plant Protection*, 45(15), 1819-1825.

Mas’ud, A. (2018). *Diversity of intraspesies of Ornithoptera croesus endemic butterflies of bacan island at various altitudes on mount sibela based on morphological characters, molecular markings-rapd and their conservation strategies and the development of reference books*. Dissertation and thesis postgraduate program, State University of Malang, Indonesia.

Mas’ud, A., Corebima, A.D., Haerullah, A., Hasan, S., & Alisi. (2019). Types of butterflies visiting mussaenda and ashoka flowers in the mount sibela nature reserve, Bacan Island. *Jurnal Biologi Tropis*, 19(2), 189-196.

Ojeda-Avila, T., Woods, H. A., & Raguso, R. A. (2003). Effects of dietary variation on growth, composition, and maturation of *Manduca sexta* (Sphingidae: Lepidoptera). *Journal of Insect Physiology*, *49*(4), 293-306.

Omura, H. & Honda, K. (2005). Priority of color over scent during flower visitation by adult *Vanessa indica* butterflies. *Oecologia*, 142(4), 588-596.

Sands, D. (2008). Conserving the Richmond Birdwing Butterfly over two decades: Where to next?. *Ecological Management & Restoration*, 9(1), 4-16.

Sands, D. & New, T.R. (2013). Conservation of the richmond birdwing butterfly in Australia. Springer, Dordrecht, Heidelberg, New York, London.

Scott, J.A. (2006). *Butterfly hostplant records, 1992-2005, with a treatise on the evolution of Erynnis, and a note on new terminology for mate-locating behavior.* Doctoral dissertation, Colorado State University, United States.

Selvey, H. (2008). Studies of the eggs and larvae of the Richmond birdwing butterfly (*Ornithoptera richmondia*). *Metamorphosis Aust Issue* 5,15-16.

Snodgrass, R.E. (1961). The caterpillar and the butterfly. *Smithsonian Miscellaneous Collections,* 143(6), 1-51.

Soekardi, H. (2012). The relationship of papilionidae butterflies and their larvae feeding host plants in the Gita Persada Butterfly Park, Proceedings of the National Seminar on Science, Mathematics and Natural Sciences, Informatics and Applications, Lampung, Indonesia.

Suwarno, Salmah, M.R., Hassan, A.A., & Norani, A. (2007). Effect of different host plants on the life cycle of *Papilio polytes cramer* (Lepidoptera: Papilionidae) (Common Mormon Butterfly). *Journal of Bioscience*, 18(1), 35-44.

Taylor, M.F.J. (1984) The dependence of development and fecundity of *Samea multiplicalis* on earlyl arval nitrogen intake. *Journal of Insect Physiology*, 30, 779-785.

Taylor, M.F.J. & Sands, D.P.A. (1986). Effects of ageing and nutrition on the reproductive system of *Samea multiplicalis* (Guenee) (Lepidoptera: Pyralidae). *Bulletin of Entomological Research*, 76, 513-517.

Zulnawati, A., Dahelmi, D., & Rahayu, R. (2018). Feeding Preference of *Papilio memnon Linnaeus*, 1758 (Lepidoptera) Larvae on Host Plants *Citrus aurantifolia* and *Citrus hystrix* (Rutaceae). Metamorfosa: *Journal of Biological Sciences*, 5(2), 266-272.

J. Entomol. Res. Soc., 24(1): 47-61, 2022
Doi: 10.51963/jers.v24i1.2111

Research Article
Online ISSN:2651-3579

DNA Barcoding Data of Aphids (Hemiptera: Aphidomorpha) in Safflower (*Carthamus tinctorius* L.) with New Host Plant Records in Turkey

Cenk YÜCEL[1*] Işıl ÖZDEMİR[2] Didem CORAL[3]

[1,2,3]Plant Protection Central Research Institute, Gayret Street, Fatih Sulltan
Mehmet Boulevard, No. 66, P.K.49 06172, Yenimahalle, Ankara, TURKEY
e-mails: [1]cenkyucel@gmail.com, [2]isilozdemir70@gmail.com, [3]didemcosahin@gmail.com
ORCID IDs: [1]0000-0001-5223-9257, [2]0000-0001-9542-7442, [3]0000-0002-4957-940X

ABSTRACT

Safflower (*Carthamus tinctorius* L.) is an important oilseed plant that is grown as vegetable oil source in the world and in Turkey due to its use as a quality edible oil and biodiesel raw material obtained from its seeds. In Ankara and Eskişehir Provinces, in areas where are safflower dense plantations, aphids were sampled from the leaves, stems and roots of each plant observed once or twice a week between May-June and September-October in 2015-2017.

As a result of the study, it was determined that there are infested with harmful aphids in safflower plantations areas. Eight aphid species were identified morphologically; *Aphis craccivora*, *A. fabae*, *Brachycaudus (Prunaphis) cardui*, *B. helichrysi*, *Myzus persicae*, *Uroleucon aeneum*, *U. carthami* and *U. jaceae*. *Brachycaudus (P.) cardui* and *Uroleucon aeneum* were recorded on safflower for the first time. In addition, DNA barcoding of safflower associated aphids were performed for confirming the identification of aphids and COI sequences for the pest aphids were uploaded to the BOLD database. Identification key to *Carthamus tinctorium*-feeding aphids in Turkey based on apterous viviparous females is provided.

Key words: Safflower, Aphididae, new host plant record, Central Anatolia, COI, mtDNA.

Yücel, C., Özdemir, I., & Coral, D. (2022). DNA Barcoding data of aphids (Hemiptera: Aphidomorpha) in Safflower (*Carthamus tinctorius* L.) with new host plant records in Turkey. *Journal of the Entomological Research Society*, 24(1), 47-61.

Received: August 04, 2021 *Accepted: February 19, 2022*

INTRODUCTION

The aphids (Hemiptera: Aphidomorpha), which represents an important group with its vector ability among plant pests, are polymorphic species and cause serious losses in terms of both quality and quantity by stinging and absorbing them in many cultured plants of economic importance. Aphids are among the important plant pests in our country as well as all over the world. Many of them feed on cereals, fruits and vineyards, forest trees, park and ornamental plants, causing significant damage. Especially the damage they give to agricultural products due to their primer and seconder damage is also economically important as product loss. Oilseed plants are plants having strategic importance in human and animal nutrition due to the vegetable oils, proteins, carbohydrates, minerals and vitamins they contain. The remaining pulps of oilseed plants after obtaining fat are an absolute source of protein for meat, milk and egg production (Anonymous, 2021). Due to these crucial properties, oilseed plants constitute the main source of both vegetable oil and mixed feed sector. In recent years, biodiesel sectors have been included in these two main sectors. The most widely known oilseed is sunflower in Turkish conditions. However, the land and climatic conditions of Turkey are extremely suitable for oilseed production in different alternatives. Soy, rapeseed and safflower stand out as alternative sources of oilseeds for Turkey (Anonymous, 2021).

The Safflower (*Carthamus tinctorius* L.) is annual oil plant with a pile root system, with between 30-50% fat in its seeds, whose oil can be used to make biodiesel, whose pulp is considered as animal feed (Öğüt & Oğuz, 2005). Turkey's agricultural potential is increasing day by day due to its characteristics such as being a potential source of raw materials for vegetable oil and mixed feed sectors, being able to grow in alternative areas, entering into debate, evaluating barren areas (İşler & Karaosmanoğlu, 2010).

Global climate change leads to negative consequences in nature, while it produces positive changes in some groups of insects, such as aphids. In order to monitor the effects of global warming and climatic changes, it is necessary to know the current status of the species, their spread, the complexes they have created in different ecologies and even their past situations (Görür, 2008). Aphids are among the important plant pests in Turkey as well as all over the world.

There are 5668 species of 733 genera in the world, while 591 aphid species are known from Turkey (Favret, 2021; Blackman & Eastop, 2021; Kök & Özdemir, 2021). The following 20 aphid species are reported on *Carthamus tinctorius* in the world: *Acyrthosiphon ilka*, *Amphorophora* sp., *Aphis craccivora, A. fabae, A. gossypii*, *Aulacorthum solani*, *Brachycaudus helichrysi*, *Capitophorus elaeagni*, *Macrosiphum euphorbiae*, *Myzus persicae*, *Protaphis anuraphoides, P. carthami, P. pseudocardui, Protrama flavescens*, *Sitobion akebiae, Uroleucon carthami, U. compositae, U. gobonis, U. jaceae* and *U. sonchi* (Holman, 2009; Blackman & Eastop, 2021).

The aphids are named afit, püseron, zenk, cute, kezbi, kevzi in Turkey. They are important due pest insect to its negative effects on the vegetative development and productivity of plants. Especially the effect due to the primary and secondary damages in agricultural plants causes harvest loss and this is economically important. Aphids secrete saliva during feeding in the plant tissue, which causes molting or over proliferation of cells. This secretion, called honey substance, offers a suitable fattening environment for the development of saprophyte fungi in temperate regions, forming blackbal disease or fumajin. Aphids are the most important vector groups carrying plant virus diseases (Toros, 1973; Blackman & Eastop, 2021).

In agricultural management and plant quarantine, a rapid and accurate aphid identification to the species level is a critical task. However, high taxonomical expertise is required and morphological identification methods are time-consuming (Hebert, Cywinska, Ball, deWaard, 2003). Polymorphism, morphological plasticity, cryptic species and damaged specimens and immature stages are the other challenges. Molecular methods with easy DNA-based identification tool called DNA barcoding have been performed to resolve this problem (Hebert et al, 2003). The DNA barcoding identifies target species using short DNA sequences as barcodes (Hebert et al, 2003), a 658-bp fragment of the mitochondrial cytochrome c oxidase (CO1) gene. Since DNA Barcoding is an emerging tool, databases should be constructed on the basis of specimens identified by specialists to make identification comprehensive and reliable (Jalali, Rakshit, & Venkatesan, 2015).

MATERIAL AND METHODS

Material

In 2015-2017, aphids were sampled from the areas where safflower plant cultivation is carried out in Ankara and Eskişehir province. The specimens were studied using a LEICA DM LB2 compound light microscope and morphological characters were measured using LAS 4.1 version software. Measurements of morphological characters were done according to Blackman & Eastop (2006, 2021).

Methods

Field studies

Sampling was carried out in Ankara and Eskişehir Provinces and districts, where safflower plant were sowed densely. Collected specimens localities were given. Each locality shows safflower field areas which were given with their size in the result part. The samples were collected from the leaves, stems and root parts of each plant observed infested with pests. The samplings were performed once a week or every two weeks between May-June and September-October, and in other months when necessary according to suitable the climatic situation. The samplings were performed between 2015 and 2017.

The aphid and infested host plant organs were cut with pruning shears, first wrapped in a piece of paper to remove moisture. Then the samples were put in polyethylene bags and wrote the date, place and host plant number on them, and these bags were placed in the ice box and brought to the laboratory. The adults in the samples were placed into %96 alcohol and the nymphs were maintained in the cages with the plant parts (16:8) under controlled conditions (16: 8 D/L, 25±2°C and a 70±10% H) , until they became adults. After the alate and aptera individuals reached to the adult period, they were placed into 96% ethyl alcohol for identification.

Laboratory studies

Slide mounting of aphids were done using the method applied by Hille Ris Lambers (1950).

Molecular studies

DNA of the species was extracted by dissecting abdomen and three pairs legs and using a Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) with slight modifications as described by Magoga et al (2016). Extracted DNA was used as template for a 658-bp fragment of the mitochondrial CO1 gene amplified by PCR using universal primers LCO1490 (5’-GGT CAA CAA ATC ATA AAG ATA TTG G) and HCO2198 (5’-TAA ACT TCA GGG TGA CCA AAA AAT CA) (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994). PCR reactions were performed in a 25µl final volume reaction mix and PCR thermal profile as in Montagna et al (2017). Successful amplifications were determined by gel electrophoresis and sequenced bidirectionally by ABI Technology (Applied Biosystems, Foster City, CA, USA). The electropherograms obtained were manually edited, checked for double peaks and frameshifts by using Geneious Pro 5.5 (Biomatters Ltd., Auckland, New Zealand), and primers were removed. Each sequence was translated to protein in the EMBOSS transeq tool (www.ebi.ac.uk/Tools/emboss/transeq) to be sure that they complied with an open reading frame. All the COI sequences obtained in this study were clustered using the Barcode Index Number (BIN) in BOLD (Ratnasingham & Hebert, 2013). Furthermore, a dataset composed of our sequences and sequences of close species to our three species mined from BOLD database was built. A neighbor joining cluster analysis (NJ; Saitou & Nei, 1987) was performed for a representing the genetic differences between sequences and clusters of sequences in the dataset by using MEGA 6 (Molecular Evolutionary Genetics Analysis Version 6.0). *Myzus persicae* (Sulzer, 1776) was included as the outgroup. Tamura & Nei (1993) was used as the model of nucleotide substitution, and 1000 bootstrap replicates were performed. Finally, the sequences were deposited in the BOLD system (sequence page from CENK001-21 to CENK005-21). based on K2P distances using MEGA 6 (Tamura, Stecher, Peterson, Filipski, Kumar, 2013). Sequences were aligned using MUSCLE (Edgar, 2004), implemented in MEGA 6. Nonparametric bootstrap support values were obtained by resampling and analyzing 1000 replicates (Felsenstein, 1985).

RESULTS

List of investigated aphids and new aphid-plant interactions is as follows.

Aphis craccivora **Koch, 1854 (Aphidinae: Aphidini)**

Detected for the first time in safflower in Turkey (Yücel, Özdemir, Ertürk, Şahin, 2014).

Material examined: Oltan, Ayaş, Ankara, 27.05.2015, Area 1, 39°57′13 N 32°09′55 E 827 m, 35 da; Area 2, 39°59′16 N 32°12′30 E 798 m, 30 da; Ortabereket, 27.05.2015, Area 1, 40°06′54 N 32°24′53 E 1028 m, 28 da; Sinanlı, 27.05.2015, Area 1 39°59′23 N 32°18′37 E 823 m, 25 da; Oltan, 25.05.2016, Area 1, 39°57’17 N 32°08’56 E 860 m, 15 da; Area 2, 39°57’18 N 32°07’59 E 893 m, 15 da; Sinanlı, 25.05.2016, Area 1, 40°00’25 N 32°15’46 E 932 m, 17 da; Kaçarlı, Şereflikoçhisar, 28.05.2015, Area 1, 39°05′10 N 33°31′16 E 980 m, 70 da; Area 2, 39°05′53 N 33°31′55 E 1010 m, 30 da; Gülhüyük, 28.05.2015, Area 3, 39°06′54 N 33°34′39 E 970 m, 60 da; Area 4, 39°07′26 N 33°34′48 E 914 m, 20 da.

Comments on coloration and morphology: *Aphis craccivora* is one of the bright black Aphids and is quite complex in relation with its hosts. Young individuals are seen as slightly waxy secreted. In wingless viviparous females, the head and body are black in color and have dark and typical patterned sclerotization, starting from methanotum and covering the abdomen dorsum. In winged viviparous females, the head and body are black in color. Their antennae are half the length of the body. Abdomen is bright brownish black.

Host plants of this species detected in Turkey: *Acacia* sp., *Alhagi pseudalhagi*, *Amaranthus retroflexus, Anthemis* sp., *Begonville* sp., *Brassica oleracea, Bromus japonicus, Capsicum annuum, Capsella bursa pastoris, Cardaria draba, Catalpa* sp., *Centaurae* sp., *Chenopodium album, Cichorium intybus, Cicer arientinum, Convolvulus arvensis, Crepis foetida, Cucumis melo, Cucurbita pepo, Cydonia vulgaris, Dipsacus laciniatus, Galium aparine, Gossypium herbaceum, Heracleum* sp., *Hibiscus esculentus, Lens esculentum, Lycopersicum esculentum, Malus domes tica, Medicago sativa, Portulaca oleraceae, Prunus* sp., *Pyrus communis, Ribes rubrum, Rosa* sp., *Rumex* sp., *Sanguisorba minor, Senecio vulgaris, Solanum lycopersicum, S. melongena, Taraxacum officinale, Urtica urens, Vicia faba* and *Vitex agnus castus* (Düzgüneş & Toros, 1978; Tuatay, 1993; Ölmez & Ulusoy, 2002; Toros, Yaşar, Özgökçe, Kasap, 2002; Aslan, 2002; Özdemir, 2004; Ayyıldız & Atlıhan, 2006; Kocadal, 2006; Kaygın, Görür, & Çota, 2008).

Virus transmission: This species is known to be a vector of approximately 30 plant viruses (Blakman & Eastop, 1984, 2000). Examples of nonpersistent viruses include bean yellow mosaic virus, beet mosaic virus, cucumber mosaic virüs and pea mosaic virüs. Peanut badge virüs and peanut mottle virus as examples of viruses transmitted in a persistent way (Kennedy, Day, Eastop, 1962; Blakman & Eastop, 2021).

Aphis fabae **Scopoli, 1763 (Aphidinae: Aphidini)**

It was recorded for the first time on *Carthamus* in Ankara and Eskişehir Provinces.

Material examined: Aşağıhacıbekir, Bala, Ankara, 28.05.2015, Area 1, 39°23′44 N 33°17′38 E 844 m, 60 da; Area 2, 39°24′16 N 33°17′59 E 826 m, 75 da; Bala, Tarım, 28.05.2015, Area 3, 39°30′46 N 33^{0}16′14 E 1007 m, 20 da; Area 4, 39°29′43 N, 33^{0}17′01 E 940 m, 400 da; Kesikköprü, Area 1, 39°22’33 N 33°24’03 E 762 m, 30 da; Area 2, 39°25’54 N 33°21’00 E 910 m, 20 da; Aşağıhacıbekir, 28.05.2016, Area 1, 39°28’08 N 33°21’07 E 876 m, 30 da; Mahmudiye, Işıklar, Eskişehir, 20.05.2015, Area 1, 39°27’54 N 30°58’21 E 933 m, 30 da; Area 2, 39°26’43 N 30°57’50 E 904 m, 50 da; Mesudiye, 20.05.2015, Area 1, 39°31’50 N 30°55’10 E 891 m, 30 da

Comments on coloration and morphology: In the winged viviparous female, the body is of colors ranging from brown to black. There are with areas dark green blackish irregular pattern on the abdomen. In the wingless viviparous female, the body is brown, greenish. There are irregular dark pigmented areas on the abdomen. *Aphis fabae* is matte black or very dark coffee color in living individuals. White pleural candle secretions are present in dots and almost always present in wingless individuals and rarely seen in young individuals. Winged individuals have sclerit in the abdomen 4th and 5th tergit, and the cauda is dark in color.

Host plants of this species detected in Turkey: *Pimpinella anisum, Vitis vinifera, Vibirnum* sp. (Bodenheimer & Swirski, 1957), *Solanum dulcamara* (Tuatay & Remaudiere, 1964), *Arbitus verachne* (Canakçıoğlu, 1967), *Solanum lycopersicum, Cucurbita pepo, Ranunculus* sp., *Zea mais* (Canakçıoğlu, 1966; 1975), *Papaver* sp., *Nicotiana tabacum, Vicia fabae, Philadelphus coronarius, Matricaria* sp., *Amaranthus* sp., *Lactuca sativa* (Giray, 1974), *Foeniculum vulgare* and *Ferula* sp. (Tuatay, Kalkandere, Aysev, 1972; Düzgüneş, Toros, Kılınçer, Kovancı, 1982); *Papaver* sp., *Phaseolus* sp., *Pimpinella anisum*, *Vicia faba*, *Beta vulgaris*, *Phaseolus vulgaris*, *Solanum lycopersicum*, *Solanum nigrum*, *Urtica urens*, *Heracleum sphondylium*, *Beta vulgaris*, *Portulaca oleracea*, *Anthemis arvensis*, *Cucurbita pepo*, *Zea mays*, *Papever somniferum*, *Nicotiana tabacum*, *Foeniculum vulgare*, *Matricaria* sp., *Amaranthus* sp., *Lactuca sativa*, *Chrysanthemum* sp., *Dianthus* sp., *Impatiens balsamina*, *Kniphofia hybrida*, *Portulaca grandiflora* ve *Zinnia elegans* were registered as hosts of this species (Tuatay & Remaudiere, 1964; Çanakçıoğlu, 1967; Giray, 1974; Çanakçıoğlu, 1975; van Harten, 1975; Düzgüneş et al, 1982; Zeren, 1989; Akkaya & Uygun, 1996; Toros, Yaşar, Özgökçe, & Kasap, 1996; Özdemir & Toros 1997; Ölmez, 2000; Özdemir, Toros, Kılınçer, & Gürkan, 2006; Toros et al, 2002).

In European conditions, it chooses other woody shrubs as primary hosts like *Euonymus europaeus* and *Viburnum opulus* and *Philadelphus coronarius* and switch to numerous herbaceous secondary hosts (Stroyan, 1984). Regularly visited by ants (Blackman & Eastop, 1984, 2021).

Virus transmission: Examples of viruses that transplanting nonpersistent way are yellow mosaic, sugar beet mosaic, Dahlia mosaic, cucumber mosaic viruses. Beet yellow mesh viruses, and potato leaf curly viruses as examples of viruses transplanted in a persistent way (Kennedy et al, 1962).

***Brachycaudus* (*Prunaphis*) *cardui* (Linnaeus, 1758) (Aphidinae: Aphidini)**

This aphid is recorded for the first time on Safflower in the world (Blackman & Eastop, 2021; Holman, 2009).

Material examined: Gölbaşı, Günalan, 21.05.2018, Area 1 39°36’55 N 32°52’52 E 930 m, 15 da; Area 2 39°37’13 N 32°54›05 E 901 m, 10 da; İkizce, 21.05.2018, Area 1 39°36’33 N 32°40›22 E 862 m, 50 da; Area 2 39°35’52 N 32°41’59 E 844 m, 15 da; Dikilitaş, 21.05.2018, Area 1 39°33’56 N 32°43’54 E 870 m, 20 da; Mahmudiye, Işıklar, Eskişehir, 20.05.2015, Area 1, 39°27’54 N 30°58’21 E 933 m, 30 da; Area 2, 39°26’43 N 30°57’50 E 904 m, 50 da; Mesudiye, 20.05.2015, Area 1, 39°31’50 N 30°55’10 E 891 m, 30 da

Comments on coloration and morphology: The first record in Turkey was made in Ankara in 1939 through *Prunus domestica* and *Carduus* sp. (Bodenheimer & Swirski, 1957). There are with areas dark brownish and dark green blackish irregular pattern on the abdomen. In the wingless viviparous female, the body is brown, greenish

Host plants of this species detected in Turkey: Düzgüneş & Tuatay (1956) were found on *Carduus* sp. It chooses many plants from the Malvaceae family as hosts. In Turkey, it is reported on *Heliotropium, Achillea* (Tuatay & Remaudiere, 1964), *Eryngium* sp. (Canakçıoglu, 1966), *Cynara scolymus* (Giray, 1974), *Prunus domestica, Carduus crispus, Prunus mahalep*, thorns and weeds (Düzgüneş et al, 1982), *Anchusa* sp., *Artemisia* sp., *Carlina* sp., *C. vulgaris, Cirsium cephalotes, C. arvense* i*nconium* and *Carduus acanthoides* (Tuatay, 1988). It is found on *Salix* sp., *Centaurea* sp., *Circium* sp., *Onapardium illyricum, Prunus spinosa, Silybum marianum, Cistus crveicus, Circus benedicus, Circium spinasissinum* and *Circium* in the Eastern Mediterranean region (Toros et al, 2002).

B. (P.) cardui, which is heavily visited by ants, feeds on plum leaves in spring, causing longitudinal severe curls along the middle vein. In summer it forms dense colonies in the pots and flower heads of secondary hosts.

Virus transmission: It also transferring beans yellow mosaic, cabbage black-ringed stain, cucumber mosaic, onion yellow dwarfism (Kennedy et al, 1962) and Sharka viruses in a non-persistent way (Blackman & Eastop, 1984, 2000, 2021).

***Brachycaudus helichrysi* (Kaltenbach, 1843) (Aphidinae: Aphidini)**

It was recorded for the first time on *Carthamus* in Ankara and Eskişehir Provinces (Yücel et al, 2014).

Material examined: Mahmudiye, Işıklar, Eskişehir, 20.05.2015, Area 1, 39°27'54 N 30°58'21 E 933 m, 30 da; Area 2, 39°26'43 N 30°57'50 E 904 m, 50 da; Mesudiye, 20.05.2015, Area 1, 39°31'50 N 30°55'10 E 891 m, 30 da.

Comments on coloration and morphology: *B. helichrysi*, which is about 2 mm in size, has a slightly waxy bright appearance on its primary host, *Prunus domestica,* as in wingless form of the spring population. These wingless individuals are green, coffee or brownish yellow in color. The population in secondary hosts are very small in size and are green, pale yellow or whitish (Blackman & Eastop, 1984). The cornicle is light, short and flat and it does not have any pattern on it.

Host plants of this species detected in Turkey: In this study of *B. helichrysi*, first mentioned by Tuatay & Remaudiere (1964) in Turkey.

Some hosts in Turkey are *Taraxacum officinalis, Carthomus tintorius, Carthamus dentatus, Chrysanthemum leucanthemum, Prunus* sp., *Senecio vernalis, Centaurea* sp., *Chrysanthemum* sp., *Helianthus annuus, Matricaria* sp., *Prunus* sp., *Prunus persicae, Senecio* sp. and *Helianthus* sp. (Giray, 1974; Tuatay, 1988), *Aster alpinus, Dahlia hybrida, Zinnia elegans* (Özdemir & Toros, 1997). Ölmez (2000) reported on *Prunus domestica* and *Calendula officinalis* as the first record for Diyarbakır province. It was determined on *Matricaria chamomilla, Prunus spinosa, Prunus domestica* in the eastern Mediterranean region (Toros et al, 2002).

B. helichrysi causes significant damage to plums due to toxic saliva. It is also known as an important pest of *Chrysanthemum* in the greenhouse. It is not visited by ants.

Virus transporting: It is known as a vector of Cineraria mosaic, cucumber mosaics, Dahlia mosaics and Sharka viruses (Kennedy, Day, & Eastop, 1962). Sometimes it can also transmit nonpersistent viruses to plants that do not have hosts due to heavy flights (Blackman & Eastop, 1984; 2000).

Myzus (Nectarosiphon) persicae **(Sulzer, 1776) (Aphidinae: Aphidini)**

Yücel, Özdemir, Ertürk, & Şahin (2014) determined this aphid species on Safflower for the first time in Turkey.

Material examined: Sivrihisar, Mülkköy, Eskişehir, 21.05.2015, Area 1 39°29'57 N 31°47'59 E 897 m, 20 da; Hamamkarahisar, 21.05.2015, Area 1 39°29'25 N 31°47'16 E 835 m, 15 da; Area 2 39°29'06 N 31°46'41 E 821 m, 15 da; Çifteler, Eminekin, Eskişehir, 21.05.2015, Area 1, 39°21'55 N 31°08'54 E 852 m, 10 da; Area 2, 39°20'53 N 31°07'17 E 921 m, 20 da; Şereflikoçhisar, Yazısöğüt, 26.05.2015, Area 1 39°05'26 N 33°35'26 E 1169 m, 25 da; Kaçarlı, 13.06.2017, Area 1 39°07'03 N 33°31'09 E 843 m, 15 da

Comments on coloration and morphology: In wingless viviparous females, body color varies from whitish yellowish green to grayish-green, pale yellow green or pinkish and reddish green. Their antennae are slightly shorter than the body, and the antennae tubercels are distinct. The antennae and cornical ends are dark shaded and cornical is cylindrical or slightly bulging. Cauda is sharp and shorter than corniculus. Winged viviparous females also have a rather bright abdomen, head and thorax is in blackish color. Antennae are dark brown or black and about the average body length. The cornicle color is dark brownish and cylindrical in shape. Cauda is slightly muffled and has three pairs of hairs on the sides (Düzgüneş & Tuatay, 1956; Blackman & Eastop, 1984).

Host plants of this species detected in Turkey: Some hosts of this species in Turkey are *Allium sativum, Althea rosa, Antirrhinum* sp., *Asparagus* sp., *Atropa belladona, Beta vulgaris, Brassica oleraceae, Capsella bursa-pastoris, Capsicum annuum, Cardaria draba, Carduus pycnocephalus, Carthamus tinctorus, Cirsium arvense, Crataegus* sp., *Cucurbita pepo, Cucumis melo, Daucus carota, Foeniculum vulgare, Helianthus annus, Hordeum vulgare, Lactuca sativa, Lycopersicum esculentum, Malus communis, Malva neglacta, Nicotiana tabacum, Petroselinum hortense, Phaseolus vulgaris, Portulago oleraceae, Prunus avium, Pyrus communis, Rhaphanus raphanistrum, Sesamum indicum, Solanum melongena, Spinacia oleracea, Triticum* sp., *Tulipa* sp., *Viola tritocolar* and *Zea mays* (Düzgüneş & Tuatay, 1956; Bodenheimer & Swirski, 1957; Tuatay & Remaudiere, 1964; Giray, 1974; Çanakçıoğlu, 1975; Düzgüneş & Toros 1978; Düzgüneş et al, 1982; Karaat & Göven, 1986; Zeren, 1989; Tuatay, 1991; Önuçar & Ulu, 1993; Kıran, 1994; Akkaya & Uygun, 1996; Toros et al, 1996; Özdemir & Toros, 1997; Ölmez, 2000; Çobanoğlu, 2000; Toros et al, 2002; Özdemir, 2004; Ayyıldız & Atlıhan, 2006; Kocadal, 2006).

Virus transmission: It is stated that this species can transmit more than 100 plant virus diseases through persistent and nonpersistent ways. Of these, it is especially important by transporting potato and tobacco viruses that cause economic damage. *M.*

persicae, a polyfag species, has been reported that it transfers tobacco leaf bending virüs and tomato yellow web virüs as persistent viruses. In addition, potato acuba mosaic virus, potato A virus, potato Y virus and tobacco wilt virüs are transferred in a nonpersistent way (Kennedy et al, 1962).

Uroleucon **(*Uromelan*)** ***aeneum*** **(Hille Ris Lambers, 1939) (Aphidinae: Aphidini)**

This aphid is recorded for the first time on Safflower in the world (Blackman & Eastop, 2021; Holman, 2009). It was recorded for the first time on *Carthamus* in Ankara and Eskişehir Provinces (Yücel et al, 2014).

Material examined: Güzelyurt, Kalecik, Ankara, 29.05.2015, 40°08′26 N 33°23′59 E 721 m; 40°08′54 N 33°25′13 E 773 m; Güzelyurt, Hacıköy, Ankara, 26.06.2015, 40°11′39 N 33°26′47 E 846 m; 40°11′39 N 33°26′47 E 846 m; 40°10′53 N 33°26′01 E 861 m.

Comments on coloration and morphology: Wingless and winged are shiny black in color, have a metallic appearance. Adults are large and striking. It is dark brown in color in the pre-adult period.

Host plants of this species detected in Turkey: Its hosts are *Carduus crispus, Carduus* sp., *Cirsium arvense* and *Cirsium* sp. (Canakçıoğlu, 1975). In addition, It was reported in Van/Tatvan on *Cirsium* sp. (Tuatay, 1967).

Virus transporting: *U.* (*U.*) *aeneum* was transmit Moroccan watermelon mosaic virus (MWMV; Potyvirus, Potyviridae) (Chatzivassiliou, Papapanagiotou, Mpenardis, Perdikis, Menexes, 2016).

Uroleucon (Uromelan) jaceae **(Linnaeus, 1758) (Aphidinae: Aphidini)**

Yücel et al (2014) recorded this aphid species on Safflower for the first time in Turkey.

Material examined: Eskikarsak, Polatlı, Ankara, 27.05.2015, Area 1 39°53′30 N 32°04′43 E 692 m, 40 da; Sarıoba 27.05.2015; 25.06.2015), Area 1 39°51′40 N 32°05′39 E 706 m, 30 da; Eskipolatlı 25.05.2016, Area 1 39°33′36 N 32°11′43 E 892 m, 18 da; Area 2 39°32′14 N 32°10′15 E 816 m, 10 da; Şeyhali 25.05.2016, Area 1 39°30′58 N 32°17′02 E 875 m, 10 da; Area 2 39°29′17 N 32°18′24 E 937 m, 15 da; Şabanözü 25.05.2016, Area 1 39°43′01.5″N 32°03›50.5″E 762 m, 20 da

Comments on coloration and morphology: Its color is ranging from dark reddish brown to black. The antennae, legs, cornicle and cauda are black. Wingless individuals are 2.5- 3.5 mm long (Blackman & Eastop, 1984; 2000).

Host plants of this species detected in Turkey: *Acroptilon pieris, A. repens, Carduus nutans, Carduus* sp., *Centaurea calcitrapa, C. coroniformis, C. solstitialis, Centaurea* spp., *Carlina vulgaris, Circium arvense, Onopordium anatolicum* and Compositae (Boschma, 1939; Bodenheimer & Swirski, 1957; Tuatay, Gül, Demirtola, Kalkandelen, & Çağatay, 1967; Giray, 1974; Tuatay, 1991; Aslan, 2002; Özdemir, 2004; Yücel et al, 2014).

Virus transmission: This species transmits the cucumber mosaic virus in a nonpersistent way (Kennedy et al, 1962).

***Uroleucon* (*Uromelan*) *carthami* (Hille Ris Lambers, 1948) (Aphidinae: Aphidini)**

It was recorded for the first time on *Carthamus* in Eskişehir provinces (Yücel et al, 2014).

Material examined: Güzelyurt, Kalecik, Ankara, 26.06.2015, Area 2, 40°08′54 N 33°25′13 E 773 m, 25 da; Hacıköy 26.06.2015, Area 3 40°11′39 N 33°26′47 E 846 m, 70 da; Area 4 40°10′53 N 33°26′01 E 861 m, 35 da; Oltan, Ayaş, Ankara, 27.05.2015, Area 1 39°57′13 N 32°09′55 E 827 m, 35 da; Area 2 39°59′16 N 32°12′30 E 798 m, 30 da; Ortabereket, 27.05.2015, Area 1 40°06′54 N 32°24′53 E 1028 m, 28 da; Sinanlı, 27.05.2015, Area 1 39°59′23 N 32°18′37 E 823 m, 25 da; Oltan, 25.05.2016, Area 1 39°57′17 N 32°08′56 E 860 m, 15 da; Büyükboyalık, Area 1, 39°33’06 N 33°13’47 E 897 m, 25 da; Üçem, Area 1, 39°32’17 N 33°12’54 E 928 m, 15 da; Ergin, Area 1, 39°37’50 N 33°09’32 E 914 m, 15 da; Area 2, 39°39’14.6 N 33°07’35 E 865 m, 18 da; Kerişli, 14.06.2017, Area 1, 39°41’51 N 33°07’19 E 935 m, 20 da; Karaali, 14.06.2017, Area 1, 39°39’40 N 32°57’50 E 945 m, 20 da; Area 2, 39°39’59 N 32°58’28 E 912 m, 15 da.

Comments on coloration and morphology: The color of the aptherapy varies from dark brown to blackish-brown according to Papapanagiotou et al (2012), the body length is 2.1-3.3 mm. *Carthamus* spp. in southern and central Europe. Algeria (Laamari, Coeur d’acier, & Jousselin, 2013), Israel, Lebanon, Turkey and Pakistan and India (Kashmir) in the east. Most of the records from *C. tinctorius* in India are probably referred to as *U. compositae*. There is also confusion in European literature with the very closely related *U. jaceae* and *U. aeneum*, which are normally on the other Cynareae but can sometimes also be fed in *Carthamus* (Nieto Nafria, Remaudiere, & Mier Durante, 1986). Sexual morphs and life cycle are unknown. In Turkey, It was determined on 29.6.1963 in Ankara, Beypazarı-Nallıhan road; on 5.6.1963 in Mersin, Narlıkaya and on *C. dentatus* in Muğla, Marmaris on 11.6.1963 (Tuatay & Remaudiere, 1964).

Host plants of this species detected in Turkey: It was determined on 29.06.1963 in Ankara, Beypazari-Nallıhan road; on 5.6.1963 in Mersin, Narlıkaya and on *C. dentatus* in Muğla, Marmaris on 11.6.1963 (Tuatay & Remaudiere, 1964).

Virus transmission: There is no record of this aphid being a virus vector.

Molecular analysis

In molecular studies, four CO1 sequences (ranged from 619 to 661 bp) were successfully obtained after performing quality control analysis. As it is known for arthropods, our sequence data also revealed a high AT-content for the DNA barcode region with a base composition of A = 35%; C = 13.7%; G = 10.1%; T = 41.1%. This study provided CO1 sequences of *Brachycaudus helichrysi* (1 sequence), *Uroleucon aeneum* (1 sequence) and two *Uroleucon jaceae* (2 sequences) species. In BIN analysis, unique BINs were revealed for our sequences with less than 1% nucleotide distances. In addition, certain morphological identifications of our specimens performed by the second author, which supports the accuracy of the sequences. Dataset includes for neighbour joining tree 56 close sequences to our species and one outgroup as *M. persicae*. The neighbour joining tree confirmed the results previously obtained on the basis of the BIN analyses, and all the species possessing at least one conspecific were monophyletic and supported with high bootstrap values (Fig. 1).

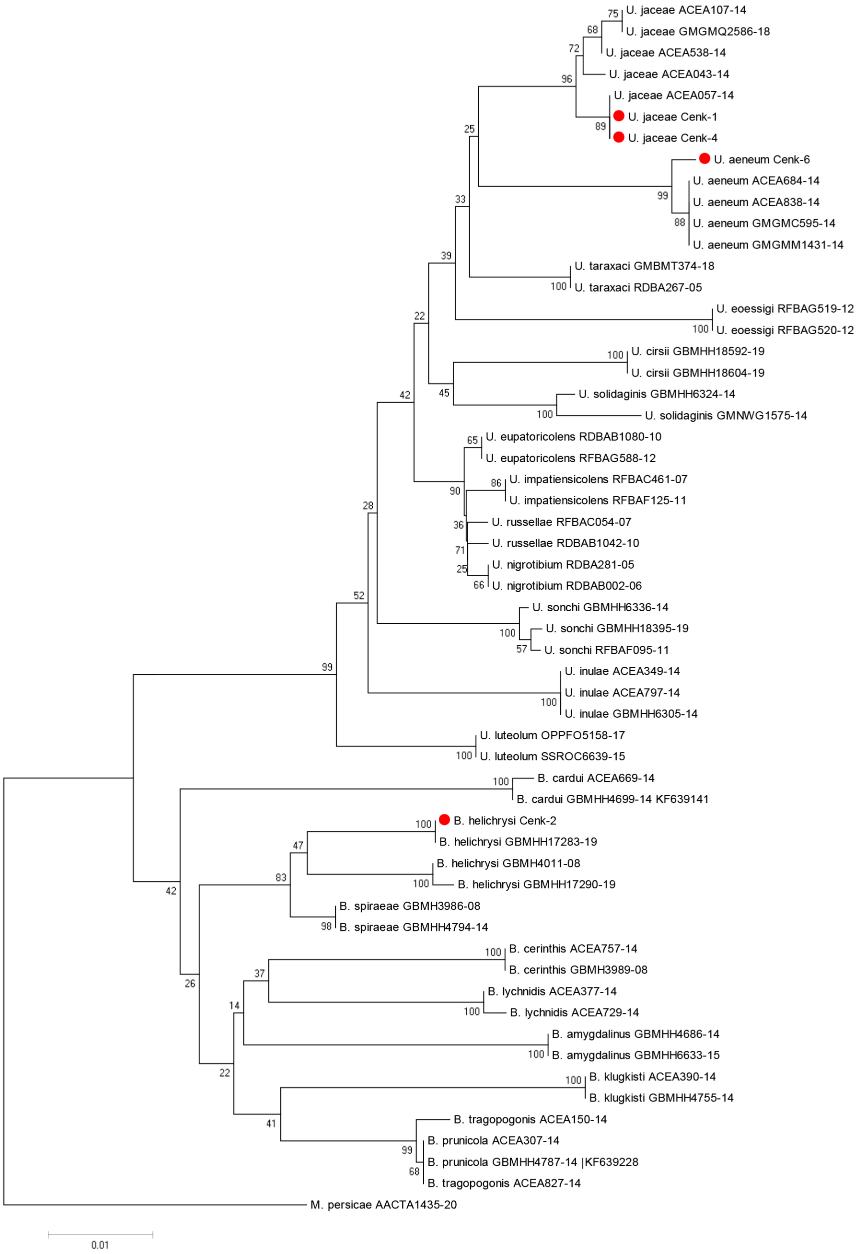


Fig. 1. Neighbour-joining tree inferred on dataset representative sequences of close species available in BOLD. Bootstrap values are reported above the main lineages; sequences with red dot are our sequences in this study.

DISCUSSION

In this study, for providing confirmation of aphid species identification, DNA barcoding was performed. As a result, confirmation of our DNA sequences and neighbour-joining tree results with the morphological identifications gives promising results for future studies. Aphids are a group of plant sap sucking insects, including many pests in agriculture. Therefore, an accurate and rapid taxonomic identification of individuals must to be regarded as crucial for management strategies for crop pests in Turkey. Due to morphological plasticity aphid identification is considered to be difficult. Traditional morphological identification requires high quality slides and specimens; however, even for taxonomists, their small body size and difficulty in slide making procedure cause to a time consuming process of species identification. Moreover, morphological characteristics of aphids can be influenced by biotic factors such as life cycle, natural enemy, feeding site, and host plant, as well as abiotic factors (Tamura et al, 2013; Li et al, 2020). In addition, high phenotypical similarity and intraspecific variation among species could interrupt the identification of aphid species. For these reasons, supporting and checking the identification of aphid species with the DNA barcoding is vital with new prospective informations, valuable for future studies and applications.

Fourteen aphid species are given in the world aphid host plant catalogue (Holman, 2009). Eight species were identified in this study and eight species were detected morphologically; *Aphis craccivora* Koch, *Aphis fabae* Scopoli, *Brachycaudus (Prunaphis) cardui* (Linnaeus), *Brachycaudus helichrysi* (Kaltenbach), *Myzus (Nectarosiphon) persicae* (Sulzer), *Uroleucon (Uromelan) jaceae* (Linnaeus), *Uroleucon (Uromelan) aeneum* (Hille Ris Lambers) and *Uroleucon (Uromelan) carthami* (Hille Ris Lambers) are reported from Safflower (*Carthamus tinctorius* L.). All of these species is associated with Asteraceae plants worldwide (Holman, 2009; Blackman & Eastop, 2021).

ACKNOWLEDGEMENTS

We would like to thank the General Directorate of Agricultural Research and Policies of the Ministry of Agriculture and Forestry of Turkey.

REFERENCES

Akkaya, A. & Uygun, N. (1996). Diyarbakır ve Şanlıurfa illeri yazlık sebze ekosistemindeki insecta faunası. Türkiye III. Entomoloji Kongresi Bildirileri, 24-28 Eylül 1996, Ankara, 423-430.

Anonymous (2021, September 10). Retrieved from https://arastirma.tarimorman.gov.tr /ttae/Sayfalar/Detay.aspx?SayfaId=59.

Aslan, M. (2002). *Kahramanmaraş ilinde Aphidoidea (Homoptera) türleri ile bunların parazitoid ve predatörlerinin saptanması*. Phd Thesis, Çukurova Universitesi, Fen Bilimleri Enstitüsü, Konya, Turkey.

Ayyıldız, Y. & Atlıhan, R. (2006). Balıkesir ili sebze alanlarında görülen yaprak biti türleri ve doğal düşmanları. *Yüzüncü Yıl Üniversitesi Ziraat Fakültesi Tarım Bilimleri Dergisi*, 16(1), 1-5.

Blackman, R.L. & Eastop, V.F. (1984). A*phids on the World's crops: An identification quide*. (2nd ed.). A Wiley, Intenscience Publication, New York.

Blackman, R.L. & Eastop, V.F. (2000). *Aphids on the World's crops: An identification quide*. (2nd ed.). A Wiley, Intenscience Publication, New York.

Blackman, R.L. & Eastop, V.F. (2006). *Aphid's on the World's herbaceous plants and shrubs: An identification and information guide*. Vol 2, A Wiley, Chichester, New York.

Blackman, R.L. & Eastop, V.F. (2021, February 01). *Aphids of the World's plants: an online identification and Information Guide*. Retrieved from www.aphidsonworldsplants.info

Boschma, H. (1939). *Temminckia. A Journal of systematic zoology*. Vol. IV, Brill Academic Publishers, Nederland.

Çanakçıoğlu, H. (1967). *Türkiye'de orman ağaçlarına arız olan yaprakbitleri (Aphidoidea) üzerine araştırmalar*. T.C. Tarım Bakanlığı, Orman Genel Müdürlüğü 466(22), 8, Ankara.

Chatzivassiliou, E.K., Papapanagiotou, A.P., Mpenardis, P.D., Perdikis, D.C., & Menexes, G. (2016). Transmission of Moroccan watermelon mosaic virus (MWMV) by Aphids in Greece. *Plant Disease*, 100(3), 601-606.

Çobanoğlu, S. (2000). Aphididae (Hom.) species of Edirne province (Thrace part of Turkey). *Entomologist's Monthly Magazine*, 136, 45-52.

Düzgüneş, Z. & Tuatay, N. (1956). *Türkiye aphidleri.* Ziraat Vekaleti, Ankara Ziraat Enstitüsü Müdürlüğü. Sayı 4, Ankara.

Düzgüneş, Z. & Toros, S. (1978). Ankara ili ve çevresinde elma ağaçlarında bulunan yaprakbiti türleri ve kısa biyolojileri üzerinde araştırmalar. *Türkiye Bitki Koruma Dergisi*, 1(3), 151-175.

Düzgüneş, Z., Toros, S. Kılınçer N., & Kovancı, B. (1982). Ankara ilinde bulunan Aphidoidea türlerinin parazit ve predatörlerinin tespiti. Tarım ve Orman Bakanlığı Zirai Mücadele ve Zirai Karantina Genel Müdürlüğü, Yayın Şubesi.

Edgar, R.C. (2004). Muscle: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32, 1792-1797.

Favret, C. (2021, May 2). Aphid species file. Version 5.0/5.0. Retrieved from http://Aphid.SpeciesFile.org.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39, 783-791.

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294-299.

Giray, H. (1974). İzmir ili ve cevresinde Aphididae (Homoptera) familyasi türlerine ait ilk liste ile bunların konukcu ve zarar şekilleri hakkında notlar. *Ege Ünivesitesi Ziraat Fakültesi Dergisi*, 11(1), 39-69.

Görür, G. (2008). Türkiye afit faunasının son durumu ve afitlerin ekonomik önemi. *Türk Bilimsel Derlemeler Dergisi,* 1(1), 17-22.

Hebert, P.D.N., Cywinska, A., Ball, S.L., & deWaard, J.R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*. 270(1512), 313-321.

Hille Ris Lambers, D. (1950). On mounting aphids and other softskinned insects. *Entomologische Berichten*, 13, 55-58.

Holman, J. (2009). *Host plant catalog of aphids, Palaearctic region*. Springer, Branisovska.

İşler, A. & Karaosmanoğlu, F. (2010). Biofuels in Turkey, in rethinking structural reform in Turkish agriculture: beyond the World Bank's strategy, In Karapınar, B., Adaman, F., & Özertan, G. (Eds.), Agriculture issues and policies series (pp 319-340). Nova Science Publishers, New York.

Jalali, S.K., Rakshit, O., & Venkatesan, T. (2015). DNA barcoding for identification of agriculturally important insects. In A.K., Chakravaryhy (Ed.). *New horizons in insect science: Towards sustainable pest management.* (pp: 13-22), Springer, New Delhi, India.

Karaat, Ş. & Göven, M.A. (1986). Güneydoğu Anadolu Bölgesinde tütün dikim alanlarında Şeftali yaprakbiti (*Myzus persicae* Sulz.)' nin doğal düşmanlarının genel durumu. Türkiye I. Biyolojik Mücadele Kongresi Bildirileri, Adana, s. 162-172.

Kaygin, A.T., Görür, G., & Çota, F. (2008). Contribution to the aphid (Homoptera: Aphididae) species damaging on woody plants in Bartın, Turkey. *International Journal of Natural and Engineering Sciences*, 2(1), 83-86.

Kennedy, J.S., Day, M.F., & Eastop, V.F. (1962). *A conspectus of Aphids as vector of plant viruses*. Commonwealth Institute of Entomology, London, UK.

Kıran, E. (1994). Güneydoğu anadolu bölgesi hububat ekiliş alanlarında görülen yaprakbiti türleri ve doğal düşmanları üzerinde çalışmalar. 3. Biyolojik Mücadele Kongresi Bildirileri, Ankara, s.29-37.

Kocadal, E. (2006). Kuzey Kıbrıs Türk Cumhuriyeti'ndeki Aphidoidea (Homoptera) türleri, bunların konukçuları, parazitoit ve predatörlerinin belirlenmesi. Yüksek Lisans Tezi. Çukurova Üniversitesi, Fen Bilimleri Enstitüsü, Konya, Turkey.

Kök, Ş. & Özdemir, I. (2021). Annotated systematic checklist of the aphids (Hemiptera: Aphidomorpha) of Turkey. *Zootaxa*, 4925, 1-74.

Laamari, M., Coeur d'acier, A., & Jousselin E. (2003). New data on aphid fauna in Algeria. *Zookeys*, 319, 223-229. doi: 10.3897/zookeys.319.4340.

Li, Q., Deng, J., Chen C., Zeng, L., Lin, X., Cheng Z., Qiao G., & Huang X. (2020). DNA barcoding subtropical aphids and implications for population differentiation. *Insects*, 11(11), 1-17.

Magoga, G., Sassi, D., Daccordi, M., Leonardi, C., Mirzaei, M., Regalin, R., Lozzia, G., & Montagna, M. (2016). Barcoding Chrysomelidae: a resource for taxonomy and biodiversity conservation in the Mediterranean Region. *Research on Chrysomelidae*. 6, *ZooKeys*, 597, 27-38.

Montagna, M., Kubisz, D., Mazur, M.A., Magoga, G., Ścibior, R., & Kajtoch, L. (2017). Exploring species-level taxonomy in the *Cryptocephalus flavipes* species complex (Coleoptera: Chrysomelidae). *Zoological Journal of the Linnean Society*, 179, 92-109.

Nieto Nafria, J.M., Remaudiere, G., & Mier Durante, M.P. (1986). Contribucion al conocimiento de la fauna de pulgones de Andalucia. Actas de las VIII Jornadas de la Asociacion Espariola de Entomologia, Seville, Octubre, 382-395.

Ölmez, S. & Ulusoy, M.R. (2002). Diyarbakır ilinde Aphidoidea üst familyasına bağlı türlerin predatörlerinin saptanması. Türkiye 5. Biyolojik Mücadele Kongresi, Erzurum, s. 237-246.

Öğüt, H. & Oğuz, H. (2005). Biodiesel: Third millennium fuel. (2nd ed., pp: 55-60). Nobel Publishing House , Ankara, Turkey.

Ölmez, S. (2000). *Diyarbakır ilinde Aphidoidea (Homoptera) türleri ile bunların parazitoit ve predatörlerinin saptanması.* Yüksek Lisans Tezi. Çukurova Üniversitesi, Fen Bilimleri Enstitüsü, Konya, Turkey.

Önuçar, A. & Ulu, O. (1993). Ege bölgesi meyve fidanlıklarındaki zararlılar üzerinde faunistik çalışmalar. *Bitki Koruma Bülteni*, 33(1-2), 23-37.

Özdemir, I. & Toros, S. (1997). Ankara parklarında mevsimlik süs bitkilerinde zararlı Aphidoidea (Hom.) Türleri. *Turkish Journal of Entomology*, 21(4), 283-298.

Özdemir, I., (2004). *Ankara ilinde otsu bitkilerde Aphidoidea türleri üzerinde taksonomik araştırmalar*. Phd Thesis. Graduate School of Science and Engineering of Ankara University, Ankara, Turkey.

Özdemir, I., Toros, S., Kılıncer, A.N., & Gürkan, M.O. (2006). A survey of Aphididae (Homoptera) on wild plants in Ankara, Turkey. *Ecology*, 15(58), 1-6.

Papapanagiotou, A.P., Nathanailidou, M., Taylor, M., Zarpas, K.D., Voudouris, K., Tsitsipis, J.A., & Margaritopoulos, J.T. (2012). New records of aphid species (Hemiptera: Aphididae) in Greece. *Entomologia Hellenica*, 21(2), 54-68.

Ratnasingham, S. & Hebert, P.D.N. (2013). A DNA-based registry for all animal species: The Barcode Index Number (BIN) System. *PLoS One*, 8, e66213. h

Saitou, N. & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4, 406-425.

Stroyan, H.L.G. (1984). Aphids-Pterocommatinae and Aphidinae (Aphidini) Homoptera: Aphididae. Handbooks for The Identification of British Insects. *Royal Entomological Society of London*, 2(6), 210-218.

Tamura, K. & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10(3), 512-26.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30, 2725-2729.

Toros, S. (1973). Bitki patojen viruslarının aphidlerle nakil mekanizması. *Bitki Koruma Bülteni*, 13(2), 83-105.

Toros, S., Yaşar, B., Özgökçe, M.S., & Kasap, İ. (1996). Van ilinde Aphidoidae (Homoptera) üstfamilyasına bağlı türlerin saptanması üzerine çalışmalar. Türkiye 3. Entomoloji Kongresi, Ankara,Türkiye, 549-556.

Toros, S., Uygun, N., Ulusoy, R., Satar, S., & Özdemir, I. (2002). *Doğu Akdeniz Bölgesi Aphidoidea t*ürleri. T. C. Tarım ve Köyişleri Bakanlığı, Tarımsal Araştırmalar Genel Müdürlüğü, Ankara.

Tuatay, N. & Remaudiere, G. (1964). Premiere contribution au catalogue des Aphididae (Hom.) de la Turquie. *Rev. Path. Vegveale et D'entomologie Agricole de France*. 43(4), 243-278.

Tuatay, N., Gül, S., Demirtola, A. Kalkandelen, A., & Çağatay, N. (1967). *Nebat koruma müzesi kataloğu* (1961-1966). (pp: 66). Türkiye Cumhuriyeti Tarım Bakanlığı Zirai Mücadele Zirai Karantina Genel Müdürlüğü Yayınları, Mesleki Kitaplar Serisi. Ayyıldız Matbaası, Ankara.

Tuatay, N., Kalkandelen, A., & Aysev, N. (1972). *Nebat koruma müzesi böcek kataloğu* (1961-1971). Türkiye Cumhuriyeti Tarım Bakanlığı Zirai Mücadele Zirai Karantina Genel Müdürlüğü Yayınları, Mesleki Kitaplar Serisi. Mesleki Kitaplar Serisi. Yenigün Matbaası, Ankara.

Tuatay, N. (1991). Türkiye yaprakbitleri (Homoptera: Aphididae): III. Aphidinae: Macrosiphini (III. Kısım). *Bitki Koruma Bülteni*, 31(1-4), 3-18.

Tuatay, N. (1993). Türkiye yaprakbitleri (Homoptera: Aphididae): IV. Aphidinae: Macrosiphini (I. Kısım). *Bitki Koruma Bülteni*, 33(3-4), 83-106.

Van Harten, A. (1975). Notes on a small collection of Aphids from continental portugal (Homoptera, Aphidoidea). *Agronomia Lusitana*, 36(3), 217-222.

Yücel C., Özdemir I., Ertürk S., Şahin, D.C. (2014). *Aspir (Carthamus tinctorius)'de ilk kayıt yaprakbiti (Hemiptera: Aphididae) türleri*. 22.Ulusal Biyoloji Kongresi, Eskişehir, s. 1165.

Zeren, O. (1989). *Çukurova Bölgesinde sebzelerde zararlı olan yaprakbitleri (Aphidoidea) türleri, konukçuları, zararları ve doğal düşmanları üzerinde araştırmalar. T*arım Orman ve Köyişleri Bakanlığı Araştırma Yayınları *Serisi* Yayın No.59, Ankara.

J. Entomol. Res. Soc., 24(1): 63-74, 2022
Doi: 10.51963/jers.v24i1.2136

Research Article
Online ISSN:2651-3579

New Records on the Ichneumonidae Fauna (Hymenoptera) of the Black Sea Coast of Turkey

Janko KOLAROV[1] Saliha ÇORUH[2]*

[1]Faculty of Pedagogy, University of Plovdiv, Plovdiv, BULGARIA
[2]Atatürk University, Faculty of Agriculture, Department of Plant Protection, 25240 Erzurum, TURKEY
e-mails: [1]j.kolarov@abv.bg, [2]*spekel@atauni.edu.tr
ORCID IDs: [1]0000-0002-0027-4162, [2]0000-0002-6822-6677

ABSTRACT

In this study, Ichneumonidae (Hymenoptera) species collected from Black Sea Coast (Rize) of Turkey are presented. In total 15 species belonging to the subfamilies Metopiinae, Ophioninae, Orthocentrinae, Oxytorinae, Pimplinae and Tryphoninae were determined. Of these, *Exochus alpinus* (Zetterstedt, 1838), *Cylloceria borealis* (Roman, 1925), *Orthocentrus patulus* Holmgren, 1858 and *Zatypota albicoxa* (Walker, 1874) are new records for the Turkish fauna. In addition, new localities for the other species are recorded. We discuss the geographical and zoogeographical records of all the species.

Key words: Darwin wasps, Hymenoptera, Ichneumonidae, new records, Rize, Turkey.

Kolarov, J., Çoruh, S. (2022). New records on the Ichneumonidae fauna (Hymenoptera) of the Black Sea Coast of Turkey. *Journal of the Entomological Research Society*, 24(1), 63-74.
Received: September 13, 2021 *Accepted: March 08, 2022*

INTRODUCTION

Hymenoptera is one of the four large insect orders exceeding 100,000 species in the world, the other major orders being Coleoptera, Lepidoptera and Diptera (Gauld & Bolton, 1988; Wahl & Micheal, 1993).

Ecologically and economically few groups of insects are so important to mankind as the Hymenoptera. Bees provide the vital ecosystem service of pollination in both natural and managed systems (Gallai, Salles, Settele, & Vaissiere, 2009) while parasitic Hymenoptera control populations of phytophagous insects (Tscharntke, Bommarco, Clough, Crist, & Kleijn, 2007) and can be effective agents for control of pest insects (Bale, van Lenteren, & Bigler, 2008; Brodeur & Boivin 2004; Jonsson, Wratten, Landis, & Gurr, 2008).

Ichneumonidae is the first megadiverse Apocrita family in the world with 25,285 species (Yu, Achterberg, & Horstmann, 2016). The family is divided into more than 40 subfamilies (Broud, Shaw, & Fitton, 2018). Consequently, the biology of ichneumonids is extremely diverse. Ichneumonids mostly parasitize the immature stages of the Holometabola, and are frequently associated with Lepidoptera and sawflies (Hymenoptera). Ectoparasitism is considered the primitive condition and endoparasitism has evolved several times independently within the family (Rasplus, Villemant, Paiva, Delvare, & Roques, 2010).

Studies on Ichneumonidae of Turkey have gained acceleration in recent years. Çoruh, Gürbüz, Kolarov, Yurtcan, & Özdan (2013), reported 975 species in 282 genera for Turkey Ichneumonidae fauna. Now, the number of Ichneumonidae species has reached 1316 with valuable studies: Yaman, 2014; Özdan, 2014; Çoruh, Kolarov, & Çoruh, 2014a; Çoruh, Kolarov, & Özbek, 2014b; Kolarov, Çoruh, & Çoruh, 2014a; Kolarov, Yıldırım, Çoruh & Yüksel, 2014b; Riedel, Kolarov, Çoruh & Çoruh, 2014; Kolarov, Çoruh ,& Çoruh, 2015; Kolarov, Çoruh, & Çoruh, 2016; Çoruh, 2017; Kolarov, Çoruh, & Çoruh, 2017; Narmanlıoğlu & Çoruh, 2017; Çoruh, Kolarov, & Çoruh, 2018; Riedel, Diller, & Çoruh, 2018; Sarı & Çoruh, 2018; Çoruh, 2019a, b; Vas, 2019a, b; Özdan & Gürbüz, 2019; Çoruh, Kolarov & Ercelep, 2019; Çaylak & Çoruh 2020a, b; Kolarov, Çoruh, & Çoruh, 2020; Kıraç & Gürbüz, 2020; Schwarz, 2020, Teymuroğlu, & Çoruh 2021, Kolarov, Çoruh & Ercelep, 2021, Yurtcan, Çoruh, Kolarov, Özdan, Gürbüz, & Erkaya, 2021; Çoruh, Kolarov, & Ercelep, 2022).

The purpose of this study is to identify the Ichneumonidae species collected from Black sea region, to make this data available to researchers and relevant people and to contribute the biodiversity.

MATERIAL AND METHODS

Data sampling

Adult ichneumonid samples were collected by sweeping net on flowering plants in six different localities and altitudes at above level (10-200 m) (Table 1) of Rize (Fig. 1, 2) in Turkey (Anatolia), during summer of 2016-2017. Collected specimens were preserved in 70 % ethanol. All examined material was collected by the second author and Halil Çoruh and determined by authors and deposited in the Entomology Museum Erzurum, Turkey (EMET). New record of species is marked by an asterisk (*). General distributions (Table. 2), hosts and associated plants of the species were taken from Yu et al, (2016).

Table 1. Data of collected species.

	Locality	**Year**	**Altitude (M.)**
Rize	Ardeşen	2016-2017	10
	Çayeli		10
	Derepazari		50
	Güneysu		150
	İyidere		210
	Pazar		37

Table 2. Zoogeographic distribution of collected species.

Zoogeographical Regions	Afrotropical	Eastern Palearctic	European Region	Nearctic	Neotropical	Oceanic	Oriental	Western Palearctic
Colpotrochia cincta		•	•					•
Exochus alpinus				•				
Exochus prosopius		•	•					•
Enicospilus ramidulus	•	•	•				•	•
Cylloceria borealis		•	•				•	•
Orthocentrus patulus			•					•
Oxytorus luridator			•					•
Clistopyga rufator		•	•					•
Pimpla aquilonia		•	•	•				•
Pimpla rufipes			•		•			•
Pimpla spuria		•	•				•	•
Scambus nigricans		•	•					•
Zatypota albicoxa		•	•				•	•
Ctenochira angulata		•	•					•
Oedemopsis scabricula		•	•	•			•	•

Fig. 1. Map of study area.

Fig 2. Photographs of study area.

Study area

Rize is a province located in the northeastern Turkey and has a coast to the Black Sea. Located in the Black Sea Region, Rize is surrounded by Trabzon in the west, Artvin in the east, Bayburt in the southwest, and Erzurum in the south (Anonymous, 2021).

In general, the land of Rize province are covered with a very diverse and very lush vegetation. Nearly 48% of Rize provincial lands are covered with forest and healts. Forest are found up to an altitude of 2800 m. 24% is covered with cultivated and plated areas.

RESULTS

During the investigation, we found 15 species belonging to Metopiinae, Ophioninae Ophioninae, Orthocentrinae, Oxytorinae, Pimplinae and Tryphoninae. The species list is given below.

Subfamily Metopiinae Förster,1869

Colpotrochia cincta **(Scopoli, 1763)**

Material examined: Rize: İyidere, 210 m, 23.07.2016, 2 ♀♀; 02.08.2017, 1 ♂.

Distribution: Palaearctic, known from Turkey (Kolarov, Çoruh, Yurtcan, & Gürbüz, 2009; Kolarov & Çalmaşur, 2011; Çoruh & Özbek, 2011; Çoruh, Kolarov, & Çoruh, 2014a; Çoruh, Kolarov, & Özbek, 2014b; Kolarov, Yıldırım, Çoruh, & Yüksel, 2014b).

Hosts: *Acronicta aceris*, *Acronicta rumicis*, *Aletia conigera*, *Axylia*, *Mythimna turca*, *Papestra biren.*

Associate plant: *Peucedanum oreoselinum.*

****Exochus alpinus* (Zetterstedt, 1838)**

Material examined: Rize: Ardeşen, 02.08.2017, 2 ♀♀; Güneysu, 150 m, 16.06.2016, 1 ♀.

Distribution: Palaearctic, new for Turkey.

Hosts: *Acleris rhombana*, *Apotomis sauciana*, *Archips xylosteana*, *Epinotia solandriana*, *Epinotia tetraquetrana*, *Hedya dimidiana*, *Phauda flammans*, *Triaxomera parasitella.*

***Exochus prosopius* Gravenhorst, 1829**

Material examined: Rize: İyidere, 210 m, 29.06.2016, 1 ♀; 23.07.2017, 2 ♂♂; 02.08.2017, 1 ♂.

Distribution: Palaearctic, known from Turkey (Kolarov, Çoruh, Yurtcan & Gürbüz, 2009; Çoruh et al, 2014b; Kolarov, Çoruh, & Çoruh 2017).

Subfamily Ophioninae Shuckard, 1840

***Enicospilus ramidulus* (Linnaeus, 1758)**

Material examined: Rize: Güneysu, 150 m, 05.07.2017, 1 ♀; İyidere, 210 m, 09.06.2017, 2 ♂♂ 5♀♀.

Distribution: Afrotropical, Palaearctic, Oriental, known from Turkey (Kolarov, 1995; Kolarov, Pekel, Özbek, Yıldırım, & Çalmaşur, 2000; Gürbüz, 2004, 2005; Beyarslan, Yurtcan, Erdoğan, & Aydoğdu, 2006; Okyar & Yurtcan, 2007; Çoruh & Çoruh, 2008, Gürbüz, Kırtay & Birol, 2009; Kıraç & Gürbüz, 2020; Çoruh et al, 2014b; Kolarov, Çoruh & Çoruh, 2014a; Çoruh & Çalmaşur, 2016; Kolarov et al, 2017; Kolarov et al, 2020).

Hosts: *Agrotis segetum*, *Anarta myrtilli*, *Axylia putris [Nicotiana tabacum]*, *Catocala electa*, *Cucullia absinthii*, *Dendrolimus pini*, *Dypterygia scabriuscula*, *Eriogaster lanestris*, *Hadena bicruris*, *Hadena rivularis*, *Hyles euphorbiae*, *Hyphantria cunea [Corylus avellana]*, *Lacanobia oleracea*, *L. pisi*, *Melanchra persicariae*, *Nematus salicis*, *Ochropleura praecox*, *Panolis flammea*, *Phalera bucephala*, *Phlogophora meticulosa*, *Poecilocampa populi*.

Associate plants: *Alnus glutinosa*, *Carum carvi*, *Oryza sativa*, *Salvia glutinosa*, *Seseli libanotis*.

Subfamily Orthocentrinae Förster, 1869

****Cylloceria borealis* (Roman, 1925)**

Material examined: Rize: İyidere, 210 m, 09.06.2017, 1 ♂.

Distribution: Holarctic, new for Turkey.

****Orthocentrus patulus* Holmgren, 1858**

Material examined: Rize: Pazar, 37 m, 31.08.2017, 1 ♀.

Distribution: Europe, new for Turkey.

Subfamily Oxytorinae Thomson, 1883

Oxytorus luridator **(Gravenhorst, 1820)**

Material examined: Rize: Pazar, 37 m, 31.08.2017, 1 ♀.

Distribution: Europe, known for Turkey (Kolarov, Çoruh & Çoruh, 2015; Kolarov, Çoruh & Çoruh, 2016).

Associate plants: *Sparganium* sp.

Subfamily Pimplinae

Clistopyga rufator **Holmgren, 1856**

Material examined: Rize: Çayeli, 10 m, 16.06.2017, 1 ♀.

Distribution: Palaearctic, known from Turkey (Yurtcan, 2004, 2007; Çoruh, 2005; Çoruh, Özbek, Kolarov, 2007; Buncukçu, 2008; Çoruh & Özbek, 2008; Gürbüz, Ljubomirov, Kolarov, Yurtcan, Tabur, Çoruh & Buncukçu, 2008; Gürbüz et al, 2009; Çoruh, 2009, 2010; Birol, 2010; Çoruh & Kolarov, 2010; Çoruh, Çalmaşur & Tozlu, 2010; Özdan, 2014; Çoruh et al, 2014b; Çoruh, 2016; et al, 2021).

Host: *Clubiona juvenis.*

Pimpla aquilonia **Cresson, 1870**

Material examined: Rize: İyidere, 210 m, 09.06.2017,1 ♂; Pazar, 37 m, 11.07.2017, 1 ♂.

Distribution: Holarctic, known from Turkey (Yurtcan & Beyarslan, 2005, Çoruh & Kolarov, 2010; Çoruh et al, 2014a; Çoruh, 2016)

Hosts: *Archips fuscocupreanus*, *Argiope argentata*, *Choristoneura diversana*, *Coleophora serratella*, *Croesia semipurpurana*, *Cymolomia hartigiana*, *Dendrolimus albolineatus*, *Epinotia nigricana*, *Grapholita molesta*, *Lambdina fiscellaria [Abies balsamea]*, *Lambdina fiscellaria lugubrosa*, *Magdalis frontalis*, *Orgyia leucostigma*, *Orthosia hibisci*, *Parasteatoda tepidariorum*, *Psilocorsis cryptolechiella*, *Rheumaptera hastata*, *Spilonota ocellana*, *Teleiodes saltuum*, *Tortrix viridana*, *Zeiraphera griseana*,

Associate plants: *Listera ovata*.

Pimpla rufipes **Brullé, 1846**

Material examined: Rize: İyidere, 210 m, 23.06.2017, 1 ♀.

Distribution: Palaearctic, Neotropical, known from Turkey (Çoruh, 2005, 2009; Okyar & Yurtcan, 2007; Çoruh & Özbek, 2008; Kolarov & Çalmaşur, 2011; Özbek & Çoruh, 2012, Çoruh, 2016).

Hosts: *Hyphantria cunea*, *Malacosoma neustria*, *Plusia* sp.

Remarks: This species obtained from *Malacosoma neustria* and *M. franconica, Yponomeuta malinella* in our previous study (Çoruh & Özbek, 2008).

***Pimpla spuria* Gravenhorst, 1829**

Material examined: Rize: Derepazarı, 50 m, 23.06.2016, 1 ♂; Pazar, 37 m, 04.07.2017,1 ♂.

Distribution: Palaearctic, Oriental, known for Turkey (Fahringer, 1922; Özdemir & Kılınçer, 1990; Öncüer, 1991; Kolarov & Beyarslan, 1994; Kolarov, Yurtcan, & Beyarslan, 1997a; Kolarov, Beyarslan & Yurtcan, 1997 b; Kolarov, Özbek, & Yıldırım, 1999; Kolarov, Yurtcan & Beyarslan, 2002; Gürbüz, 2005; Kolarov & Gürbüz, 2004, Çoruh, 2005; Gürbüz, 2005; Yurtcan & Beyarslan, 2005; Kırtay, 2008; Çoruh, Çalmaşur, & Tozlu, 2010; Çoruh & Kolarov, 2010; Eroğlu, Kıraç & Birol, 2011; Kolarov et al, 2014b; Çoruh et al, 2014a; Çoruh, 2016; Kolarov et al, 2016; Sarı & Çoruh, 2018; Kıraç & Gürbüz, 2020).

Hosts: *Agonopterix heracliana*, *Agonopterix propinquella*, *Archips rosana*, *Calliteara pudibunda*, *Canephora hirsuta*, *Cydia funebrana*, *Cydia pomonella*, *Cynaeda dentalis*, *Depressaria pastinacella*, *Enarmonia formosana*, *Etiella zinckenella*, *Eupithecia vulgata*, *Eupoecilia ambiguella*, *Gypsonoma aceriana*, *Hyphantria cunea*, *Larentia clavaria*, *Lobesia botrana*, *Loxostege sticticalis*, *Lymantria dispar*, *Naranga aenescens*, *Ostrinia nubilalis*, *Pempelia genistella*, *Psoricoptera gibbosella*, *Sesamia nonagrioides*, *Tebenna bjerkandrella*, *Yponomeuta malinella*, *Yponomeuta padella.*

Associated plants: *Acer campestre*, *Anethum graveolens*, *Chaerophyllum bulbosum*, *Daucus carota*, *Daucus carota sativus*, *Euphorbia nicaeensis*, *Heracleum sphondylium*,*Tamarix* sp.

Remarks: This species obtained from *Malacosoma neustria* and *Yponomeuta malinella* in our previous study (Çoruh & Özbek, 2008).

***Scambus nigricans* Thomson, 1877**

Material examined: Rize: İyidere, 210 m, 05.08.2017, 1 ♀; Pazar, 37 m, 23.07.2017, 1 ♂, 01.08.2017, 1♂.

Distribution: Palaearctic, known from Turkey (Kolarov & Beyarslan, 1994; Kolarov et al, 1997a; Kolarov et al, 1999; Kolarov et al, 2002; Kolarov & Gürbüz, 2004; Çoruh, 2005; Yurtcan, 2007; Çoruh et al, 2007, Çoruh & Kolarov, 2010; Çoruh et al, 2010; Kolarov & Çalmaşur, 2011; Özbek & Çoruh, 2012; Çoruh et al, 2014b; Çoruh, 2016; Kolarov et al, 2017; Kolarov et al, 2020; Yurtcan et al, 2021).

Hosts: *Adaina microdactyla*, *Aethes francillana, Aethes williana, Agonopterix heracliana, Alsophila aescularia, Apocheima hispidaria,Cnephasia stephensiana, Cochylis atricapitana, Cydia delineana, Cydia discretana, Cydia pactolana, Depressaria daucella, Depressaria pastinacella, Dioryctria simplicella, Epiblema cirsiana, Epiblema scutulana, Gortyna flavago, Hartigia linearis, Heptamelus ochroleucus, Lampronia morosa, Lipara lucens, Lycaena dispar batava, Lycia pomonaria, Malacosoma neustria, Mompha conturbatella, Myelois circumvoluta, Noeeta pupillata, Oxyna parietina*, *Phigalia pilosaria, Platyptilia nemoralis, Pseudaletia unipuncta, Rhyacionia buoliana, Scrobipalpa clintoni,Triedris paleana*.

Associated plants: *Anethum graveolens*, *Chaerophyllum bulbosum*, *Daucus carota*, *Euphorbia nicaeensis*, *Heracleum sphondylium*, *Peucedanum oreoselinum*.

Remark: This species was collected on *Heracleum pastinacifolium* in our previous study (Kolarov et al, 2020).

***Zatypota albicoxa* (Walker, 1874)**

Material examined: Rize: Pazar, 37 m, 19.07.2017, 2 ♀♀.

Distribution: Palaearctic, Oriental, new for Turkey.

Hosts: *Parasteatoda lunata*, *Parasteatoda merapiensis*, *Parasteatoda oculiprominens*, *Parasteatoda simulans*, *Parasteatoda tabulata*, *Parasteatoda tepidariorum*, *Theridion limatum.*

Associate plants: *Oryza sativa*.

Subfamily Tryphoninae Shuckard, 1840

***Ctenochira angulata* (Thomson, 1883)**

Material examined: Rize: Pazar, 37 m, 27.08.2017, 1 ♂.

Distribution: Palaearctic, known from Turkey (Yurtcan & Beyarslan, 2002; Yaman, 2014; Kolarov et al, 2016; Çoruh, 2019).

***Oedemopsis scabricula* (Gravenhorst, 1829)**

Material examined: Rize: Ardeşen, 10 m, 05.08.2017, 3 ♀♀.

Distribution: Holarctic, Oriental, known from Turkey (Çoruh, Özbek, Kolarov, 2005; Beyarslan, Erdoğan, Çetin, & Aydoğdu, 2006; *Çoruh et al*, 2014a; 2014b; Yaman, 2014; Çoruh, 2019, Kolarov et al, 2017).

Hosts: *Acleris variegana*, *Aleimma loeflingiana*, *Ancylis apicella*, *Archips oporana*, *Archips rosana*, *Blastobasis lignea*, *Cacoecimorpha pronubana*, *Choristoneura rosaceana*, *Cladius difformis*, *Clepsis costana*, *Croesia holmiana*, *Ditula angustiorana*, *Epinotia albangulana*, *Epinotia solandriana*, *Epiphyas postvittana*, *Eudemis profundana*, *Euproctis similis*, *Pandemis cerasana*, *Pandemis heparana*, *Ptycholoma lecheana*, *Rhyacionia buoliana*, *Selania leplastriana*, *Spilonota ocellana*, *Tortrix viridana*, *Yponomeuta cagnagella*.

Zoogeograpgical notes

Enicospilus ramidulus is the species with the multiregional ranges is the species *Enicospilus ramidulus*, distributed in Afrotropical, Palaearctic and Oriental zoogeographical regions.

Three species have distributional ranges in two zoogeographical regions of them: *Pimpla rufipes* distributed in Western Palaearctic and Neotropical regions; *Pimpla spuria* and *Zatypota albicoxa* distributed in Palaearctic and Oriental regions.

Two species, *Cylloceria borealis* and *Pimpla aquilonia* have Holarctic distribution. Most of the species have Palaearctic distribution. Of them, *Colpotrochia cincta*, *Exochus alpinus*, *E. prosopius*, *Clistopyga rufator*, *Scambus nigricans* and *Ctenochira angulata*.

European distribution ranges have 2 species - *Orthocentrus patulus* and *Oxytorus luridator*.

REFERENCES

Anonymous, 2021(September, 1), *Rize.* Retrieved from https://tr.wikipedia.org/wiki/Rize_(il)

Bale, J. S., Van Lenteren, J. C. & Bigler, F. (2008). Biological control and sustainable food production. *Philosophical Transactions of the Royal Society B-Biological Sciences*, 363, 761-776.

Beyarslan, A., Yurtcan, M., Erdoğan, Ö.Ç., & Aydoğdu, M. (2006). A study on Braconidae and Ichneumonidae from Ganos Mountains (Thrace Region, Turkey) (Hymenoptera, Braconidae, Ichneumonidae). *Linzer Biologische Beitrage*, 38(1), 409-422.

Birol, O. (2010). *Isparta İli Davraz Dağı Ichneumonidae (Hymenoptera) Faunası Üzerine Bir Araştırma.* Süleyman Demirel Üniversitesi, Fen Bilimleri Enstitüsü, Isparta, 71 s.

Buncukçu, A., 2008, *Isparta İli Merkez ve Adana, Yumurtalık İlçesi-Halep Çamlığı Ichneumonidae Türlerinin Tespiti ve Kültüre Edilebilen Türlerin Biyolojilerinin Araştırılması*. Süleyman Demirel Üniversitesi, Fen Bilimleri Enstitüsü, Isparta, 74 s.

Brodeur, J. & Boivin, G. (2004). Functional ecology of immature parasitoids. *Annual Review of Entomology*, 49, 27-49.

Broud, G., Shaw, M., & Fitton, M. (2018). Ichneumonid Wasps (Hymenoptera: Ichneumonidae): their Classification and Biology. *Handbooks for the Identification of Brithish Insects*, 7(12), 1-418.

Çaylak, F.Z. & Çoruh, S. (2020a). First record of *Woldstedtius citropectoralis* Schmiedeknecht, 1926 (Hymenoptera: Ichneumonıdae: Diplazontinae) from Turkey. *Munis Entomology & Zoology*, 15(2), 457-462.

Çaylak, F.Z. & Çoruh, S. (2020b). Contribution to the knowledge of Ichneumonidae (Hymenoptera) of Bursa Uludağ National Park area including new records. *Turkish Journal of Entomology*, 43(4), 503-517.

Çoruh, S. (2005). Erzurum ve Çevre İllerdeki Pimplinae (Hymenoptera, Ichneumonidae) Türleri Üzerinde Faunistik, Sistematik ve Ekolojik Çalışmalar. Atatürk Üniversitesi, Fen Bilimleri Enstitüsü, Erzurum, 212 s.

Çoruh, S. (2009). Two little-known Ichneumonidae (Hymenoptera) from Turkey, including one new for the Turkish fauna. *Zoology in the Middle East*, 48: 106-107.

Çoruh, S. (2010). Composition, habitat distribution and seasonal activity of Pimplinae (Hymenoptera: Ichneumonidae) in North-East Anatolia region of Turkey. *Anadolu Tarım Bilimleri Dergisi*, 25(1): 28-36.

Çoruh, S. (2016). Biogeography and Host Evaluation of the Subfamily Pimplinae (Hymenoptera: Ichneumonidae) in Turkey. *Journal of the Entomological Research Society*, 18(2), 33-66.

Çoruh, S. (2017). Taxonomical and biogeographical evaluation of the subfamily Ichneumoninae (Hymenoptera: Ichneumonidae) in Turkey. *Entomofauna*, 38(21), 425-476.

Çoruh, S. (2019a).Taxonomic and biogeographic evaluations of the subfamily Cryptinae (Hymenoptera: Ichneumonidae). *Turkish Journal of Entomology*, 43(3), 313-337.

*Çoruh, S. (2019b).*Taxonomical and biogeographical evaluation of the subfamily Tryphoninae (Hymenoptera: Ichneumonidae) in Turkey. *Journal of the Entomological Research Society*, 21(3), 301-321.

Çoruh, İ. & Çoruh, S. (2008). Ichneumonidae (Hymenoptera) species associated with some Umbelliferae plants occuring in Palandöken Mountains of Erzurum, Turkey. *Turkish Journal of Zoology*, 32(2), 121-124.

Çoruh, S. & Özbek, H. (2008). New and rare Ichneumonidae (Hymenoptera) species from Turkey. *Zoology in the Middle East*, 43, 114-116.

Çoruh, S. & Kolarov, J. (2010). A review of the Turkish Orthopelmatinae (Insecta: Hymenoptera: Ichneumonidae). *Scientific Research and Essays*, 5(22), 3518-3521.

Çoruh, S. and Özbek, H. (2011). New and little known some Ichneumonidae (Hymenoptera) species from Turkey with some ecological notes. *Turkish Journal of Entomology*, 35(1), 119-131.

Çoruh, S. & Çalmaşur, Ö. (2016). A new and additional records of the Ichneumonidae (Hymenoptera) from Turkey. *Turkish Journal of Zoology*, 40(4), 625-629.

Çoruh, S., Özbek, H. & Kolarov, J. (2005). A contribution to the knowledge of Tryphoninae (Hymenoptera: Ichneumonidae) from Turkey. *Zoology in the Middle East*, 35: 93-98.

Çoruh, S., Özbek, H., & Kolarov, J. (2007). Aras Vadisi (Kars)'ne yerleştirilen malezya tuzağından elde edilen Ichneumonidae (Hymenoptera) türleri. Türkiye II. Bitki Koruma Kongresi Bildirileri, 27-29 Ağustos 2007, Isparta, Türkiye, 209.

Çoruh, S., Çalmaşur, Ö., & Tozlu, G. (2010). Artvin (Yusufeli)'in Tenthredinidae ve Ichneumonidae (Hymenoptera) türleri. Geçmişten Geleceğe Yusufeli Sempozyumu Bildirileri, 9-11 Haziran 2010, Yusufeli, Artvin, Türkiye, 317-321.

Çoruh, S., Gürbüz, M.F., Kolarov, J., Yurtcan, M., & Boncukçu Özdan, A. (2013). New and Little Known Species of Ichneumonidae (Hymenoptera) for the Turkish Fauna. *Journal of the Entomological Research Society*, 15(3), 71-83.

Çoruh, S., Kolarov, J., & Çoruh, İ. (2014a). Ichneumonidae (Hymenoptera) from Anatolia. II. *Turkish Journal of Entomology*, 38, 279-290.

Çoruh, S., Kolarov, J., & Özbek, H. (2014b). The fauna of Ichneumonidae (Hymenoptera) of eastern Turkey with zoogeographical remarks and host data. *Journal of Insect Biodiversity*, 2(16), 1-21.

Çoruh, S., Kolarov, J., & Çoruh, İ. (2018). Ichneumonidae (Hymenoptera) from Anatolia II. *Linzer Biologishce Beitrage*, 50(1), 217-224.

Çoruh, S., Kolarov, J., & Ercelep, Ö. S. (2019). A contribution to the Ichneumoninae (Hymenoptera: Ichneumonidae) of Trabzon, Turkey. *Munis & Entomology Zoology*, 14(2), 584-590.

Çoruh, S., Kolarov, J., & Ercelep, Ö. S. (2022). A Contribution to the Ichneumonidae (Hymenoptera) of Trabzon, III. Cryptinae. *Atatürk University, Journal of Agricultural Faculty*, 53(1), 8-13.

Eroğlu, F., Kıraç, A. & Birol, O. (2011). A Faunistic study on Ichneumonidae (Hymenoptera) in Türkmen Mountain, Turkey. *Linzer Biologische Beitrage*, 43(2), 1219-1228.

Fahringer, J. (1922). Hymenopterologische Ergebnisse einer wissenschaftlichen Studienreise nach der Türkei und Kleinasien (mit Ausschluß des Amanusgebirges). *Archiv für Naturgeschichte*, A(88), 149-222.

Gallai, N., Salles, J. M., Settele, J., & Vaissiere, B. E. (2009). Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological Economics*, 68, 810-821.

Gauld, I. D. & Bolton, B. (1988). *The Hymenoptera*. Oxford University Press/British Museum National History. Oxford University Press, Oxford, New York & Toronto. 332 pp.

Gürbüz, M. F. (2004). *Isparta İli Ichneumonidae (Hymenoptera) Familyası Türleri Üzerine Faunistik ve Sistematik Çalışmalar*. Süleyman Demirel Üniversitesi, Fen Bilimleri Enstitüsü, Isparta, 68 s.

Gürbüz, M. F. (2005). A survey of the Ichneumonidae (Hymenoptera) of Isparta in Turkey. *Linzer Biologische Beiträge*, 39(2), 1809-1912.

Gürbüz, M.F., Kırtay, H., & Birol, O. (2009). A study of Ichneumonidae (Hymenoptera) of Kasnak Oak Forest Nature Reserve in Turkey with new records. *Linzer Biologische Beiträge*, 41(2), 1985-2003.

Gürbüz, M.F., Ljubomirov, T., Kolarov, J., Yurtcan, M., Tabur, M.A., Çoruh, S., & Buncukçu, A. (2008). Investigation of the Ichneumonidae, Ampulicidae, Crabronidae and Sphecidae (Hymenoptera, Insect) Fauna in Natural Protection Zones of East Mediteranean Region in Turkey. *Tübitak TBAGU/ 168 (106T189) No'lu Proje,* 127 s.

Jonsson, M., Wratten, S.D., Landis, D.A., & Gurr, G.M. (2008). Recent advances in conservation biological control of arthropods by arthropods. *Biological Control*, 45, 172-175.

Kırtay, H. (2008). An Invastigation on Ichneumonidae (Hymenoptera) Fauna ın Kasnak Oak (*Quercus vulcanica* Boiss. and Heldr. ex Kotschy) Forest Nature Protect Area, Isparta. Süleyman Demirel Üniversity, Institute of Science, Isparta, Türkiye, 77 s.

Kıraç, A. & Gürbüz, M. F. (2020). Honaz Dağı Milli Parkı Ichneumonidae (Insecta, Hymenoptera) Faunası. *Bilge International Journal of Science and Technology and Research*, 4(2), 150-159.

Kolarov, J. & Beyarslan, A. (1994). Investigations on the Ichneumonidae (Hym.) Fauna of Turkey. 1. Pimplinae and Tryphoninae. *Turkish Journal of Entomology*, 18(3),133-140.

Kolarov, J. (1995). A cataloque of the Turkish Ichneumonidae (Hymenoptera). *Zeitschrift für Entomologie*, 7, 137-188.

Kolarov, J. & Gürbüz, M F. (2004). A study of the Turkish Ichneumonidae (Hymenoptera) I. Pimplinae. *Linzer Biologische Beiträge*, 36(2), 841-845.

Kolarov, J. & Çalmaşur, Ö. (2011). A study of Ichneumonidae (Hymenoptera) from North Eastern Turkey. *Linzer Biologische Beiträge*, 43(1), 777-782.

Kolarov, J., Yurtcan, M. & Beyarslan, A. (1997a). New and rare Ichneumonidae (Hym.) from Turkey. 1. Pimplinae, Tryphoninae, Phygadeuontinae, Banchinae and Ctenopelmatinae. *Acta Entomologica Bulgarica*, 3(3/4), 10-12.

Kolarov, J., Beyarslan, A., & Yurtcan, M. (1997b). Ichneumonidae (Hym.) from the Gökçeada and Bozcaada islands-Turkey. *Acta Entomologica Bulgarica*, 3(3/4), 13-15.

Kolarov, J., Özbek, H. & Yıldırım, E. (1999). New distributional data of the Turkish Ichneumonidae (Hymenoptera). I. Pimplinae and Tryphoninae. *Journal of the Entomological Research Society*, 1(2), 9-15.

Kolarov, J., Yurtcan, M., & Beyarslan, A. (2002). Ichneumonidae Species of the Turkish Aegean Region. Parasitic Wasps: Evolution, Systematics. *Biodiversity and Biological Control*, 299- 305.

Kolarov, J., Çoruh, S., & Çoruh, İ. (2014a). Ichneumonidae (Hymenoptera) from Anatolia. III. *Turkish Journal of Entomology*, 38, 377-388.

Kolarov, J., Çoruh, S., & Çoruh, İ. (2015). Oxytorinae, a new subfamily for the Turkish fauna (Hymenoptera: Ichneumonidae). *Turkish Journal of Zoology*, 39, 832-835.

Kolarov, J., Çoruh, S., & Çoruh, İ. (2016). Contribution to the knowledge of the Ichneumonidae (Hymenoptera) fauna of Turkey from northeastern Anatolia, Part I. *Turkish Journal of Zoology*, 40(1), 40-56.

Kolarov, J., Çoruh, S., Çoruh, İ. (2017). A study of Ichneumonidae (Hymenoptera) from Northeastern Anatolia III, with new records and description male of *Temelucha pseudocaudata* Kolarov, 1982. *Turkish Journal of Entomology*, 41(2): 125-146.

Kolarov, J., Çoruh, S., & Çoruh, İ. (2020). Ichneumonidae (Hymenoptera) from Anatolia III. *Atatürk University Journal of Agricultural Faculty*, 5(2), 162-168.

Kolarov, J., Çoruh, S., & Ercelep, Ö. S. (2021). A contribution to the Ichneumonidae (Hymenoptera) of Trabzon, Turkey. II. Campopleginae. *Munis Entomology & Zoology*, 16(2), 745-750.

Kolarov, J., Çoruh, S., Yurtcan, M., & Gürbüz, M. F. (2009). A study of Metopiinae from Turkey with description of a new species (Hymenoptera: Ichneumonidae). *Zoology in the Middle East*, 46, 75-82.

Kolarov, K., Yıldırım, E., Çoruh, S., & Yüksel, M. (2014b). Contribution to the knowledge of the Ichneumonidae (Hymenoptera) fauna of Turkey, *Zoology in the Middle East*, 60(2),154-161.

Kolarov, J., Pekel, S., Özbek, H., Yıldırım, E., & Çalmaşur, Ö. (2000). New distributional data of Turkish Ichneumonidae (Hymenoptera). III. The subfamily Ophioninae. Türkiye IV Entomoloji Kongresi, 12-15 Eylül 2000, Kuşadası-Aydın, Türkiye, 349-356.

Narmanlıoğlu, H.K. & Coruh, S. (2017). Parasitoids of the apple ermine moth, *Yponomeuta malinellus* Zeller, 1838 (Lepidoptera: Yponomeutidae), in the Çoruh Valley, Erzurum Province, Turkey. *Turkish Journal of Entomology*, 41(4), 357-365.

Okyar, Z. & Yurtcan, M. (2007). Phytophagous Noctuidae (Lepidoptera) of the Western Black Sea Region and their ichneumonid parasitoids. *Entomofauna*, 28, 377-388.

Öncüer, C. (1991). *Türkiye Bitki Zararlısı Böceklerinin Parazit ve Predatör Kataloğu*. Ege Üniversitesi, Ziraat Fakültesi Yayınları, 505, 354.

Özdemir, Y. & Kılınçer, H. (1990). The species of Pimplinae and Ophioninae from Central Anatolia. Proceedngs of the Second Turkish National Congress of Biological Control, 1990, Ankara, Turkey, 309-318.

Özbek, H. & Çoruh, S. (2012). Larval parasitoids and larval diseases of *Malacosoma neustria* L. (Lepidoptera: Lasiocampidae) detected in Erzurum Province, Turkey. *Turkish Journal of Zoology*, 36(4), 447-459.

Özdan, A. (2014). *Gelincik Dağı Tabiat Parkı ve Kovada Gölü Milli Parkı (Isparta) Ichneumonidae (Hymenoptera) Faunası.* Süleyman Demirel Üniversitesi, Fen Bilimleri Enstitüsü, Isparta, 150 s.

Özdan, A., Gürbüz, M.F. (2019). Ichneumonidae (Hymenoptera) fauna of Kovada Lake National Park, Isparta, Turkey. *Turkish Journal of Entomology*, 43(3): 301-312.

Rasplus, J-Y., Villemant, C., Paiva, M.R., Delvare, G., Roques, A. (2010). Hymenoptera. Chapter 12. In Roques A et al (Eds.). *Arthropod invasions in Europe* (page range). *BioRisk*, 4(2)**,** 669-776.

Riedel, M., Diller E., & Çoruh, S. (2018). New Contributions to the Ichneumoninae (Hymenoptera, Ichneumonidae) from Turkey. *Journal of the Entomological Research Society*, 20(1): 57-70.

Riedel, M., Kolarov, J., Çoruh, S. & Özbek, H. (2014). A contribution to the Mesochorinae (Hymenoptera: Ichneumonidae) of Turkey. *Zoology in the Middle East*, 60, 217-221.

Sarı, Ü. & Çoruh, S. (2018). Ichneumonidae (Hymenoptera) from Northeastern Anatolia Region (Erzurum, Aşkale). *Turkish Journal of Entomology*, 42(3): 215-228.

Schwarz, M. (2020). Zer Kenntnis der palaarktischen *Meringopus* -Arten (hymenoptera, Ichneumonidae, Cryptinae). *Linzer Biologishe Beitrage*, 52(1): 583-682.

Teymuroğlu, E. & Çoruh, S. (2021). Ichneumonidae (Hymenoptera) species associated with sugar beet plants occurıng in Erzincan (Çayırlı) of Turkey. *Munis Entomology & Zoology*, 16(2), 985-990.

Tscharntke, T., Bommarco, R., Clough, Y., Crist, T.O., & Kleijn, D. (2007). Conservation biologi-cal control and enemy diversity on a landscape scale. *Biological Control*, 43, 294-309.

Wahl, D.B. & Micheal, J.S. (1993). Family Ichneumonidae. In Goulet H., Huber J. (Eds.), *The Hymenoptera of the world, an identification guide to families* (pp. 395-442) , Canada Agriculture, Canada.

Vas, Z. (2019a). Contributions to the taxonomy, identification, and biogeography of the Western Palaearctic species of *Campoletis* Förster (Ichneumonidae: Campopleginae). *Zootaxa*, *4565*(3), 373-382**.**

Vas, Z. (2019b). New species and new records of Ichneumon wasps from the Eastern Mediterranean and the Black Sea Regions (Hymenoptera: Ichneumonidae). *Acta Zoologica Academiae Scentiarum Hungaricae*, 65(1),19-30.

Yaman, G. (2014). Türkiye Tryphoninae (Hymenoptera: Ichneumonıdae) türlerinin kontrol listesi. Trakya University, Fen Bilimleri Enstitüsü, Edirne, 88 s.

Yu, D. S. Ki, Achterberg, C. Van, & Horstmann, K. (2016). Taxapad 2016, Ichneumonoidea 2015. Database on flash-drive. www.taxapad.com, Nepean, Ontario, Canada.

Yurtcan, M. (2004). Trakya Bölgesi Pimplinae (Hymenoptera: Ichneumonidae) Faunasının Taksonomik ve Faunistik Yönden Araştırılması, Trakya Üniversitesi Fen Bilimleri Enstitüsü, Edirne,

Yurtcan, M. (2007). Ephialtini tribe (Hymenoptera, Ichneumonidae, Pimplinae) of Turkish Thrace region, Entomofauna, 28, 389-404.

Yurtcan, M. & Beyarslan, A. (2002). The species of Tryphoninae (Hymenoptera: Ichneumonidae) in Turkish Thrace. *Turkish Journal of Zoology*, 26(1), 77-95.

Yurtcan, M. & Beyarslan, B. (2005). Polysphinctini and Pimplini (Hymenoptera: Ichneumonidae: Pimplinae) from the thrace Region of Turkey. *Fragmenta Faunistica*, 48(1), 63-72.

Yurtcan, M., Çoruh, S., Kolarov, J., Özdan, A.B., Gürbüz, M.F. & Erkaya, İ. (2021). Ichneumonidae (Hymenoptera) fauna of natural protectıon areas in the east mediteranean region of Turkey, Part II. *Entomological News*, 129(5), 453-472.

J. Entomol. Res. Soc., 24(1): 75-87, 2022 *Research Article*
Doi: 10.51963/jers.v24i1.2097 *Online ISSN:2651-3579*

Effects of Selected Plant Secondary Metabolites in Mulberry, Apple, Plum, and Walnut on the Pupal Parameters of *Hyphantria cunea* Drury, 1773 (Lepidoptera: Arctiidae) Larvae Infected by *Bacillus thuringiensis* subsp*. kurstaki*

Elif Fatma TOPKARA

Department of Biology, Science and Art Faculty, Ondokuz Mayıs University, 55139, Samsun, TURKEY
e-mail: topkaraelif@hotmail.com, ORCID ID: 0000-0002-4743-2914

ABSTRACT

In this study, the effects of various secondary metabolites in the economically important plants on the pupal weight, the pupal total protein and the pupal total lipid contents of *Hyphantria cunea* larvae infected by *Bacillus thuringiensis* subsp. *kurstaki* were investigated. In order to find out their effects on the larvae, the phenolic compounds present in *Morus alba* (mulberry), *Malus pumila* (apple), *Prunus dometica* (plum), and *Juglans regia* (walnut) leaves samples, which are the most preferred by *H. cunea* and have economic importance, were determined by phytochemical methods. The changes observed in the pupae's weights, total protein and lipid contents were related to the plant leaves' chemical contents. Among the non-infected groups, the mulberry-fed group had the highest both pupal weight and the pupal protein content. The minimum amount of gallotannin, as well as the catechin and rutin contents, was present in the mulberry leaves. The minimum pupal weight and the pupal protein contents were obtained in the larvae fed by the apple leaves with the highest rosmarinic acid and protocatechuic acid. The pupal weights and the pupal total protein contents were decreased by the bacterial infection, while the pupal total lipid contents increased by the bacterial infection. As a result of this study, the effects of both plant secondary metabolites experimentally used and *B. thuringiensis* infection on the pupal parameters of *H. cunea* were shown to be statistically significant.

Key words: *Bacillus thuringiensis*, gallotannin, *Hyphantria cunea*, phenolic compound, pupal weight.

Topkara, E.F. (2022). Effects of selected plant secondary metabolites in mulberry, apple, plum, and walnut on the pupal parameters of *Hyphantria cunea* Drury, 1773 (Lepidoptera: Arctiidae) larvae infected by *Bacillus thuringiensis* subsp*. kurstaki*. *Journal of the Entomological Research Society*, 24(1), 75-87.
Received: July 08, 2021 *Accepted: January 04, 2022*

INTRODUCTION

Insects dominate more than half of the known living organisms in the world in terms of their number of species. Herbivore insects, which obtain their nutrients through various plant parts, and make up the greatest part of the total biomass in the world (Tek & Okyar, 2017). Among these, the herbivorous lepidopteran larvae consume large amounts of plant material throughout their development from the first instar to the last instar (Esperk & Tammaru, 2004; Gotthard, 2004). To prevent their attacks, plants defend themselves against herbivores through various physical and chemical defenses (Chen, Kim, Klinkhamer, & Escobar-Bravo, 2020). While physical defenses such as the leaf hardness and the trichomes adversely affect the performance and preference of herbivores (Chen et al, 2020); however, the chemical defenses involve various plant secondary metabolites (PSMs) biochemically generated in plants (Mazid, Khan, & Mohammad, 2011; Rosa, Woestmann, Biere, & Saastamoinen, 2018). PSMs are widely distributed in many plant taxa (e.g. phenolic acids, flavonoids). The phenolic compounds have a great importance to defend plants parts against herbivores and microbial attacks. PSMs function by interfering with the basic metabolic, biochemical, physiological functions of the cells, and even behavior of herbivorous insects (Kessler & Baldwin, 2002; Tan & Luo, 2011; Tangtrakulwanich & Reddy, 2014). In studies, chlorogenic acid (Kundu & Vadassery, 2019), rosmarinic acid (Khan et al, 2019), rutin (Silva et al, 2016), protocatechuic acid (Syafni, Putra, & Arbain, 2012), benzoic acid (Beran, Kollner, Gershenzon, & Tholl, 2019), and tannic acid (Ma et al., 2019) have been shown to biological activities against herbivores.

The autumn webworm, *Hyphantria cunea* Drury (Lepidoptera: Arctiidae), being a polyphagous species that feed on a vast number of plant species and is a serious invader in a wide variety of habitats. This insect larvae causes significant damage to both forests and orchard trees in urban areas throughout its range (Liao et al, 2010). As in many countries, this pest causes significant damage to the crop in Turkey too. Control measures are required especially to prevent economic damages to orchards and ornamental trees. In our study, *Bacillus thuringiensis* (*Bt*), the most commonly used, the most cost-effective and containing many spore-crystal toxins in its formulation, has been used in biological control of *H. cunea* larvae (Weinzierl, Henn, Koehler, & Tucker, 2005).

Plants contain PSMs with the complex chemical composition (Guerriero et al, 2018). PSMs formed more than once from various structural classes, rather than a single compound, differ in both content and quantity. How these compounds affect the herbivores is important because it is essential to know the biology of the species in the combat against harmful species. Studies on insect feeding in the literature generally evaluate insect performance in terms of survival and development (Sousa et al, 2016; Rosa et al, 2018; Huang, Lv, Zhang, & Chang, 2020). In addition to these studies, studies addressing the parameters that affect the fitness and fecundity of insects are also crucial. Our aim in this study has been to determine how the phenolic compounds present in the leaves of *Juglans regia* (walnut), *Malus pumila* (apple), *Prunus dometica* (plum), and *Morus alba* (mulberry), which are the most preferred food items by *H. cunea* and economically important for local people, affects the pupal weight, the pupal total protein contents, and pupal total lipid contents of the insect fed on them. Besides, we have aimed to determine how *B. thuringiensis* subsp. *kurstaki* (*Btk*) effects on these parameters.

MATERIAL AND METHODS

Collection of the larvae and the feeding experiments

The 1st generations of *H. cunea* larvae were collected from Çarşamba District of Samsun, Turkey, in June 2020 (N41°30'-E36°05'). The larvae collected were placed into the plastic containers (5×10×2 cm) one by one with 50 larvae in each group and were let to feed on the leaf samples of walnuts, mulberries, apples, and plums plants until they reached the pupal stage. In this study, the larvae of the 2nd generation obtained as the offspring of the 1st generations' adults were used. The larvae of 2nd generation were allowed to feed on the leaf samples of these four plant species until the pupal stage. The feeding experiments were carried out at 25±2°C, 70% humidity, 16 h light/8 h dark. The plant leaves used in the feeding experiments were collected daily; each leaf sample was sterilized with 50% ethyl alcohol, and then given to the larvae.

Preparing bacterial suspension

Bacillus thuringiensis subsp. *kurstaki* (*Btk*) was used in larval infection. The strain was obtained from culture collection of microbiology laboratory at Karadeniz Technical University. The *Btk* was grown overnight at 30°C in nutrient broth (AppliChem, Darmstadt, Germany). The optical density of the growing culture was measured at a wavelength of 600 nm and set to $OD_{600} = 1.89$ (Ben-Dov, Boussiba, & Zaritsky, 1995).

Experimental setup

Each group consisted of 50 *H. cunea* larvae. The larvae in the non-infected groups were fed with the non-contaminated leaves for five days. For infected groups, each plant leaf used for feeding the larvae was contaminated by 1 ml of the bacterial suspension. The larvae continued to feed until they became pupae. Since deaths also occurred due to infection, 30 pupae were used for analysis to keep the number constant in each group.

The phenolic and gallotannin contents of the plant leaf samples

The determination of PSMs was made with HPLC brand Thermo Finnigan Surveyor (Thermo Finnigan, San Jose, CA, USA). HPLC-UV analyses were performed on a reverse phase C18 column (150 mm × 4.6 mm id, 5 µm particle; Fortis, France) using a Thermo Finnigan Surveyor HPLC and UV detector which is simultaneously operating dual-UV wavelength. Gradient elution was used for HPLC analyses. The mobile phase was (A) 2% acetic acid in water and (B) 70:30 acetonitrile:water. The following gradient was used; 0-3 min 5% B; 3-8 min 5-15% B; 8-10 min 15-20% B; 10-12 min 20-25% B; 12-20 min 25-40% B; 20-30 min 40-80% B. The injection volume was 25 µl, the column temperature was 30°C and the flow rate was 1.2 ml/min.

The method used to determine gallotannin contents of the leaf samples was described by Bate-Smith (1977). For gallotannin analysis, a certain amount of leaf samples from each plant species were taken daily and dried in an oven until reaching constant weights. The dried leaves were ground. 4 tubes with 10 ml were used for each sample. A 5% KIO_3 solution was prepared for analysis. 0.5 mg of the leaves sample was taken on the 4 tubes prepared for each sample and put into the tubes. 1 ml of the previously prepared 5% KIO_3 solution was added to three of the samples placed in the tube. For the control, only 1 ml of distilled water was added to the 4th tube. The prepared samples were kept in an ice bath for 1 hour. The samples were then removed from the ice bath and their absorbance was measured in a spectrophotometer at a wavelength of 550 nm. A standard curve was prepared with tannic acid solutions (0.1-0.7 mg/ml) to calculate the gallotannin amounts of the samples.

The determination of the pupal total lipid contents

Hyphantria cunea pupae were kept in the incubator at 50°C for one month to achieve constant weights and thus calculate their dry weights. To calculate the lipid amounts of the pupae reaching constant weights, the pupae were placed in glass tubes and kept in pure chloroform for 24 hours on a rotary shaker. After this treatment, the supernatants of the tubes were discarded. This process was repeated three times so that the lipid contents of the pupae were removed. The pupae were put back into an oven and dried again to constant weights, and then the lipid-free weights of the pupae were determined by weighing out. The analyses were performed individually for each pupae. The total lipid contents of the pupae were calculated from the obtained data (Simpson, 1983).

The determination of the pupal total protein content

The nitrogen content determination of the lipid-free *H. cunea* pupae was made by semi-micro Kjeldahl method with Kjeltec Auto 1030 analyzer (Tecator, Sweden). The analyses were performed individually for each pupae. Total protein contents of the pupae were calculated by multiplying the nitrogen content of each pupal sample found as a result of the process by the constant 6.25 (Oonincx, van Broekhoven, van Huis, & van Loon, 2015).

Statistical analysis

In the study, whether the pupal weights, pupal lipid and protein contents of *H. cunea* were statistically different from each other were determined by ANOVA followed by post hoc Dunnet test. SPSS 21.0 software was used for statistical analysis.

RESULTS

The phenolic and gallotannin contents of the leaf samples

While the highest amount of chlorogenic acid contents was in the plum leaves, the lowest content was present in the mulberry leaves. Catechin and rutin were only present in the mulberry leaves. Benzoic acid was determined to be present only in the walnut leaves. Protocatechuic acid and rosmarinic acid contents were the highest in the apple lowest content and the lowest content of them were found to be in the plum leaves (Fig. 1). Among all plant leaf samples, the highest gallotannin content was determined in the walnut leaves and the lowest content of it was in the mulberry leaves (Fig. 2).

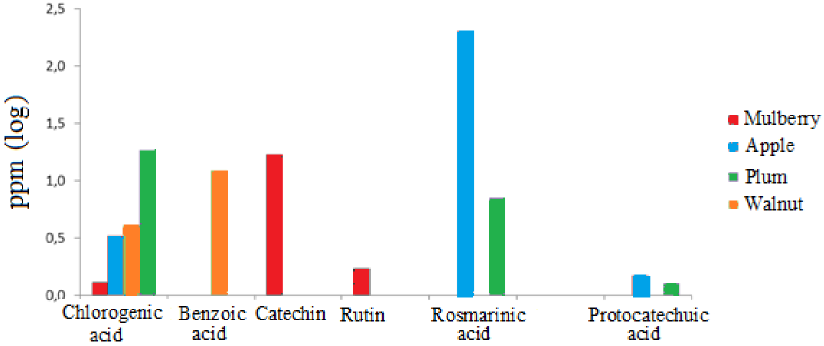


Fig. 1. The contents of phenolic compounds in leaves.

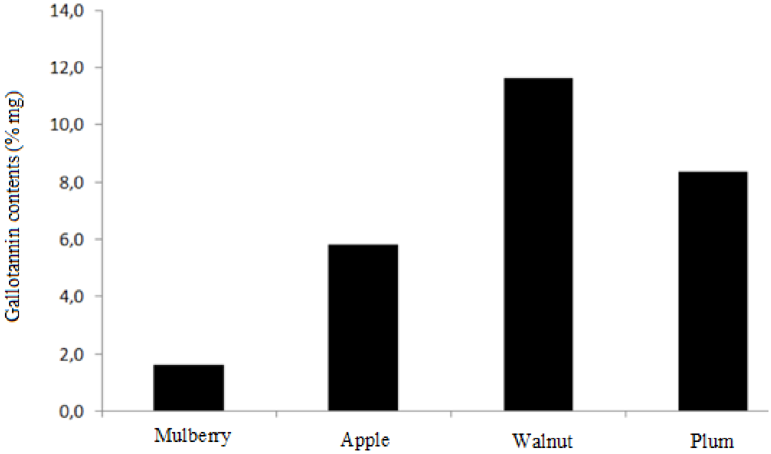


Fig. 2. The gallotannin contents in leaves.

The pupal weights

Among the non-infected groups, while the lowest pupal weight was obtained in the apple-fed group (21.7±1.4 mg, t=-0.7, P>0.05), and the highest pupal weight was found to be in the mulberry-fed group (25±1.7 mg, t=-40, P>0.05). The pupal weights of all groups infected with bacteria decreased compared to the non-infected ones. Among the infected groups, the lowest pupal weight was in the walnut-fed group (18.8±1.2 mg, t=-2.3, P<0.05) and the highest in the mulberry-fed group (23.2±0.8 mg, t=-40, P>0.05) (Fig. 3).

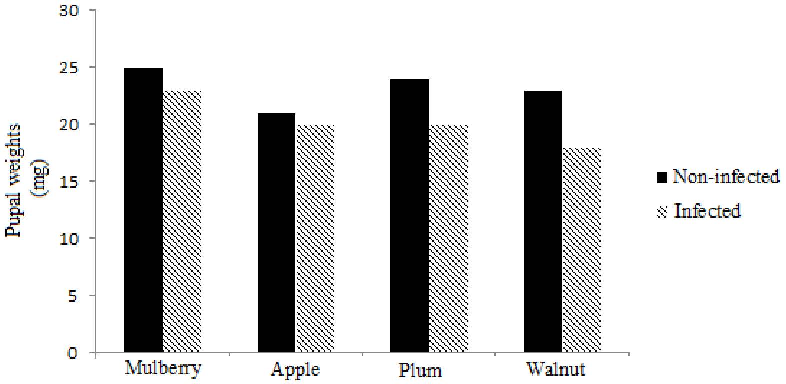


Fig. 3. The pupal weights of *Hyphantria cunea* according to different plant groups.

The pupal total protein contents

Among the non-infected groups, the pupal total protein contents were 13.3±1.1 mg, t=-2.1, P<0.05 in the mulberry, 11.2±0.7 mg, t =-2.5, P<0.001 in the apple, 13.1±0.7 mg, t=-5, P<0.001 in the plum, and 12.3±0.6 mg, t=-3.2, P<0.05 in the walnut-fed groups. Among the bacteria infected groups, while the lowest pupal total protein content was found in the plum-fed group (8.7±0.6 mg, t=-5, P<0.001) and the highest one in the mulberry-fed group (10.7±0.4 mg, t =-2.1, P<0.05). The pupal total protein contents of all groups were found to be decreased by the bacterial infection compared to non-infected groups (Fig. 4).

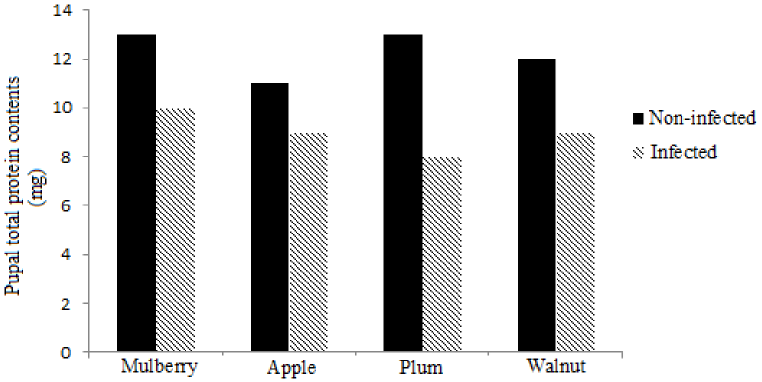


Fig. 4. The pupal protein contents of *Hyphantria cunea* according to different plant groups.

The pupal total lipid contents

The pupal total lipid contents in the non-infected groups were respectively in the plum (7.4±0.5, t=1.3 mg, P>0.05)> mulberry (7.3±0.5 mg, t=2.3, P<0.05)> apple (6.8±0.6 mg, t=1.6, P>0.05) > walnut (6.6±0.6 mg, t=-1, P>0.05). In the infected groups, the pupal total lipid contents were 9.0±0.6 mg, t=2.3, P<0.05 in mulberry, 8.1±0.5 mg, t=1.6, P>0.05 in apple, 8.5±0.6 mg, t=1.3, P> 0.05 in plum, and 5.8±0.5 mg, t=-1, P>0.05 in walnut-fed group. It was determined that the pupal total lipid contents in all groups (except walnut) increased by the bacterial infection compared to the non-infected ones (Fig. 5).

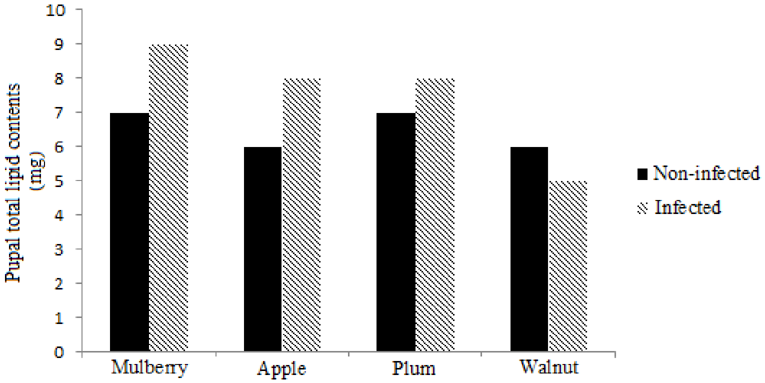


Fig. 5. The pupal lipid contents of *Hyphantria cunea* according to different plant groups.

DISCUSSION

Host preference by herbivorous animals is determined according to the characteristics of the plants (Stam et al, 2014), and the characteristics of the plants depend on the PSMs as well as the primary metabolites (Coyle, Clark, Raffa, & Johnson, 2011). Many phenolic compounds have antagonistic feeding effects on various taxa and other biological processes (Lindroth & St Clair, 2013). In the early stages of lepidopteran larvae, many phenolic compounds have growth inhibitory activities (Bhattacharya & Chenchaiah, 2007), which also affects subsequent biological parameters. The finding of the least pupal weight was in the apple-fed group among the non-infected groups in our study was supported by above mentioned studies. The apple leaves had maximum rosmarinic acid in our study, which adversely affected the pupal weight. In a study performed with *Anastrepha ludens*, Aluja et al (2014), found that the larvae fed by a cultivar containing the maximum amount of catechin and rutin had a minimum pupal weight. The result of this study contradicted with the result that we found in our study that the catechin and rutin only present in the mulberry leaves caused maximum pupal weight. Besides, in the mulberry leaves gallotannin content was minimal. Since tannins are astringent and bitter polyphenols (Ashok & Upadhyaya, 2012), the larvae may have reached maximum pupal weight by preferring more to be fed with the mulberry leaves containing minimum tannins. Tayal, Somavat, Rodriguez, Martinez, & Kariyat (2020) in a study with *Manduca sexta* larvae found out that the larvae fed on maize leaves with more tannin had lower pupal weights. Also, studies conducted with various species have shown that the tannin decreases pupal weight (Barbehenn et al, 2009; Topkara, 2019). Based on the results obtained from these studies, a possible reason may be that the emergence of semiquinone radicals in the presence of tannins increases the metabolic cost and decreases the efficiency of the conversion of consumed food into body mass (Barbehenn et al, 2009).

Exposure to sublethal concentrations of insecticides affects insect population dynamics by altering the biological parameters of individuals (Stark & Banks, 2003; Desneux, Decourtye, & Delpuech, 2007; Qu et al, 2015). Abedi, Saber, Vojoudi, Mahdavi, & Parsaeyan, (2014) showed that *Btk* infection decreased pupal weights of *Heliothis armigera*. Nouri-Ganbalani, Borzoui, Abdolmaleki, Abedi, & Kamita (2016) noted that *Bt* strains cause a low pupal weight in *Plodia interpunctella*. In our study, we found that the pupal weights of all groups infected with *Btk* decreased compared to the non-infected groups; these results may have likely to adversely affect the fitness of *H. cunea*. Besides this, the pupal weight and the fecundity are also related. Also in the current study, *Btk* toxicity may lead to decreased fecundity. In a study (Pineda et al, 2009), several Coleopteran pests have been reported to have decreased fecundity after exposure to pesticides. The fecundity of the adult females is greatly affected by bioinsecticides. Due to the strong correlation between the fecundity and the pupal mass in females (Bauerfeind & Fischer, 2009), it can be understood that low female pupal weight can directly affect reproductive abilities. Since oviposition is an important phenomenon, reduction in the fecundity may be a management strategy against the herbivore pests (Ketoh, Glitho, Koumaglo, & Garneau, 2000; Zhao, Yang, Wang-Pruski, & You, 2008).

The accumulation of the storage proteins and the use of the proteins by insects in later stages are important events associated with the metamorphosis of holometabolous insects. The total protein contents of insect are essential for all stages of development of the insect. The assessment of changes in the total protein contents is important to determine whether the ingredients in the diets are used effectively by the insect and whether it effects on the development of the insects (Sak, Uçkan, & Ergin, 2006; 2011). Among the non-infected groups, we found that the pupal protein content was the lowest in the apple-fed group. The highest contents of rosmarinic acid and protocatechuic acid were present in the apple leaves adversely affected the pupal total protein content. These results in our study are supported by Dixit, Praveen, Tripathi, Yadav, & Verma (2017) research which was found that the increasing amount of PSMs caused a decrease in the total protein contents of *Helicoverpa armigera* and *Spodoptera litura*. Also, we found that the least of chlorogenic acid content in the mulberry leaves caused the highest pupal total protein content. Chlorogenic acid is known to be an antifeedant substance (Ikonen, Tahvanainen, & Roininen, 2001). Owing to its ability to bind proteins covalently, this substance can be especially harmful to the protein digestion in insects. Therefore, it is not surprising that a low chlorogenic acid content in the leaf samples causes the highest pupal protein content. Besides, the content of gallotannin in the mulberry leaves was the lowest among the leaf samples. Considering the interaction between tannins and proteins (Adamczyk, Simon, Kitunen, Adamczyk, & Smolander, 2017), the highest pupal total protein content in the mulberry-fed group with low tannins supports this result.

Considering that the pupal total protein content is an important parameter for the development of insects, it is a disadvantage for the insects that the pupal total protein contents of all groups obtained by the bacterial infection were decreased compared to the non-infected groups. The studies have demonstrated a relationship between the toxic properties of insecticides and the total protein amount (Ahmed, Wilkins, & Mantle, 2002; Guedes et al, 2006). It is known that *Bt* toxicity causes a decrease in the protein contents of insects (Abedi et al, 2014). Our study also is supported by the research of Elgizawy & Ashry (2019) showed that *Bt* infection reduced the total protein contents of insects compared to the control. Among the infected groups in our study, the lowest pupal protein content was determined in the plum-fed group. The plum leaves had the highest amount of chlorogenic acid among the plant leaves analyzed. Chlorogenic acid increases the toxicity of bacteria (Ludlum, Felton, & Duffey, 1991). Therefore, it is not surprising that the lowest total protein content was found in this group.

Lipids are the main component of all living organisms (Yi et al, 2013) and an important energy sources (Smit, Muskiet, & Boersma, 2004). Lipids are used by insects for various physiological functions such as development, flight, reproduction, in the structure of cell membranes, communication via pheromones, etc. (Beenakkers, Vanderhorst, & Vanmarrewijk, 1985; Lease & Wolf, 2011). The total lipid content of the insects is affected by their diets (Oonincx et al, 2015). In our study, it has been determined that the highest pupal total lipid content in the non-infected groups has found in the plum-fed group having the highest chlorogenic acid content. In the pupal stage, the lipid stores during the larval stages will be used to facilitate the metamorphosis and also to support the energy demands of reproduction and flight in adulthood (Ziegler, 1991), so that chlorogenic acid will be advantageous for the species. Furthermore, it was observed that the lowest pupal total lipid content was in the walnut-fed group among both the non-infected and infected groups. The highest amount of tannin content was found in the walnut leaves. Tannins can bind a wide variety of natural polymeric compounds, including lipids *in vitro* (Barbehenn & Constabel, 2011). In this case, the highest gallotannin content in the walnut leaves is a disadvantage for the insect feeding. Also, benzoic acid, present only in walnut leaves, may have had a detrimental effect on the total lipid contents of pupae.

Many insecticides have been shown to reduce biochemical components in the animal body (Khosravi, Sendi, & Ghadamyari, 2010; Zhao et al, 2016). In a study performed with *Agrotis ipsilon*, Xu et al (2016) observed that when insecticide was applied to the larvae, the lipid contents of them decreased compared to the control. Our study is also contradicted by the study of Elgizawy & Ashry (2019) in which *Bt* infection decreased the total lipid content of *Tribolium castaneum* compared to the control. It was determined that the pupal total lipid contents in all groups (except walnut) increased by the bacterial infection compared to the non-infected groups. It is surprising as the bacterial infection plays a triggering role on the lipid.

CONCLUSION

Holometabolous insects use the proteins and lipids they take and store in the larval stage for metamorphosis and for adult stage. In our study, it has been shown that the PSMs present in the plants consumed by the larvae affect the biological properties of *Hyphantria cunea* at the pupal stage. Our study has determined that PSMs affect the body weight of the insect by altering the total pupal lipid and total protein contents of

H. cunea. Furthermore, the effect of the bacteria used against the insect pests on the pupal parameters should not be ignored. It should be evaluated that the effects of both the PSMs and the infection can have a strong impact on the population dynamics of lepidopteran pests and contribute to their control.

ACKNOWLEDGEMENT

I thank for Assoc. Prof. Oğuzhan Yanar and Prof. Dr. Mahmut Bilgener from Ondokuz Mayis University for their contributions.

REFERENCES

Abedi, Z., Saber, M., Vojoudi, S., Mahdavi, V., & Parsaeyan, E. (2014). Acute, sublethal, and combination effects of azadirachtin and *Bacillus thuringiensis* on the cotton bollworm, *Helicoverpa armigera*. *Journal of Insect Science*, 14, 30.

Adamczyk, B., Simon, J., Kitunen, V., Adamczyk, S., & Smolander, A. (2017). Tannins and their complex interaction with different organic nitrogen compounds and enzymes: old paradigms versus recent advances. *Chemistry Open*, 6(5), 610-614.

Ahmed, S., Wilkins, R., & Mantle, D. (2002). Comparative effect of various insecticides on intracellular proteases in an insecticide-resistant and susceptible strains of *Musca domestica*. *Journal of Biological Sciences*, 2(3), 183-185.

Aluja, M., Birke, A., Ceymann, M., Guillén, L., Arrigoni, E., Baumgartner, D., Pascacio-Villafán, C., & Samietz, J. (2014). Agroecosystem resilience to an invasive insect species that could expand its geographical range in response to global climate change. *Agriculture, Ecosystems & Environment*, 186, 54-63.

Ashok, P.K. & Upadhyaya, K. (2012). Tannins are astringent. *Journal of Pharmacognosy and Phytochemistry*, 1(3), 45-50.

Bate-Smith, E.C. (1977). Astringent tannins of *Acer* species. *Phytochemistry*, 16, 1421-1426.

Barbehenn, R.V. & Constabel, P.C. (2011). Tannins in plant-herbivore interactions. *Phytochemistry*, 72(13), 1551-1564.

Barbehenn, R.V., Jaros, A., Lee, G., Mozola, C., Weir, Q., & Salminen, J.P. (2009). Hydrolyzable tannins as “quantitative defenses”: limited impact against *Lymantria dispar* caterpillars on hybrid poplar. *Journal of Insect Physiology*, 55, 297-304.

Bauerfeind, S.S. & Fischer, K. (2009). Effects of larval starvation and adult diet-derived amino acids on reproduction in a fruit-feeding butterfly. *Entomologia Experimantalis et Applicata*, 130(3), 229-237.

Beenakkers, A.M.T., Vanderhorst, D.J., & Vanmarrewijk, W.J.A. (1985). Insect lipids and lipoproteins, and their role in physiological processes. *Progress in Lipid Research*, 24(1), 19-67.

Ben-Dov, E., Boussiba, S., & Zaritsky, A. (1995). Mosquito larvicidal activity of *Escherichia coli* with combinations of genes from *Bacillus thuringiensis* subsp. *israelensis*. *Journal of Bacteriology*, 177(10), 2851-2857.

Beran, F., Kollner, T.G., Gershenzon, J., & Tholl, D. (2019). Chemical convergence between plants and insects: biosynthetic origins and functions of common secondary metabolites. *New Phytologist*, 223, 52-67.

Bhattacharya, A.K. & Chenchaiah, K.C. (2007). Seed coat phenolic compounds of *Cajanus cajan* as chemical barrier in formulation of artificial diet of *Spodoptera litura* (F.). *Annals of Plant Protection Sciences* 15(1), 92-96.

Chen, G., Kim, H.K., Klinkhamer, P.G.L., & Escobar-Bravo, R. (2020). Site-dependent induction of jasmonic acid-associated chemical defenses against western flower thrips in *Chrysanthemum. Planta*, 251, 1-14.

Coyle, D.R., Clark, K.E., Raffa, K.F., & Johnson, S.N. (2011). Prior host feeding experience influences ovipositional but not feeding preference in a polyphagous insect herbivore. *Entomologia Experimentalis et Applicate*, 138(2), 137-145.

Desneux, N., Decourtye, A., & Delpuech, J.M. (2007). The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology*, 52, 81-106.

Dixit, G., Praveen, A., Tripathi, T., Yadav, V.K., & Verma, P.C. (2017). Herbivore-responsive cotton phenolics and their impact on insect performance and biochemistry. *Journal of Asia-Pacific Entomology*, 20, 341-351.

Elgizawy, K.K. & Ashry, N.M. (2019). Efficiency of *Bacillus thuringiensis* strains and their Cry proteins against the red flour beetle, *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae). *Egyptian Journal of Biological Pest Control*, 29(1), 1-9.

Esperk, T. & Tammaru, T. (2004). Does the ‘investment principle’ model explain moulting strategies in lepidopteran larvae? *Physiological Entomology*, 29, 56-66.

Gotthard, K. (2004). Growth strategies and optimal body size in temperate *Pararginii* butterflies. *Integrative and Comparative Biology*, 44, 471-479.

Guedes, R.N.C., Oliveira, E.E., Guedes, N.M.P., Ribeiro, B., & Serrao, J.E. (2006). Cost and mitigation of insecticide resistance in the maize weevil, *Sitophilus zeamais*. *Physiological Entomology*, 31(1), 30-38.

Guerriero, G., Berni, R., Muñoz-Sanchez, J.A., Apone, F., Abdel-Salam, E.M., Qahtan, A.A., Alatar, A.A., Cantini, C., Cai, G., Hausman, J.F., Siddiqui, K.S., Hernández-Sotomayor, S.M.T., & Faisal, M. (2018). Production of plant secondary metabolites: examples, tips and suggestions for biotechnologists. *Genes*, 9(6), 309.

Huang, X., Lv, S., Zhang, Z., & Chang, B.H. (2020). Phenotypic and transcriptomic response of the grasshopper *Oedaleus asiaticus* (Orthoptera: Acrididae) to toxic rutin. *Frontiers in Physiology*, 11, 52.

Ikonen, A., Tahvanainen, J., & Roininen, H. (2001). Chlorogenic acid as an antiherbivore defence of willow against leaf beetles. *Entomologia Experimentalis et Applicata*, 99, 47-54.

Kessler, A. & Baldwin, I.T. (2002). Plant responses to insect herbivory: the emerging molecular analysis. *Annual Review of Plant Biology*, 53, 299-328.

Ketoh, G.K., Glitho, A.I., Koumaglo, K.H., & Garneau, F.X. (2000). Evaluation of essential oils from six aromatic plants in togo for *Callosobruchus maculatus* F. Pest control. *International Journal of Tropical Insect Science*, 20(1), 45-49.

Khan, S., Taning, C.N.T., Bonneure, E., Mangelinckx, S., Smagghe, G., Ahmad, R., Fatima, N., Asif, M., & Shah, M.M. (2019). Bioactivity-guided isolation of rosmarinic acid as the principle bioactive compound from the butanol extract of *Isodon rugosus* against the pea aphid, *Acyrthosiphon pisum. Plos One*, 14(6), e0215048.

Khosravi, R., Sendi, J., & Ghadamyari, M. (2010). Effect of *Artemisia annua* L. on deterrence and nutritional efficiency of lesser mulberry pyralid (*Glyphodes pylolais* Walker) (Lepidoptera: Pyralidae). *Journal of Plant Protection Research*, 50, 423-428.

Kundu, A. & Vadassery, J. (2019). Chlorogenic acid-mediated chemicaldefence of plants against insect herbivores. *Plant Biology*, 21(2), 185-189.

Lease, H.M. & Wolf, B.O. (2011). Lipid content of terrestrial arthropods in relation to body size, phylogeny, ontogeny and sex. *Physiological Entomology*, 36(1), 29-38.

Liao, F., Wang, L., Wu, S., Li, Y.P., Zhao, L., Huang, G.M., Niu, C.J., Liu, Y.Q., & Li, M.G. (2010). The complete mitochondrial genome of the fall webworm, *Hyphantria cunea* (Lepidoptera: Arctiidae). *International Journal of Biological Sciences*, 6(2), 172-186.

Lindroth, R.L. & St Clair, S.B. (2013). Adaptations of quaking aspen (*Populus tremuloides* Michx.) for defense against herbivores. *Forest Ecology and Management*, 299, 14-21.

Ludlum, C.T., Felton, G.W., & Duffey, S.S. (1991). Plant defenses: chlorogenic acid and polyphenol oxidase enhance toxicity of *Bacillus thuringiensis* subsp. *kurstaki* to *Heliothis zea*. *Journal of Chemical Ecology*, 17, 217.

Ma, K., Li, F., Tang, Q., Liang, P., Liu, Y., Zhang, B., & Gao, X. (2019). CYP4CJ1-mediated gossypol and tannic acid tolerance in *Aphis gossypii* Glover. *Chemosphere*, 219, 961-970.

Mazid M., Khan, T.A., & Mohammad, F. (2011). Role of secondary metabolites in defense mechanisms of plants. *Biology and Medicine*, 3, 232-249.

Nouri-Ganbalani, G., Borzoui, E., Abdolmaleki, A., Abedi, Z., & Kamita, S.G. (2016). Individual and combined effects of *Bacillus thuringiensis* and azadirachtin on *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae). *Journal of Insect Science*, 16(1), 95.

Oonincx, D.G.A.B., van Broekhoven, S., van Huis, A., & van Loon, J.J.A. (2015). Feed conversion, survival and development and composition of four insect species on diets composed of food by-products. *PLoS One*, 10, 1-20.

Pineda, S., Martinez, A.M., Figueroa, J.I., Schneider, M.I., Estal, D.P., Estal, V.E., Gomez, B., Smagghe, G., & Budia, F. (2009). Influence of azadirachtin and methoxyfenozide on life parameters of *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Journal of Economic Entomology*, 102(4), 1490-1496.

Qu, Y.Y., Xiao, D., Li, J., Chen, Z., Biondi, A., Desneux, N., Gao, X., & Song, D. (2015). Sublethal and hormesis effects of imidacloprid on the soybean aphid *Aphis glycines*. *Ecotoxicology*, 24(3), 479-487.

Rosa, E., Woestmann, L., Biere, A., & Saastamoinen, M. (2018). A plant pathogen modulates the effects of secondary metabolites on the performance and immune function of an insect herbivore. *Oikos*, 127, 1539-1549.

Sak, O., Ergin, E., Uçkan, F., Rivers, D.B., & Er, A. (2011). Changes in the hemolymph total protein of *Galleria mellonella* (Lepidoptera: Pyralidae) after parasitism and envenomation by *Pimpla turionellae* (Hymenoptera: Ichneumonidae). *Turkish Journal of Biology*, 35(4), 425-432.

Sak, O., Uçkan, F., & Ergin, E. (2006). Effects of cypermethrin on total body weight, glycogen, protein, and lipid contents of *Pimpla turionellae* (L.) (Hymenoptera: Ichneumonidae). *Belgian Journal of Zoology*, 136(1), 53-58.

Silva, T.R.F.B., de Sousa Almeida, A.C., de Lima Moura, T., de Silva, A.R. de Sousa Freitas, S., & Jesus F.G. (2016). Effect of flavonoid rutin on the biology of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Acta Scientiarum*, 38(2), 165-170.

Simpson, S.J. (1983). Changes during the fifth instar of *Locusta migratoria* in the rate of crop emptying and their relationship to feeding and food utilization. *Entomologia Experimentalis et Applicata*, 33, 235-243.

Smit, E.N., Muskiet, F.A.J., & Boersma, E.R. (2004). The possible role of essential fatty acids in the pathophysiology of malnutrition: a review. *Prostaglandins, Leukotrienes & Essential Fatty Acids*, 71(4), 241-250.

Sousa, F.F., Mendes, S.M., Santos-Amaya, O.F., Araujo, O.G., Oliveira, E.E., & Pereira, E.J.G. (2016). Life-history traits of *Spodoptera frugiperda* populations exposed to low-dose *Bt* maize. *Plos One*, 11, e0156608.

Stam, J.M., Kroes, A., Li, Y., Gols, R., van Loon, J.J.A., Poelman, E.H., & Dicke, M. (2014). Plant interactions with multiple insect herbivores: from the community to genes. *Annual Review of Plant Biology,* 65(1), 689-713.

Stark, J.D. & Banks, J.E. (2003). Population-level effects of pesticides and other toxicants on arthropods. *Annual Review of Entomology* 48, 505-519.

Syafni, N., Putra, D.P., & Arbain, D. (2012). 34-Dihydroxybenzoic acid and 34-dihydroxybenzaldehyde from the fern *Trichomanes chinense*; isolation antimicrobial and antioxidant properties. *Indonesian Journal of Chemistry*, 12, 273-278.

Tan, Q.G. & Luo, X.D. (2011). Meliaceous limonoids: chemistry and biological activities. *Chemical Reviews*, 111, 7437-7522.

Tangtrakulwanich, K. & Reddy, G.V.P. (2014). *Advances in plant biopesticides*. Springer, New Delhi, India.

Tayal, M. & Somavat, P., Rodriguez, I., Martinez, L., & Kariyat, R. (2020). Cascading effects of polyphenol-rich purple corn pericarp extract on pupal, adult, and offspring of tobacco hornworm (*Manduca sexta* L.). *Communicate & Integrative Biology*, 13(1), 43-53.

Tek, S.E. & Okyar, Z. (2017). Biological observations on some herbivorous insects. *Trakya University Journal of Natural Sciences*, 18(1), 59-64.

Topkara, E.F. (2019). The effects of different secondary compounds on the development of *Uresiphita gilvata* (Lepidoptera: Crambidae) larvae. *Turkish Journal of Agriculture-Food Science and Technology*, 7(2), 253-257.

Weinzierl, R., Henn, T., Koehler, P.G., & Tucker, C.L. (2005). *Microbial insecticides.* University of Florida Institute of Food and Agricultural Sciences Extension, Gainesville, Florida.

Xu, C., Zhang, Z., Cui, K., Zhao, Y., Han, J., Liu, F., & Mu, W. (2016). Effects of sublethal concentrations of cyantraniliprole on the development, fecundity and nutritional physiology of the black cutworm *Agrotis ipsilon* (Lepidoptera: Noctuidae). *PLoS One*, 11(6), e0156555.

Yi, L.Y., Lakemond, C.M.M., Sagis, L.M.C., Eisner-Schadler, V., van Huis, A., & van Boekel, M.A.J.S. (2013). Extraction and characterisation of protein fractions from five insect species. *Food Chemistry*, 141(4), 3341-3348.

Zhao, Y., Yang, G., Wang-Pruski, G., & You, M. (2008). *Phyllotreta striolata* (Coleoptera: Chrysomelidae): arginine kinase cloning and RNAi-based pest control. *European Journal of Entomology*, 105(5), 815-822.

Zhao, Y.H., Xu, C.M., Wang, Q.H., Wei, Y., Liu, F., & Mu, W. (2016). Effects of the microbial secondary metabolite benzothiazole on the nutritional physiology and enzyme activities of *Bradysia odoriphaga* (Diptera: Sciaridae). *Pesticide Biochemistry and Physiology*, 129, 49-55.

Ziegler, R. (1991). Changes in lipid and carbohydrate metabolism during starvation in adult *Manduca sexta*. *Journal of Comparative Physiology B*, 161(2), 125-131.

J. Entomol. Res. Soc., 24(1): 89-101, 2022
Doi: 10.51963/jers.v24i1.2028

Research Article
Online ISSN:2651-3579

Comparative lethal and sublethal toxicity of some conventional insecticides against tomato leafminer, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae)

Mohsen TALEH[1]* Hooshang RAFIEE-DASTJERDI[2] Bahram NASERI[3]
Asgar EBADOLLAHI[4] Mustafa ALKAN[5]

[1,2,3]Department of Plant Protection, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, IRAN
[4]Department of Plant Sciences, Moghan College of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, IRAN
[5]Plant Protection Central Research Institute, Department of Entomology, Ankara, TURKEY
e-mails: [1]*m.taleh@uma.ac.ir, [2]Hooshangrafiee@gmail.com, [3]bnaseri@uma.ac.ir, [4]ebadollahi@uma.ac.ir, [5]Alkan0101@gmail.com
ORCID IDs: [1]*0000-0003-3682-598X, [2]0000-0003-1278-2858, [3]0000-0001-5821-0957, [4]0000-0003-3276-1608, [5]0000-0001-7125-2270

ABSTRACT

This study was conducted to introduce effective agent(s) among some novel and conventional insecticides in the management of *Tuta absoluta* (Meyrick, 1917), the most destructive insect pest of tomato throughout the world, in the lethal and sublethal terms of features. Toxicity of five chemical pesticides, including azadirachtin, emamectin benzoate, imidacloprid, lambda-cyhalothrin, and thiacloprid, was determined on 2nd-instar larvae of *T. absoluta* by leaf-dipping method in a growth chamber with 25 ± 2 °C, 65 ± 5% relative humidity, and a photoperiod of 16:8 h (L:D). The least LC_{50} value was attained with emamectin benzoate (0.52 mg A.I./L) among tested insecticides. Demographic parameters of the pest, including *GRR* (gross reproductive rate), R_0 (net reproductive rate), r_m (intrinsic rate of increase), T (mean generation time), and λ (finite rate of increase) along with larval, pupal, and pre-adult periods were more affected by emamectin benzoate and azadirachtin. Adult pre-oviposition period (APOP) (1.04 days), female longevity (18.17 days), male longevity (17.88 days), oviposition period (4.21 days), and fecundity (38.67 egg/female) were low by emamectin benzoate. In the treatments by emamectin benzoate and azadirachtin, the survival of fourth-instar larvae, pupae, and adults of insect was reduced in comparison to control. Accordingly, based on the highest adverse possessions of emamectin benzoate and azadirachtin on survival, life history, and demographic parameters of *T. absoluta,* the applicability of other tested insecticides imidacloprid, lambda-cyhalothrin, and thiacloprid may be limited.

Key words: Demographic parameters, *Tuta absoluta*, emamectin benzoate, Azadirachtin, imidacloprid.

Taleh, M., Rafiee-Dastjerdi, H., Naseri, B., Ebadollahi, A., & Alkan, M. (2022). Comparative lethal and sublethal toxicity of some conventional insecticides against tomato leafminer, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae). *Journal of the Entomological Research Society*, 24(1), 89-101.
Received: March 01, 2021 *Accepted: January 04, 2022*

INTRODUCTION

Arthropod pests have a critical role in diminishing food production and cause high loss of crops (Lietti, Botto, & Alzogaray, 2005). Tomato leafminer [*Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae)], as detracutive insect pest, annually reduces the quantity and quality of the tomato (Terzidis, Wilcockson, & Leifert, 2014). *T. absoluta* with the origin of South America quickly dispersed to European and Mediterranean countries (Guillemaud et al, 2015), and recently to Afro-Eurasia and Southwest and Central Asian countries (Biondi, Guedes, Wan, & Desneux, 2018). *T. absoluta* damages some of the most important crops of the Solanaceae family, such as tomatoes, potatoes, eggplant and sweet peppers, and even some weeds (Desneux et al, 2010; Smith et al, 2018). Although this pest mainly harms the leaves and fruits of tomato, buds, stems, and flowers can also be affected by decreasing the photosynthetic capacity and production surface area and indirect damages with secondary pathogenic contaminations (Lietti, Botto, & Alzogaray, 2005; Tropea, Siscaro, Biondi, & Zappalà, 2012).

The management of *T. absoluta* is generally dependent on multiple applications of chemical insecticides (Guedes & Picanco, 2012; Urbaneja, González-Cabrera, Arnó, & Gabarra, 2012; Tomé et al, 2013). However, over-reliance on the synthetic chemicals' application results in insecticide resistance in *T. absoluta* and insecticide residues on the environment (Guedes & Siqueira, 2013; Gontijo et al, 2013). Because tomatoes are consumed freshly, it is needed to use insecticides that have low residual and less adverse effects on human health (Silva, Berger, Bass, & Balbino, 2015). Therefore, researchers have recently focused on the discovery of insecticides that, in addition to being environmentally friendly features, have effective control on *T. absoluta* with the possible lowest doses (Soares, 2019).

In addition to the direct toxicity assessed based on the mortality of insect pests, insecticides have adverse effects on their developmental stages, physiology, and behavior (Desneux, Decourtye, & Delpuech, 2007). Investigation of the life table parameters, as one of the useful tools providing an in-depth understanding of the survival and development of insect populations, is a well-recognized method in determining sublethal impacts of insecticides (Chi & Yang, 2003). Demographic toxicology is a noble method to examine the general effects of insecticides on population parameters in insect pests, including finite rate of increase (λ), intrinsic rate of increase (r_m), mean generation time (T), and net reproductive rate (R_0) (Yin et al, 2008; Cloyd, 2012; Rasheed et al, 2020). The intrinsic rate of increase, along with other life table parameters, has been recommended as an applicable ecological parameter for demographic toxicology (Stark, Sugayama, & Kovaleski, 2007).

Several studies have conducted on the toxicity of different insecticides on *T. absoluta* (Santos, Bueno, Vieira, & Bueno, 2011; Gacemi & Guenaoui, 2012; Michaelides, Seraphides, Pitsillou, & Fenthourakis, 2019; Kandil et al, 2020). For example, Mahmoud, Soliman, Abdel-Moniem, & Abdel-Raheem (2013) examined the lethal effects of emamectin benzoate on the larvae of cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), and found that the insecticide caused a significant decline in the population of pest. The effect of azadirachtin was investigated on different stages of the *T. absoluta*, and the highest lethal effect was shown on the larval stage of this pest (Branherotto & Vendraim, 2010). The lethal effects of imidacloprid, phenoxycarb, fentoat, thiocyclam, and dinotefuran were studied on *T. absoluta* larvae and the highest and the lowest lethal percentages were recognized with imidacloprid and phenoxycarb, respectively (Eman, Redwan, & Hanan, 2012). Imidacloprid was reported as a toxic compound for the larval stages of *T. absoluta* (Mahmoud et al, 2014). The adaptability of the emamectin benzoate, imidacloprid, and azadirachtin with the IPM programs were revealed in some studies (Iwasa, Motoyama, Ambrose, & Roe, 2003; Charleston, Kafir, Dicke, & Vet, 2006; Tomé et al, 2013; Jin et al, 2014). Further, previous studies discovered that life table parameters of *T. absoluta* could be influenced by chemical insecticides (Nozad-Bonab, Hejazi, Iranipour, & Arzanlou, 2017; Zibaee & Esmaeily, 2017).

Due to the necessity of investigating the lethal and sub-lethal effects of insecticides and introduce more effective ones, the current study was conducted to assess the lethal and sublethal effects of conventional insecticides azadirachtin, lambda-cyhalothrin, emamectin benzoate, imidacloprid, and thiacloprid on the mortality, demographic parameters, and life history of *T. absoluta*.

MATERIALS AND METHODS

Host plant

The seeds of tomato, *Solanum lycopersicum* L. (Solanaceae, cultivar Super strain B), were obtained from the Ardabil Agricultural and Natural Resources Research and Education Center, AREEO, Ardabil, Iran. The seeds were cultivated in plastic pots (20 cm diameter and 19 cm height) containing sand, soil, and perlite at greenhouse with 20 ± 3 °C, 55 ± 10% relative humidity, and natural photoperiod. They were irrigated once every three days and covered with nets to protect from other pests. Plants of about 30 cm in height were carried to the growth chamber to be infested with Tomato leafminer.

Rearing of insect

The initial population of Tomato leafminer was collected from non-sprayed tomato greenhouses around Ardabil city (38.2514° N, 48.2973° E) in Northwest of Iran, and transferred to the above-mentioned greenhouse condition. To maintain the population, old plants were replaced by fresh ones every few days. Adults were transferred to shelves containing tomato pots to begin spawning on tomato leaves. Insects were reared in the growth chamber for up to three generations and the second-instar larvae of the third generation were selected for bioassays. Adults of pest were fed with 10% solution of water and honey. Insect rearing, along with all experiments, were performed in a growth chamber with 25 ± 2 °C, 65 ± 5% relative humidity, and a photoperiod of 16:8 h (L:D).

Insecticides

Commercial formulations of the following chemicals were used: azadirachtin (Neem Azal 1% EC; Trifolio, Germany), emamectin benzoate (Proclaim 5% SG; Syngenta, Switzerland), imidacloprid (Imidacloprid 35% SC; Ariashimi, Iran), lambda-cyhalothrin

(Lambda-cyhalothrin 10% SC; Ariashimi, Iran), and thiacloprid (Biscaya 240% OD; Bayer, Germany).

Toxicity bioassay

Due to the nature of the studied insecticides, which are most effective through digestive and contact routes, bioassays were performed by leaf-dipping method. Preliminary experiments performed to distinguish the suitable concentration ranges of the insecticides, determining concentrations responsible for mortalities between 20% and 80%, and the intermediate concentrations selected by logarithmic intervals. Finally, the main experiment was done using five concentrations (0.3, 0.35, 0.47, 0.72, and 1.25 mg A.I./L for emamectin benzoate, 1.87, 3.09, 5.25, 8.91, and 15 mg A.I./L for azadirachtin, 10.85, 17.85, 30.45, 51.8, and 87.5 mg A.I./L for imidacloprid, 88.8, 148.8, 252, 427. 2, and 720 mg A.I./L for thiacloprid, and 400, 512.9, 645.6, 812.8, and 1000 mg A.I./L for lambda-cyhalothrin) with distilled water as the solvent. Only distilled water was utilized in the control groups. Then, tomato leaves were dipped at mentioned concentrations for 15 s and kept for one hour to dry in the laboratory conditions. For each replicate, group of 20 larvae of 2nd instar were placed in a Petri dish (9 cm diameter). A hole was made on the lid of each Petri dish and covered with a mesh to ventilate. Petri dishes were covered thoroughly with parafilm to prevent larval escape. Experiments were replicated four times for each insecticide concentration. The dishes were then retained inside the growth chamber at the above-mentioned conditions, and the larval mortality was recorded after 72 h (Galdino et al, 2011).

Life table experiments

The leaves of tomato were immersed in sublethal LC_{30} concentrations of insecticides which were obtained from toxicity bioassays (Dong, Wang, Li, & Wang, 2017). Seventy second-instar larvae were transferred on the leaves after drying within 30 minutes in the Petri dishes. A damp cotton pad was placed on the petioles inside the aluminum foil. The Petri dishes were then transferred to the growth chamber under the mentioned conditions and the survived larvae were transferred onto untreated leaves after 24 h. They were monitored daily, and the mortality and developmental period were recorded in each treatment. After the adults' emergence and to measure the fecundity and longevity, each male was paired with a female moth and retained in rectangular plastic containers (14 × 10 cm × 4 cm). The number of eggs deposited per female was daily documented pending the death of all individuals for calculation of fecundity. The longevity of adult male and female was also documented separately. Demographic parameters, including finite rate of increase (λ), gross reproductive rate (GRR), intrinsic rate of increase (r_m), mean generation time (T), net reproductive rate (R_0) along with age-stage specific survival rate (s_{xj}) were calculated.

Data analysis and statistical calculations

The Shapiro-Wilk test (Moscardini et al, 2013) was used to check the normality of data. Mortality of the *T. absoluta* larvae was modified using Abbott's formula (Abbott, 1925). Lethal concentrations with 95% fiducial limits were calculated using Probit analysis obtain in SPSS software (version 24). Data of life table parameters were analyzed through the TWO-SEX-MS Chart program, based on the two-sex theory and the method prescribed by Chi et al, (2020). Life table parameters, including GRR, R_0, r_m, λ, T, and s_{xj} were measured according to Carey method (1993). Their means and standard errors were evaluated using the bootstrap technique according to Efron and Tibshirani (1993). Variance analysis and mean comparisons via the Paired bootstrap test were done in the TWOSEX-MS Chart program (Chi et al, 2020).

RESULTS AND DISCUSSION

Toxicity bioassay

The LC_{30} and LC_{50} values along with regression lines details for the insecticidal activity of emamectin benzoate, azadirachtin, imidacloprid, thiacloprid, and lambda-cyhalothrin against the second-instar larvae of insect pest are displayed in Table 1. Toxicity bioassays revealed that, based on the LC_{50} values, emamectin benzoate (0.52 mg A.I./L) and azadirachtin (5.19 mg A.I./L) were more toxic to *T. absoluta* than the other insecticides; imidacloprid (44.63 mg A.I./L), thiacloprid (293.92 mg A.I./L), and lambda-cyhalothrin (610.08 mg A.I./L). The calculated LC_{30} of each chemical such as emamectin benzoate (0.31 mg A.I./L) and azadirachtin (2.39 mg A.I./L), imidacloprid (18.64 mg A.I./L), thiacloprid (133.48 mg A.I./L), and lambda-cyhalothrin (438.79 mg A.I./L) was used to evaluate its sublethal effects. According to r^2 values represented in table 1, the mortality of insect pests was positively attributed to the tested concentrations of insecticides.

Table 1. Toxicity of emamectin benzoate, azadirachtin, imidacloprid, thiacloprid, and lambdacyhalotrin against second-instar larvae of *Tuta absoluta* under laboratory conditions.

	Concentration mg A.I/L (95% CL)		X^2 (df)	Slope ± SE	Sig.*	r^2
	LC_{30}	LC_{50}				
Emamectin benzoate	0.31 (0.25 - 0.36)	0.52 (0.46 - 0.59)	14.54 (18)	2.30 ± 0.13	0.69	0.88
Azadirachtin	2.39 (1.75 - 2.97)	5.19 (4.35 - 6.17)	3. 50 (18)	1.56 ± 0.09	0.99	0.86
Imidacloprid	18.64 (13.91 - 23.01)	44.63 (36.75 - 57.00)	14.30 (18)	1.38 ± 0.09	0.70	0.98
Thiacloprid	133.48 (100.10 - 163.80)	293.92 (246.90 - 354.90)	2.68 (18)	1.53 ± 0.09	0.99	0.93
Lambdacyhalotrin	438.79 (382.00 - 483.27)	610.08 (564.50 - 656.14)	6.44 (18)	3.66 ± 0.21	0.96	0.94

*Considering the significance level is more than 0.05, no heterogeneity factor was utilized in the estimation of fiducial limits. The number of tested insects was 480 for each insecticide.

Many studies reported the high efficiency of emamectin benzoate in the control of Lepidopteran pests (Lopez, Latheef, & Hoffmann, 2010; Baniameri & Cheraghian, 2012; Gacemi & Guenaoui, 2012; Mahmoud, Soliman, Abdel-Moniem, & Abdel-Raheem, 2013; Berxolli & Shahini, 2018). Roditakis et al, (2013) studied the effects of some insecticides, including emamectin benzoate, on second-instar larvae of *T. absoluta* with LC_{50} values of 0.08-0.26 mg/l after 72 h exposure time. In the other study, LC_{50} values of emamectin benzoate on *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) calculated as 0.46, 0.41, and 0.18 ppm within 24, 48, and 72 h, respectively (Lopez, Latheef, & Hoffmann, 2010). According to Roditakis et al (2018), no cases of control failure have reported in Europe to date for emamectin benzoate. On the contrary, Mujica, Pravatiner, & Cisneros (2000) reported that abamectin affects full-grown embryo and first-instar larvae but does not damage the embryo at earlier developmental stage. Therefore, the growth stage would affect the results of using this insecticide. Based on the low LC_{50} value reported in previous and present studies, emamectin benzoate had high efficiency in the management of insect pests, which can be attributed to its translaminar ability into plant tissue (Ishaaya, Barazani, & Horowith, 2002; Bengochea, Sánchez-Ramos, Saelices, & Amor, 2014). High effectiveness of azadirachtin was reported on *T. absoluta* and *Leucoptera coffeella* (Guérin-Méneville and Perrottet) (Lepidoptera: Lyonetiidae) larvae in the previous studies (Venzon et al, 2005; Berxolli & Shahini, 2017). Tomé et al. (2013) indicated that azadirachtin was very toxic to Brazilian populations of *T. absoluta* not only by direct toxicity but also by an egg-laying and larval movement deterrence effects, indicating its multiple modes of actions in the present and previous studies. Also, similar to findings of Mahmoud, Soliman, Abdel-Moniem, & Abdel-Raheem (2013), imidacloprid presented a good control against *T. absoluta* larvae in the present study. In disagreement with our results, imidacloprid was not considerably effective on tomato leafminer eggs in study of Nozad-Bonab, Hejazi, Iranipour, & Arzanlou, (2017). differences can be due to the different stages of the insect being tested.

Sublethal effects on demographic parameters

The studied insecticides had significantly adverse effects on demographic parameters of Tomato leafminer such as r_m, λ, R_0, *GRR*, and *T* (Table 2). The r_m rates were from 0.075 to 0.140 day^{-1} in different treatments, with the highest and lowest levels in control and those treated with emamectin benzoate, respectively. Except for lambda-cyhalothrin, the r_m values in treatments were statistically different from the control groups. The lowest amount of R_0 found in insects treated with emamectin benzoate, while it was the highest in the control group. The lowest (19.33 offspring) and the greatest (59.28 offspring) values of *GRR* were recorded in emamectin benzoate and control treatments, respectively. The *T* values increased from 28.85 days for the control group to 28.85, 29.18, 29.65, 32.91, and 33.65 days for lambda-cyhalothrin, thiacloprid, imidacloprid, azadirachtin, and emamectin benzoate, respectively. Regarding the λ parameter, the lowest value was observed for individuals treated with emamectin benzoate, whereas the highest value was recorded for the control.

We could decrease the pest populations by studying the demographic parameters of a pest and the recognition of the chief factors of population growth rate. Moreover, comprehension of the sublethal effects of insecticides can lead to the thoughtful utilization of these chemicals and decrease their side effects. Sublethal effects have been evaluated to discover the harmful and chronic impacts of pesticides on pest life-table parameters, which may also affect pest population dynamics (Stark & Banks, 2003). The parameter r_m has a suitable statistical base to describe population growth rates. However, r_m is influenced by various factors such as geographical origin, insect species, host type, climatic conditions (such as temperature, humidity, and light), the longevity of adults, and so on. The r_m values for emamectin benzoate and azadirachtin treatments, compared to the control group, decreased by almost 46 and 35%, respectively, while for imidacloprid, thiacloprid, and lambda-cyhalothrin treatments it was dropped to 28, 9, and 4%, respectively. The R_0 parameter signifies the number of offsprings created by a female, considering the probability of female survival per generation. This parameter significantly declined in all insecticide treatments compared to the control groups in the current study. The R_0 value for emamectin benzoate reduced by about 76% compared to the control, which was lower for the other treatments. Lixia, Changhui, & Xiaowei (2011) examined the effects of emamectin benzoate on the developmental periods of *H. armigera*, and noted that when third-instar larvae were treated with the LC_{25} of this insecticide, R_0, r_m, and λ declined by 77, 29, and 5%, respectively, and *T* increased by 6%. In the other study, in agreement with our results, Zibaee & Esmaeily (2017) reported a value of 22.42 for R_0 in *T. absoluta* under the influence of abamectin LC_{30}. The *T* parameter is the needed time for a population to increase by the net reproductive rate. In the current study, the amount of *T* parameter had an increasing trend from the control to emamectin benzoate treatment. This parameter by the LC_{25} of abamectin, chlorantraniliprole, and spinosad on *T. absoluta* had an increasing trend like that of our study (Nozad-Bonab, Hejazi, Iranipour, & Arzanlou, 2017). The gross reproductive rate (*GRR*) denotes the average number of eggs that a female produces in a single generation, providing that the female survives until the last possible day. The highest and lowest *GRR* were obtained in the control and emamectin benzoate treatments, respectively, which are consistent with those reported by Nozad-Bonab, Hejazi, Iranipour, & Arzanlou (2017). The parameter λ indicates the value in which a stable population will increase per day compared to the previous day. Its values with a decreasing trend ranged from 1.15 to 1.07 day^{-1} in the control and emamectin benzoate treatments, respectively. In line with our study, this parameter had a decreasing trend by sublethal effect of abamectin on *T. absoluta* in the study of Nozad-Bonab, Hejazi, Iranipour, & Arzanlou (2017). The sublethal concentration of abamectin significantly reduced the emergence, fecundity, and longevity of *T. absoluta* adults. Further, demographic parameters of the pest, including r_m, *GRR*, R_0, and λ, decreased in comparison to the control (Zibaee & Esmaeily, 2017), which is consistent with the results of the present study.

Sublethal effects on the life history parameters and the age-stage specific survival rate

The LC_{30} of studied insecticides prolonged the period of pre-adult stages and diminished the adult period and fecundity of *T. absoluta* (Table 3). Larval developmental period significantly increased by insecticides except for thiacloprid and lambda-cyhalothrin in comparison with the control group. For instance, the developmental period of forth-instar larvae in the control group was 3.02 days, which increased to 4.25 days in emamectin benzoate treatment. The pupal period was the lowest (7.83 days) in the control group while it was continued to 8.83 days in the treatment of emamectin benzoate. The longevity of adults was the lowest in the emamectin benzoate in comparison with the control. The highest and lowest adult pre-oviposition period (APOP) values were also observed in the control and emamectin benzoate treatments, respectively. The longest total pre-oviposition period, TPOP, was recorded for those treated with emamectin benzoate (32.08 days), while the lowest one occurred in the control group (25.5 days). Fecundity ranged from 38.67 offspring/female in emamectin benzoate treatment to 118.58 offspring/female in the control group. The lowest oviposition period (4.21 days) belonged to the emamectin benzoate treatment.

Table 2. Mean (±SE) demographic parameters of *Tuta absoluta* treated by sublethal concentration (LC30) of emamectin benzoate, azadirachtin, imidacloprid, thiacloprid, and lambdacyhalotrin.

Parameters (unit)	Control	Lambdacyhalotrin	Thiacloprid	Imidacloprid	Azadirachtin	Emamectin benzoate
r_m (day^{-1})	0.140±0.005^{a}	0.134±0.005^{ab}	0.127±0.005^{b}	0.100±0.005^{c}	0.090±0.005^{d}	0.075±0.005^{e}
R_0 (offspring)	56.92±8.47^{a}	50.32±7.66^{b}	44.07±6.84^{c}	27±4.43^{d}	21.22±3.50^{e}	13.64±2.41^{f}
GRR (offspring)	59.28±8.66^{a}	55.56±8.11^{a}	50.5±7.40^{b}	33.75±5.12^{c}	27.85±4.15^{c}	19.33±3.07^{d}
T (day) λ (day^{-1})	28.85±0.35^{c} 1.15±0.006^{a}	29.18±0.34^{c} 1.14±0.006^{ab}	29.65±0.34^{c} 1.13±0.006^{b}	32.91±0.34^{b} 1.10±0.005^{c}	33.65±0.34^{ab} 1.09±0.005^{c}	34.45±0.31^{a} 1.07±0.005^{d}

The same letters in each row indicate no significant difference at the 5% probability level (paired bootstrap test). rm, R0, GRR, T, and λ are the intrinsic rate of increase, net reproductive rate, gross reproductive rate, mean generation time, and finite rate of increase, respectively.

Table 3. Mean (± standard error) life-history parameters of *Tuta absoluta* treated by sublethal concentration (LC30) of emamectin benzoate, azadirachtin, imidacloprid, thiacloprid, lambdacyhalotrin.

Parameters (unit)	Control	Lambdacyhalotrin	Thiacloprid	Imidacloprid	Azadirachtin	Emamectin benzoate
2nd-instar (days)	2.84±0.09^{c}	2.92±0.0^{9}C	2.98±0.08^{c}	3.57±0.11^{b}	3.65±0.11^{ab}	4.03±0.08^{a}
3rd-instar (days)	2.28±0.06^{d}	2.38±0.06^{d}	2.43±0.07^{d}	2.92±0.07^{b}	3.02±0.07^{ab}	3.34±0.08^{a}
4th-instar (days)	3.02±0.09^{b}	3.2±0.08^{b}	3.38±0.07^{b}	3.92±0.09^{a}	4±0.09^{a}	4.25±0.08^{a}
Pupa (days)	7.83±0.10^{b}	7.96±0.09^{b}	8.02±0.09^{b}	8.56±0.07^{ab}	8.67±0.08^{a}	8.83±0.09^{a}
Pre-adult (days)	24.15±0.21^{d}	24.79±0.22^{cd}	25.42±0.2^{c}	29.02±0.23^{b}	29.92±0.23^{b}	31.23±0.22^{a}
Female longevity (days)	28±3.53^{a}	27.17±3.61^{ab}	26.04±3.68^{b}	24.33±3.79^{c}	22.17±3.85^{d}	18.17±3.94^{e}
Male longevity (days)	25.88±2.58^{a}	25.33±3.47^{ab}	24.50±3.54^{b}	23.42±3.64^{c}	21.04±3.79^{d}	17.88±3.87^{e}
APOP (days)	1.79±0.08^{a}	1.71±0.09^{ab}	1.67±0.1^{abc}	1.29±0.09^{cd}	1.21±0.08^{d}	1.04±0.04^{d}
TPOP (days)	25.5±0.33^{c}	26.08±0.35^{c}	26.83±0.35^{c}	30.42±0.3^{b}	31.25±0.28^{ab}	32.08±0.29^{a}
Fecundity (egg/female)	118.58±2.58^{a}	111.12±2.44^{a}	101±2.45^{b}	67.5±3.10^{c}	55.71±2.17^{d}	38.67±2.6^{e}
Oviposition period (days)	12.58±0.12^{a}	10.21±0.12^{b}	9.04±0.13^{c}	6.21±0.15^{d}	5.38±0.12^{e}	4.21±0.2^{f}

The same letters in each row indicate no significant difference at the 5% probability level (paired bootstrap test). APOP and TPOP are the adult pre-oviposition period and total pre-oviposition period, respectively.

Reduction in the incubation period of *T. absoluta* through sublethal concentration of a well-known insecticide pyriproxyfen reported in a recent work (Tomé, Cordeiro, Rosado, & Guedes, 2011). According to our outcomes, sublethal concentrations of tested insecticides, except for lambda-cyhalothrin, decreased the fecundity of *T. absoluta*. A 55% accumulation of spinosad in the ovaries of the parasitoid wasp *Hyposoter didymator* (Hymenoptera: Ichneumonidae) was reported (Williams, Valle, & Viuela, 2003). It is probably the main reason for the fecundity reduction of *T.*

absoluta by tested insecticides, especially emamectin benzoate. According to the study of Hamdy & El-Sayed (2013), biocompatible insecticides such as emamectin benzoate were more effective than other chemicals such as indoxacarb in controlling *H. armigera* and *T. absoluta* larvae, same to our outcomes. Tomé et al, (2013) presented evidence that azadirachtin was a suitable insecticide for organic agriculture, which could significantly reduce the *T. absoluta* population. The dynamics of populations may be affected by infertility in adults, as mating not generated fertile eggs (Desneux, Decourtye, & Delpuech, 2007). Unfertile eggs of *T. absoluta* influenced by the sublethal concentration of tested chemicals were also found in the present study.

The evaluation of age-stage specific survival rate (s_{xj}) of *T. absoluta* before insecticide treatments exhibited diverse survival rates for different stages (Fig. 1). The longevity of females (34 days) was more extended than the males (32 days) in control, but this amount was almost equal in insecticide treatments. In the insects treated with emamectin benzoate, azadirachtin, and imidacloprid, the survival of forth-instar larvae, pupae, and adults of insect pest was reduced compared to control.

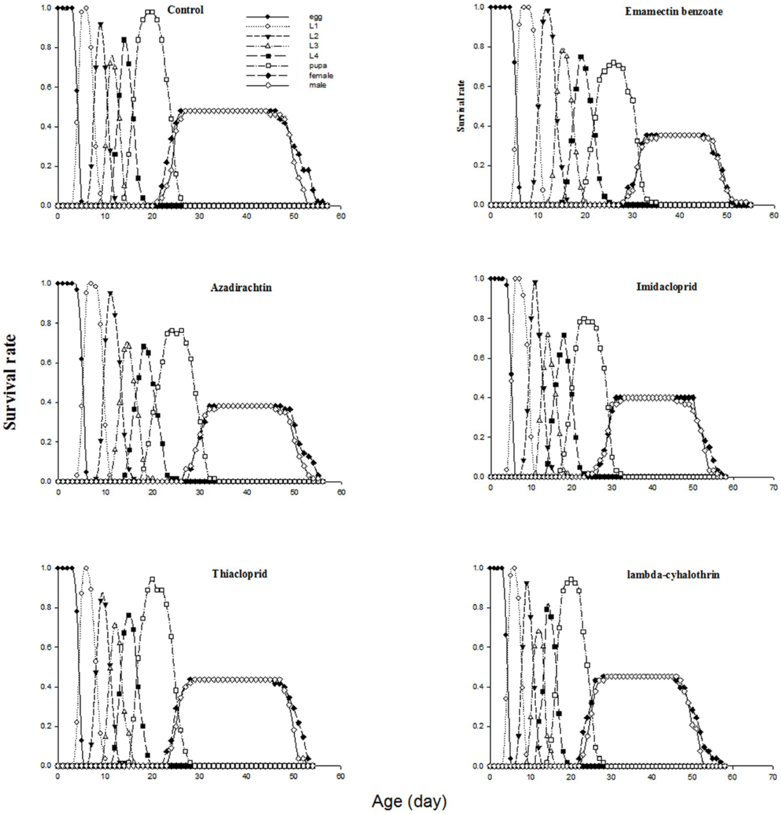


Fig. 1. Age-stage specific survival rate (sxj) curve of *Tuta absoluta* treated by the sublethal concentration of emamectin benzoate, azadirachtin, imidacloprid, thiacloprid, lambdacyhalotrin, and control. The curve of egg and first instar larvae is untreated.

Survival reduction will reduce the population and number of generations per year. Based on differences in the growth rate of treated individuals, an overlap is observed in this curve between the different stages. This curve shows the probability that treated individuals will survive to age *x* and stage *j*. Similar to this study, notable reduction was found in the survival of *T. absoluta* larval stages by sublethal concentrations of abamectin (Zibaee & Esmaeily, 2017).

CONCLUSION

According to results of the present study, the toxicity and efficiency of emamectin benzoate and azadirachtin were higher than lambda-cyhalothrin, imidacloprid, and thiacloprid in all biological stages of *T. absoluta*. Due to the insecticidal effects on the mortality, life history, and demographic parameters of *T. absoluta*, emamectin benzoate can be distinguished as a potential agent for the management of this damaging insect pest. Accordingly, emamectin benzoate, as one of the novel insecticides in Iran, would be an excellent option for operative control of *T. absoluta*, if confirmed by the further filed data.

ACKNOWLEDGMENTS

We greatly appreciate the University of Mohaghegh Ardabili for financial support.

REFERENCES

Abbott, W. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18, 265-267.

Baniameri, V. & Cheraghian, A. (2012). The first report and control of *Tuta absoluta* in Iran. *Eppo Bulletin*, 42(2), 322-324.

Bengochea, P. Sánchez-Ramos, I. Saelices, R., & Amor, F. (2014). Is emamectin benzoate effective against the different stages of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae)? *Irish Journal of Agricultural and Food Research*, 53(1), 37-49.

Berxolli, A. & Shahini, Sh. (2017). Azadirachtin, a useful alternative for controlling *Tuta absoluta* (Myerick). *European Journal of Physical and Agricultural Sciences*, 5, 40-45.

Berxolli, A. & Shahini, Sh. (2018). Controlling *Tuta absoluta* (Myerick, 1917) based on emamectin benzoate usage. *European Journal of Physical and Agricultural Sciences*, 6(2), 1-6.

Biondi, A., Guedes, R.N.C., Wan, F.H., & Desneux, N. (2018). Ecology, worldwide spread and anagement of the invasive South American tomato pinworm, *Tuta absoluta*: past, present and future. *Annual Review of Entomology*, 63, 239-258.

Branherotto, R. & Vendraim, J.J. (2010). Efeito de genotipos de tomateiro e de extratos aquosos de folhas de (*Melia azedarach*) de sementes de (*Azadirachta indica*) sobre *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Neotropical Entomology*, 39(5), 784-791.

Carey, J.R. (1993). *Applied demography for biologists with special emphasis on insects*. Oxford University Press.

Charleston, D.S., Kafir, R., Dicke, M., & Vet, L.E.M. (2006). Impact of botanical extracts derived from *Melia azedarach* and *Azadirachta indica* on populations of *Plutella xylostella* and its natural enemies: a field test of laboratory findings. *Biological Control*, 39, 105-114.

Chi, H. & Yang, T. (2003). Two-sex life table and predation rate of *Propylaea japonica* Thunberg (Coleoptera: Coccinellidae) fed on *Myzus persicae* (Sulzer) (Homoptera: Aphididae). *Environmental Entomology*, 32, 327-333.

Chi, H., You, M., Atlıhan, R., Smith, C.L., Kavousi, A., & Özgökçe, M.S. (2020). Age-Stage, two-sex life table: an introduction to theory, data analysis, and application. *Entomologia Generalis*, 40(2), 103-124.

Cloyd, R. (2012). *Indirect effects of pesticides on natural enemies.* In R.P. Soundararajan (Ed.). Pesticides: advances in chemical and botanical pesticides (pp. 127-150) Intech Open Access Publisher, Burlington, Canada.

Desneux, N., Decourtye, A., & Delpuech, J.M. (2007). The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology*, 52, 81-106.

Desneux, N., Wajnberg, E., Wyckhuys, K.A., Burgio, G., Arpaia, S., Narváez-Vasquez, C.A., González-Cabrera, J., Ruescas, D.C., Tabone, E., Frandon, J., Pizzol, J., Poncet, C., Cabello, T., & Urbaneja, A. (2010). Biological invasion of european tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. *Journal of Pest Science*, 83(3), 197-215.

Dong, J., Wang, K., Li, Y., & Wang, Sh. (2017). Lethal and sublethal effects of cyantraniliprole on *Helicoverpa assulta* (Lepidoptera: Noctuidae). *Pesticide Biochemistry and Physiology*, 136, 58-63.

Efron, B. & Tibshirani, R.J. (1993). *An introduction to the bootstrap*. Chapman and Hall, New York, USA.

Eman, M., Redwan, M., & Hanan, S.T. (2012). Toxicand biochemical effects of different insecticideson the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), *Egyptian Academic Journal of Biological Sciences*, 4(1), 1-10.

Gacemi, A. & Guenaoui, Y. (2012). Efficacy of emamectin benzoate on *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) infesting a protected tomato crop in Algeria. *Journal of Entomology and Nematology*, 5, 37-40.

Galdino, T.V.S., Picanço, M.C., Morais, E.G.F., Silva, N.R., Silva, G.A.R., & Lopes, M.C. (2011). Bioassay method for toxicity studies of insecticide formulations to *Tuta absoluta* (MEYRICK, 1917). *Ciência e Agrotecnologia*, 35(5), 869-877.

Gontijo, P., Picanço, M., Pereira, E., Martins, J., Chediak, M., & Guedes, R. (2013). Spatial and temporal variation in the control failure likelihood of the tomato leaf miner, *Tuta absoluta*. *Annals of Applied Biology*, 162(1), 50-59.

Guedes, R. & Siqueira, H. (2013). The tomato borer *Tuta absoluta*: insecticide resistance and control failure. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, 7(005), 245.

Guedes, R. & Picanço, M. (2012). The tomato borer, *Tuta absoluta* in south america: pest status, management and insecticide resistance. *EPPO Bulletin*, 42(2), 211-216.

Guillemaud, T., Blin, A., Le, G.I., Desneux, N., Reyes, M., Elisabeth, T., Tsagkarakou, A., Niño, L., & Lombaert, E. (2015). The tomato borer, *Tuta absoluta*, invading the Mediterranean Basin, originates from a single introduction from Central Chile. *Scientific Reports*, 5, 8371.

Hamdy, H.E.M. & El-Sayed, W. (2013). Efficacy of bio-and chemical insecticides in the control of *Tuta absoluta* (Meyrick) and *Helicoverpa armigera* (Hubner) infesting tomato plants. *Australian Journal of Basic and Applied Sciences*, 7(2), 943-948.

Ishaaya, I., Barazani, A., & Horowith, A.R. (2002). Emamectin, a novel insecticide for controlling field crop pests. *Pest Management Science*, 58, 1091-1095.

Iwasa, T., Motoyama, N., Ambrose, J.T., & Roe, R.M. (2003). Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*. *Crop Protection*, 23, 371-378.

Jin, T., Lin, Y-Y., Jin, Q-A., Wen, H-B., & Peng, Z-Q. (2014). Sublethal effect of avermectin and acetamiprid on the mortality of different life stages of *Brontispa longissima* (Gestro) (Coleoptera: Hispidae) and its larvae parasitoid *Asecodes hispinarum* Boucek (Hymenoptera: Eulophidae). *Crop Protection*, 58, 55-60.

Kandil, M.A.H., Sammour, A.E., Abdel-Aziz, N.F., Abdel-Maguied, E., El-Bakry, A.M., & Abdelmaksoud, V. (2020). Comparative toxicity of new insecticides generations against tomato leafminer *Tuta absoluta* and their biochemical effects on tomato plants. *Bulletin of the National Research Centre*, 44, 126.

Lietti, M.M., Botto, E., & Alzogaray, R.A. (2005). Insecticide resistance in argentine populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Neotropical Entomology*, 34(1), 113-119.

Lixia, D., Changhui, R., & Xiaowei, T. (2011). Effect of sublethal dose of emamectin benzoate on growth and development of *Helicoverpa armigera* (Hübner). *Acta Phytophylacica Sinica*, 38(6), 530-544.

Lopez, J.D., Latheef, M.A., & Hoffmann, W.C. (2010). Effect of emamectin benzoate on mortality, proboscis extension, gustation and reproduction of the corn earworm, *Helicoverpa zea*. *Journal of Insect Science*, 10(89), 1-16.

Mahmoud, M.M., Soliman, A.S.H., Abdel-Moniem, B., & Abdel-Raheem, M.A. (2013). Impact of some insecticides and their mixtures on the population of tomato borers, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in tomato crop at Upper Egypt. *Archives of Phytopathology and Plant Protection*, 47, 1764-1776.

Mahmoud, Y.A., Salem, H.A., Shalaby, S.E.M., Abdel-Razak, A.S., & Ebadah, I.M.A. (2014). Effect of certain low toxicity insecticides against tomato leaf miner, *Tuta absoluta* (Lepidoptera: Gelechiidae) with reference to their residues in harvested tomato fruits. *International Journal of Agricultural Research*, 9, 210-218.

Michaelides, G., Seraphides, N., Pitsillou, M., & Fenthourakis, S. (2019). Susceptibility of Cypriot *Tuta absoluta* populations to four targeted insecticides and control failure likelihood. *Journal of Applied Entomology*, 143, 508-517.

Moscardini, V.F., Gontijo, P.C., Carvalho, G.A., Oliveira, R.L., Maia, J.B., & Silva, F.F. (2013). Toxicity and sublethal effects of seven insecticides to eggs of the flower bug *Orius insidiosus* (Say) (Hemiptera: Anthocoridae). *Chemosphere*, 92, 490-496.

Mujica, N., Pravatiner, M., & Cisneros, F. (2000). Effectiveness of abamectin and plant-oil mixtures on eggs and larvae of the leafminer fly, *Liriomyza huidobrensis* Blanchard. CIP. Program. Report. 1999-2000, pp. 161-166, Peru.

Nozad-Bonab, Z., Hejazi, M.J., Iranipour, Sh., & Arzanlou, M. (2017). Lethal and sublethal effects of some chemical and biological insecticides on *Tuta absoluta* (Lepidoptera: Gelechiidae) eggs and neonates. *Journal of Economic Entomology*, 1-7.

Rasheed, M.A., Khan, M.M., Hafeez, M., Zhao, J., Islam, Y., Ali, S., Ur-Rehman, S., Hani, U., & Zhou, X. (2020). Lethal and sublethal effects of chlorpyrifos on biological traits and feeding of the aphidophagous predator *Harmonia axyridis*. *Insects*, 11, 491.

Roditakis, E., Skarmoutsou, C., Staurakaki, M., Martínez-Aguirre, M.R., Garc Vidal, L., & Bielza, P. (2013). Determination of baseline susceptibility of european populations of *Tuta absoluta* (Meyrick) to indoxacarb and chlorantraniliprole using a novel dip bioassay method. *Pest Management Science*, 69(2), 217-227.

Roditakis, E., Vasakis, E., Garcia-Vidal, L., Rosario Martínez-Aguirre, M., Rison, J.L., Haxaire-Lutun, M.O., & et al. (2018). A four-year survey on insecticide resistance and likelihood of chemical control failure for tomato leaf miner *Tuta absoluta* in the European/Asian region. *Journal of Pest Science*, 91,421-435.

Santos, A.C.D., Bueno, C.O.F., Vieira, S.S., & Bueno, A.F. (2011). Efficacy of insecticides on *Tuta absoluta* (Meyrick) and other pests in pole tomato. *BioAssay*, 6(4), 1-6.

Silva, W.M., Berger, M., Bass, C.H., & Balbino, V.Q. (2015). Status of pyrethoids resistance and mechanisms in Brazilian populations of *Tuta absoluta*. *Pesticide Biochemistry and Physiology*, 122, 8-14.

Smith, J.D., Dubois., T., Mallogo, R., Njau, E.F., Tua, S., & Srinivasan, R. (2018). Host range of the invasive tomato pest *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) on solanaceous crops and weeds in Tanzania. *Florida Entomologist*, 101(4), 573-579.

Soares, M.A., Passos, L.C., Campos, M.R., Collares, L.J., Desneux, N., & Carvalho, G.A. (2019). Side effects of insecticides commonly used against *Tuta absoluta* on the predator *Macrolophus basicornis*. *Journal of Pest Science*, 92, 1447-1456.

Stark, J.D. & Banks J.E. (2003). Population-level effects of pesticides and other toxicants on arthropods. *Annual Review of Entomology*, 48(1), 505-519.

Stark, J.D., Sugayama, R.L., & Kovaleski, A. (2007). Why demographic and modeling approaches should be adopted for estimating the effects of pesticides on biocontrol agents. *BioControl*, 52, 365-374.

Terzidis, A.N., Wilcockson, S., & Leifert, C. (2014). The tomato leaf miner (*Tuta absoluta*): conventional pest problem, organic management solutions? *Organic Agriculture*, 4, 43-61.

Tomé, H., Cordeiro, E., Rosado, J., & Guedes, R. (2011). Egg exposure to pyriproxyfen in the tomato leaf miner *Tuta absoluta*: ovicidal activity or behavioural-modulated hatching mortality? *Annals of Applied Biology*, 160(1), 35-42.

Tomé, H., Martins, J.C., Corrêa, A.S.T., Galdino, V.S., Picanço, M.C., & Guedes, R.N.C. (2013). Azadirachtin avoidance by larvae and adult females of the tomato leafminer *Tuta absoluta*. *Crop Protection*, 46, 63-69.

Tropea, G., Siscaro, G., Biondi, A., & Zappalà, L. (2012). *Tuta absoluta*, a south american pest of tomato now in the EPPO region: biology, distribution and damage. *EPPO Bulletin*, 42(2), 205-210.

Urbaneja, A., González-Cabrera, J., Arnó, J., & Gabarra R. (2012). Prospects for the biological control of *Tuta absoluta* in tomatoes of the Mediterranean basin. *Pest Management Science*, 68(9), 1215-1222.

Venzon, M., Rosado, M.C., Fadini, M.A.M., Ciociola, A.I., & Pallini, A. (2005). The potential of neemazal for the control of coffee leaf pests. *Crop Protection*, 24, 213-219.

Williams, T., Valle, J., & Viuela, E. (2003). Is the naturally derived insecticide Spinosad® compatible with insect natural enemies? *Biocontrol Science and Technology*, 13, 459-475.

Yin, X.H., Wu, Q.J., Li, X.F., Zhang, Y.J., & Xu, B.Y. (2008). Sublethal effects of spinosad on *Plutella xylostella* (Lepidoptera: Yponomeutidae). *Crop Protection*, 27(10), 1385-1391.

Zibaee, I. & Esmaeily, M. (2017). Effect of sublethal doses of abamectin on demographic traits of tomato leafminer *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae). *Journal of Plant Protection Research*, 57(3), 256-267.

J. Entomol. Res. Soc., 24(1): 103-110, 2022
Doi: 10.51963/jers.v24i1.2203

Research Article
Online ISSN:2651-3579

The Cockroach Wasps of Turkey, with a New Record of the Genus *Trirogma* Westwood 1841 (Hymenoptera: Ampulicidae)

İlyas CAN

Department of Biology, Faculty of Arts and Sciences, Tokat Gaziosmanpaşa University, Tokat, TURKEY
e-mail: ilyascan41@gmail.com
ORCID ID: 0000-0003-3013-6614

ABSTRACT

In this study, the genus *Trirogma*, which belongs in the subfamily Ampulicinae (Hymenoptera: Ampulicidae), was recorded for the first time from Turkey. The new record is based on a male specimen of *Trirogma caerulea* Westwood, 1841 from the Antalya province. Unlike other members of the genus, which have a limited distribution in the Oriental region and its immediate surroundings, *T. caerulea* has a relatively wider distribution in the Palearctic. With this study, the distribution area of this species was extended to the western Mediterranean coasts of Turkey, which is also the westernmost distribution point of the genus. A preliminary list of the species of Ampulicidae found in Turkey was also presented.

Key words: Ampulicidae, Hymenoptera, new record, *Trirogma*, Turkey.

Can, İ. (2022). The cockroach wasps of Turkey, with a new record of the genus *Trirogma* Westwood 1841 (Hymenoptera: Ampulicidae). *Journal of the Entomological Research Society*, 24(1), 103-110.

Received: December 15, 2021 *Accepted: March 10, 2022*

INTRODUCTION

The family Ampulicidae is a small group within Apoidea, commonly known as “cockroach wasps” with reference to their prey. It currently contains 205 valid species belonging to six genera within the subfamilies Ampulicinae and Dolichurinae (Pulawski, 2022). Members of this family mostly have a tropical distribution, except for the almost cosmopolitan genera *Ampulex* and *Dolichurus* (Ohl & Spahn, 2010). Most of these wasps are small to large sized and mostly have black integument, with some species having white or red markings, but many tropical species in *Ampulex* and all *Trirogma* have a metallic green or blue coloration (Soliman, Gadallah, Ohl & Al Dhafer, 2017). As far as is known, all species of Ampulicidae prey on cockroaches to provide food for their larvae. Some species of this group are considered as biological control agents due to their ability to hunt cockroaches (Veltman & Wilhelm, 1991; Lebeck, 1991).

Although many studies have been carried out on spheciform wasps, especially the Crabronidae and Sphecidae (Gülmez & Can, 2015; Yıldırım, Ljubomirov, Özbek & Yüksel, 2016; Can & Gülmez, 2018; 2019; 2021; Kaplan & Yıldırım, 2020; 2021), there are remarkably few published reports of the Ampulicidae (de Beaumont, 1967; 1969; Gayubo & Özbek, 2005; Bayındır, Gürbüz, Ljubomirov & Pohl, 2013; Yıldırım et al., 2016; Dollfuss, 2017; Makrousov & Proshchalykin, 2021). In Turkey, Ampulicidae has been represented by three species of the genus *Dolichurus*: *Dolichurus bicolor*, *D. corniculus* and *D. haemorrhous*. Prior to this study, there was no report of the genus *Trirogma*. Except for *Dolichurus corniculus*, which is included in the catalog by Ljubomirov & Yıldırım (2008), data on this family have never been compiled in Turkey.

The current study aims to contribute to the knowledge of Turkish fauna of Ampulicidae, with a new record of the genus *Trirogma*. This paper also presents an annotated species list of family Ampulicidae in Turkey.

MATERIAL AND METHODS

A species list of Turkish Ampulicidae is prepared based on the available literature and newly collected specimen from the Antalya province in 2021. Data on the presence of *Trirogma caerulea* in Turkey were generated with the help of citizen scientists. The specimen was identified using the key by Girish Kumar & Sheela (2018). The material is deposited in the Department of Biology, Tokat Gaziosmanpaşa University, Tokat, Turkey. The photographs of the specimens were taken using a Leica M205C stereomicroscope controlled by the Leica Application Suite 3 software. For each species, its current name, its distribution in Turkey and in the world and also some taxonomic notes are presented.

RESULTS

Family Ampulicidae Shuckard, 1840

Subfamily Dolichurinae Dahlbom, 1842

Tribe Dolichurini Dahlbom, 1842

Genus *Dolichurus* Latreille, 1809 (Fig. 1)

Dolichurus Latreille 1809: 387. Type species: *Pompilus corniculus* Spinola 1808, designated by Latreille 1810: 438.

Thyreosphex Ashmead 1904: 282. Type species: *Thyreosphex stantoni* Ashmead 1904, by monotypy.

Diagnosis: Both antennal sockets covered by a single median platform like lobe; metasternum emarginated posteriorly; metasoma inserted above and somewhat behind hind coxae; fore wing media diverging after cu-a; hind wing media diverging before cu-a; hind wing jugal lobe present; notauli well developed, complete to posterior scutal margin or nearly so; propodeal outline rather sharply bent in profile (Bohart & Menke, 1976; Girish Kumar & Sheikh, 2018; Anagha, Girish Kumar & Sureshan, 2020).

Fig. 1. Distribution map of species of *Dolichurus* in Turkey (Red: *D. bicolor*, green: *D. corniculus*, blue: *D. haemorrhous*).

Dolichurus bicolor Lepeletier de Saint Fargeau, 1845

Records from Turkey: Isparta (Bayındır et al, 2013).

Global distribution: Austria, Belgium, Finland, France, Germany, Greece, Italy, Portugal, Slovenia, Spain, Switzerland, Russia, Turkey, Ukraine (Pulawski, 2022).

Notes: This species was recorded from the province of Isparta in southwestern Turkey. Two specimens of this species were caught at an altitude of 1500 meters in the Kasnak Oak Nature Reserve (Bayındır et al, 2013).

Dolichurus corniculus (Spinola, 1807)

Records from Turkey: Hatay (de Beaumont, 1967; 1969), Erzurum (Gayubo & Özbek, 2005), Kars (Yıldırım et al, 2016), Adana, Muğla, Osmaniye, Samsun (Dollfuss, 2017).

Global distribution: Algeria, Andorra, Austria, Belarus, Belgium, Bulgaria, Croatia, Czechia, Estonia, Finland, France, Germany, Great Britain, Greece, Hungary, Italy, Iran, Latvia, Liechtenstein, Lithuania, Luxemburg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Russia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey (Pulawski, 2022).

Notes: This species, which is distributed in the Mediterranean, Eastern Anatolia, and Black Sea geographical regions of Turkey, is the most common ampulicid in this country. De Beaumont (1967) gave the first record of this species for Turkey with two male specimens from Hatay. Three more male specimens were reported by de Beaumont (1969) from the same province. He stated that the two specimens identified in 1967 were different terms of their sculpture. It was also emphasized by the author that these could be geographical or individual variations. Also, de Beaumont pointed out that the identification of the specimens from Hatay is still controversial due to the lack of females of the species reported from this province (de Beaumont, 1969).

***Dolichurus haemorrhous* A. Costa, 1886**

Records from Turkey: Muğla (Dollfuss, 2017), Antalya (Makrousov & Proshchalykin, 2021).

Global distribution: Egypt, France, Germany, Greece, Iran, Italy, Malta, Morocco, Portugal, Russia, Spain, Turkey, Ukraine (Pulawski, 2022).

Notes: This species has been detected only in the western Mediterranean region of the country. A total of ten male specimens were recorded from Turkey in the literature and no female of the species was recorded. The male of this species is diagnostically problematic as it is very similar to that of *Dolichurus corniculus*. This situation was emphasized by the researchers who carried out the study as follows. Dollfuss (2017) noted that the males of *D. hemorrhous* and *D. corniculus* are very similar, and they cannot always be clearly distinguished. Makrousov & Proshchalykin (2021) also stated that not all used distinguishing characters allow reliable distinction of these species. Makrousov & Proshchalykin (2021) suggested that studying the male genitalia of both species may be useful for species differentiation. They also studied the genitalia of male specimens collected from Turkey. In the same study, they said that both species could only be different color forms of *D. corniculus*.

Subfamily Ampulicinae Shuckard, 1840

Tribe Ampulicini Shuckard, 1840

Genus *Trirogma* Westwood, 1841

Trirogma Westwood, 152. Type species: *Trirogma caerulea* Westwood, 1841, by monotypy.

Diagnosis: Medium to large wasps, 10-33 mm long; integument of body metallic green-blue or purple, with mandibles sometimes white in males; wings not banded. Forewing media diverging at or before cu-a; hind wing media diverging after cu-a;

metasoma distinctly petiolate; antennal bases covered by a median frontal platform (Fig. 2c); metasternum emarginate posteriorly but not Y-shaped, petiole inserted above and after hind coxae (Bohart & Menke, 1976; Girish Kumar & Sheela, 2017; Soliman et al, 2017).

Trirogma caerulea Westwood, 1841

Material examined: Antalya: Konyaaltı, 50 m, 36°54'19"N, 30°39'26"E, 20.10.2021, ♂; Leg. Leyla & Samet Kılınç (citizen scientists).

Global distribution (Fig. 3): China, India, Indonesia, Iran, Iraq, Taiwan, Saudi Arabia, Singapore, Sri Lanka, United Arab Emirates (Pulawski, 2022).

Short description (Male): Length: 9.8 mm (Fig. 2). Integument shining metallic blue; antennal flagellomeres and mandible black; wings hyaline; veins brown. Setae on dorsal part of clypeus and half of inner margin of the compound eyes dense, white and appressed, completely covering integument; other parts of the body covered with scattered erect white setae.

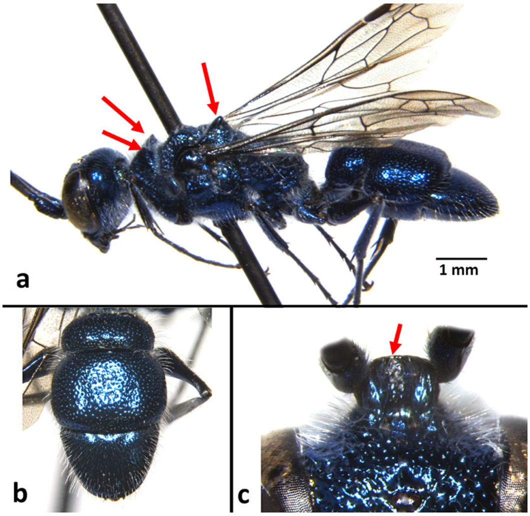


Fig 2. Some taxonomic characters of *Trirogma caerulea* ♂ a. Habitus, lateral view (scutellar tubercle and pronotum with a pair of large, pointed tubercles indicated). b. Mesosoma, dorsal view. c. Frontal lobe.

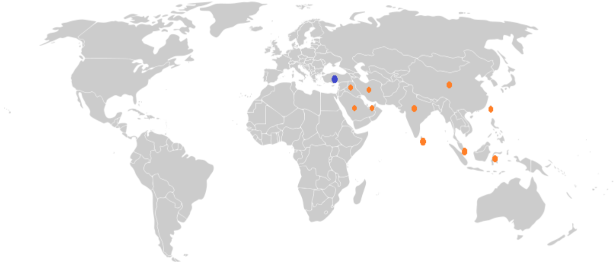


Fig. 3. Distribution map of *Trirogma caerulea* in the world (Orange: previous records, blue: new record).

Head rounded in frontal view; frons with strong rugose punctures; vertex shiny with scattered punctures; medial lobe of clypeus widely and slightly indented; with distinct rounded lateral lobs; dorsal part of clypeus uniformly weakly convex. Flagellomeres dull with rough surface; F1, F2 and F3 equal length.

Pronotum with widely crenulated lateral part; posterior border of pronotal lobe uniformly crenulated as straight line; posterolateral tubercle of pronotum sharply angled (Fig. 2a); notauli strongly crenulate; scutellum with a cone like projection at middle (Fig. 2a); mesopleural groove and sternaulus crenulate; mesopleuron shiny and sparsely punctuated; dorsal and posterior surface of propodeum broadly reticulated and shiny; the upper part of the lateral projections of the propodeum transversely striated.

Metasoma with visible three segments; tergal and sternal punctures well-defined, interspaces shiny (Fig. 2b); T2 with larger punctures than T1 and T3; S2 with a bisinuate groove interrupted at median tubercle.

DISCUSSION

The genus *Trirogma* is herewith reported for the first time from Turkey based on the record of a single specimen of *Trirogma caerulea*. Only one male of the species was caught dwelling in the urban area in the center of Antalya, and many specimens were also photographed from the same region at different times in early October 2021 (Fig. 4). It was also observed that the cockroach population was high in the region where the species was caught. In the light of the information given by the citizen scientist, it is thought that their possible prey is the American cockroach *Periplenata americana*.

This species was originally found in the Oriental region, but in the last few years has expanded its distribution westwards into the Palearctic region. The species was recently recorded in countries of the Arabian Peninsula and Middle East, such as Saudi Arabia, United Arab Emirates, Iraq, and Iran (Ebrahimi, 2008, Ohl, 2011; Dollfuss, 2017; Soliman et al, 2017). The location in this study is now the westernmost point in the Palearctic region, approximately 1300 km away from the nearest known record (Iraq, Baghdad).

Fig. 4. Photographs of different individuals of the species from the same region.

It is not yet known whether the species spread west in the Palearctic region or was transported to Turkey. Since this species has not been encountered before along the southern border of Turkey, including the border with Iraq and Iran, it is thought that the species was probably transported to Antalya by human activity. The fact that Antalya is Turkey's most important tourism center and hosts millions of foreign tourists every year supports this possibility. Furthermore, the humid and warm climate of Antalya seems to be suitable for this species to reproduce in the region. On the other hand, studies on spheciformes species in Turkey are generally carried out in natural habitats. Therefore, this species may have been overlooked until now, as there has not been enough research done in urban area, where cockroaches are densely populated. Future studies are required in order to reveal the possible way of entry of this species into Turkey.

A complete list of species belonging to the Ampulicidae is given for the first time. With the addition of this new genus record, the number of genera belonging to this family in Turkey has increased to two and the number of species to four. Considering Turkey's location, climate diversity and habitat richness, it is thought that species existing in neighboring countries such as *Ampulex assimilis*, *A. compressa*, *A. fasciata* and *Dolichurus turanicus* can also be found in Turkey. Thus, the number of species belonging to the family is expected to increase even more in the future.

ACKNOWLEDGEMENT

I would like to thank Leyla Kılınç and Samet Kılınç for capturing the individual belonging to the species and sending it together with the photographs of different individuals. I also thank Dr. Michael Ohl (Museum für Naturkunde, Berlin, Germany) for helpful contributions and comments.

REFERENCES

Anagha, S., Girish Kumar, P., & Sureshan, P.M. (2020). Additions to the knowledge of the genus *Dolichurus* Latreille (Hymenoptera: Ampulicidae) from India with the description of two new species. *Zootaxa*, 4821(1), 121-134.

Bayındır, H., Gürbüz, M.F., Ljubomirov, T., & Pohl, D. (2013). Diversity of digger wasps in Kasnak Oak Forest Nature Reserve, Isparta, Turkey, with records of eight species new to Turkey (Hymenoptera: Sphecidae, Crabronidae and Ampulicidae). *Zoology in the Middle East*, 59(2), 144-147.

Bohart, R.M. & Menke, A.S. (1976). *Sphecid wasps of the world. A generic revision.* University of California Press, Berkeley, Los Angeles, London, 695 pp.

Can, İ. & Gülmez, Y. (2018). First records of two wasp species, *Diodontus major* and *Parapiagetia tridentata* in Turkey. *Acta Zoologica Bulgarica*, 70(1), 125-127.

Can, İ. & Gülmez, Y. (2019). A new record for the Turkish Sphecidae fauna: *Prionyx radoszkowskyi* (Hymenoptera: Sphecidae). *Entomological News*, 128(4), 346-350.

Can, İ & Gülmez, Y. (2021). A faunistic study on the family Sphecidae (Hymenoptera) in the Upper Kelkit Valley with two new records and a checklist for Turkey. *Turkish Journal of Entomology*, 45(3), 305-322.

de Beaumont, J. (1967). Hymenoptera from Turkey. Sphecidae, I. *Bulletin of the British Museum (Natural History*), 19(5), 251-382.

de Beaumont, J. (1969). Sphecidae de Turquie (Hym.). *Mitteilungen Der Schweizerischen Entomologischen Gesellschaft Bulletin De La Societe Entomologique Suisse*, 62(1), 79-95.

Dollfuss, H. 2017. The Ampulicidae wasps of the "Biologiezentrum-Linz"- collection in Linz, Austria, including the genera *Ampulex* Jurine, *Dolichurus* Latreille and *Trirogma* Westwood (Hymenoptera, Apoidea, Ampulicidae). *Linzer Biologische Beiträge*, 49(1), 441-457.

Ebrahimi, E. (2008). A contribution to the sphecid wasps of Iran (Hymenoptera: Sphecidae), including first record of six species. *Journal of Entomological Society of Iran*, 28(1), 93-97.

Gayubo, S.F. & Özbek, H. (2005). A contribution to the knowledge of Spheciformes from Turkey. Part II. Ampulicidae, Sphecidae and Crabronidae (Bembicinae and Crabroninae) (Hymenoptera: Apoidea). *Journal of the Entomological Research Society*, 7(1), 1-39.

Girish Kumar, P. & Sheikh, A.H. (2018). A taxonomic review of genus *Dolichurus* Latreille (Hymenoptera: Ampulicidae) from India with description of new species. *Species*, 19, 104-116.

Girish Kumar, P. & Sheela, S. (2018). A taxonomic review of the genus *Trirogma* Westwood (Hymenoptera: Ampulicidae) from the Indian subcontinent with the description of a new species. *Devagiri Journal of Science*, 3(1), 110-120.

Gülmez, Y. & Can, İ. (2015). First record of *Sceliphron* (*Hensenia) curvatum (*Hymenoptera: Sphecidae) from Turkey with notes on its morphology and biology. *North-Western Journal of Zoology*, 11(1), 174-177.

Kaplan, E. & Yıldırım, E. (2020). New record of Crabronidae (Insecta: Hymenoptera) from Turkey. *Entomological News,* 129(2), 147-151.

Kaplan, E. & Yıldırım, E. (2021). An updated checklist of Turkish crabronid wasps (Hymenoptera: Crabronidae) with new and additional records. *Journal of Insect Biodiversity*, 21(2), 18-109.

Lebeck, L.M. (1991). A review of the hymenopterous natural enemies of cockroaches with emphasis on biological control. *Entomophaga*, 36, 335-352.

Ljubomirov, T. & Yıldırım, E. 2008. *Annotated catalogue of the Ampulicidae, Sphecidae, and Crabronidae (Insecta: Hymenoptera) of Turkey*. Pensoft, Sofia-Moscow. 316 pp.

Makrousov, M.V. & Proshchalykin, M.Yu. (2021). New and little-known digger wasps (Hymenoptera: Ampulicidae, Sphecidae, Crabronidae) from Russia. *Zootaxa*, 4952(2), 314-330.

Ohl, M. & Spahn, P. (2010). A cladistic analysis of the cockroach wasps based on morphological data (Hymenoptera: Ampulicidae). *Cladistics*, 26(1), 49-61.

Ohl, M. (2011). Order Hymenoptera, family Ampulicidae. In A. van Harten (Ed.): *Arthropod Fauna of the UAE*, (Vol. 4. pp. 475-487) Dar Al Ummah Printing, Abu Dhabi, UAE.

Pulawski, W.J. (2022, February 15). Catalog of Sphecidae. Retrieved fromandroid projeksiyon

http://research.calacademy.org/ent/catalog_sphecidae/

Soliman, A.M., Gadallah, N.S., Ohl, M. & Al Dhafer, H.M. (2017). Revision of the digger wasps of the Ampulicidae and Heterogynaidae (Hymenoptera) of Saudi Arabia, with the description of a new species of the enigmatic genus *Heterogyna* Nagy, *Journal of Natural History*, 51, 2057-2085.

Veltmann, J. & Wilhelm, W. (1991). Husbandry and display of the Jewel wasp, *Ampulex compressa*, and its potential value in destroying cockroaches. *International Zoo Yearbook*, 30(1), 118-126.

Yıldırım, E., Ljubomirov, T., Özbek, H., & Yüksel, M. (2016). New data on Spheciformes fauna (Hymenoptera: Ampulicidae, Sphecidae, Crabronidae) of Turkey. *Journal of Insect Biodiversity*, 4(3), 1-51.

AUTHOR GUIDELINES

Journal of the Entomological Research Society (J. Entomol. Res. Soc.) accepts and publishes original research articles in the all fields of entomology. The journal publishes regular research papers and review articles. Brief and timely reports may be submitted as short communications, where articles with less detailed results and evaluations sections can be accepted as short communication. The Editors first evaluate all manuscripts. At this stage, manuscripts that fail to be original, have serious scientific flaws, have poor grammar or English language, or are outside the aims and scope of the Journal will be rejected. Those that meet the minimum criteria are passed onto at least 2 experts for review. Authors should suggest four reviewers with their names, addresses and e-mail addresses who would review their manuscript. Information on the reviewers should also be uploaded as an appendix to the manuscript. Of these four reviewers, at most two should be in the author's native country and the others will be in other countries. Two reviewers are selected from these four suggested reviewers or editors may assign other reviewers. A final decision to accept or reject the manuscript will be sent to the author along with any recommendations made by the reviewers. Reviewers make recommendations to the Editor whether to accept or reject the manuscript for publishing. The Editor reaches a final decision based on the reviewers' recommendations, as well as his/her own evaluation of the manuscript.

The manuscripts should be written in Arial with 12 type size with double spacing in Microsoft Office Word. The paragraphs should not be indented. The Manuscripts in general should not exceed 30 pages.

Heading: The title of the manuscript should be informative, but preferably not exceed twenty words. Just under the heading, please provide the title, full name(s) of author(s) (The name(s) of all authors should be start with capital letter, and surname(s) should be typed in upper case), with full address and e-mails of each author on a separate line. If a genus or species name is included in the manuscript heading, these should be written in full with no abbreviations, including the author name and date; e.g. Aphodius lividus (Olivier, 1789)

Abstract: An abstract provided at the beginning of the manuscript should indicate the main aspects of the subject, not exceed 200 words, and should be followed by 5-7 key words.

Text: The standard order of sections for original manuscripts is as follows: Introduction, Material and Methods, Results, Conclusions and Discussion, Acknowledgements, References. Sub-titles should be up to the third level and Italic format should be avoided except for species names. . The scientific names (e.g. genus- and species-group names) are the only words to be italicized. References should be cited in the text by the last name(s) of the author(s) and year of publication. Atribution in main text must be given like that (Surname, 1900a; 1900b; 1991; Surname, et al, 2000, Surname1 & Surname2, 2001). Two Authors: The surname of both authors is stated with either 'and' or an ampersand (&) between. For example: Surname1 & Surname2 (2017) state… Or …(Surname1 & Surname2, 2017). Three, Four or Five Authors: For the first cite, all names should be listed: Surname1, Surname2, & Surname3 (2017) state… Or …(Surname1, Surname2, & Surname3, 2017). Further cites can be shorted to the first author's name followed by et al: Surname1 et al (2017) state… Or …(Surname1 et al, 2017). Six or more authors: Only the first author's surname should be stated followed by et al: (Surname1 et al, 2017). Works should be cited with a, b, c etc following the date. For example: (Surname1, 2017a) or (Surname1, 2017b). If these works are by the same author, the surname is stated once followed by the dates in order chronologically. For instance: Surname (2007, 2013, 2017) Or (Surname, 2007, 2013, 2017). If a page number will be given for a quote, the page number must be given after the date, for example (Surname, 2017, p.104). This rule holds for all of the variations listed. Groups of references should be listed chronologically.
For faunistic research follow this order, Distribution:.., Material examined:…, Host plant:….etc.

Example:
Sphex oxianus Gussakovskij, 1928
Distribution: Central and South West Asia, Afghanistan, Iran, Israel, Turkey (Bohart and Menke, 1976; Menke and Pulawski, 2000; Kazenas, 2001), Turkey: Artvin (De Beaumont, 1967).
Material examined: Ankara, Altındağ, Çubuk Dam Lake, 900 m, 29.06.1998, 1 ♂; Kalecik, 600 m, 24. 07. 2001, 2 ♀♀, Kalecik, 800 m, 25. 07. 2001, 3 ♀♀
Host plant: *Echinophora* sp.
Please use ♀, ♂ symbols. Please write upper genus categories with capital letters.

Illustrations: Illustrations, graphs, their caption or legends should form a separate, and a self-explanatory unit. Abbreviations in the legends should be explained but if there are too many, they should be included into a separate list. The original drawing and photographs should not be more than twice as large as when printed.

Morphological illustrations should include a scale bar. Photographs and electron micrographs should be in high-resolution JPEG file format (300 dpi). Drawings (black and white type) should be in TIFF format and their size should be no more than 10 MB. Graphs should also be in black and white and submitted in excel file format. Tables should include headings and explanations, and should be numbered consecutively. Their approximate position in the text should be indicated in the margin. Legends and titles of the graphs and tables should be in Arial with 12 type size. Please do not embed the figures, graphs and table into the text, and send them as supplementary files. In the text attribution to the figures should be given in parenthesis and must be abbreviate like this; (Fig.1).Figs. 1-10. A. marriotti sp. n. 1. Male (holotype), dorsal. 2. Female (paratype)

References:

Titles of manuscripts published in languages other than the major ones (English, German, French, Spanish, Portuguese, Turkish) should be an English translation (in parentheses) with an explanatory note at end, e.g. (in Russian). The list of references should be given at the end of the article and listed alphabetically, according to the following examples. All periodical names should be unabbreviated and italicized. In references, journal titles must be written in full (not abbreviated).

Journal Article

Beirne, B.P. & Young, D.A. (1953). The North American species of Cicadula (Homoptera, Cicadellidae). *Canadian Entomologist*, 85(1), 215-226.

Mitchell, J.A. (2017). Citation: Why is it so important. *Mendeley Journal*, 67(2), 81-95. Retrieved from https://www.mendeley.com/reference-management/reference-manager

Book

Steinmann, H.A. & Zombori, L. (1985). *An atlas of insect morphology*. (2nd ed.). Akadèmiai Kiadò, Budapest, Hungary.

Edited Book

Williams, S.T. (Ed.). (2015). *Referencing: A guide to citation rules* (3rd ed.). New York, NY: My Publisher

Edited Book Chapter

Troy, B.N. (2015). APA citation rules. In S.T, Williams (Ed.). *A guide to citation rules* (2nd ed., pp. 50-95). New York, NY: Publishers.

E-Book

Mitchell, J.A., Thomson, M., & Coyne, R.P. (2017). *A guide to citation*. Retrieved from https://www.mendeley.com/reference-management/reference-manager

Author, A. (date). *Title of book*. doi:xxxxxxxxxxxx

E-Book Chapter

Troy, B.N. (2015). APA citation rules. In S.T, Williams (Ed.). *A guide to citation rules* (2nd ed., pp. 50-95). Retrieved from https://www.mendeley.com/reference-management/reference-manager

Author, A. (date). Title of chapter. In E. Editor (Ed.). *Title of book* (pp. xx-xx). doi:xxxxxxxxxx

URLs

Mitchell, J.A. (2017, May 21). *How and when to reference*. Retrieved from https://www.howandwhentoreference.com.

Thesis

Ersoy, D. E. (2014). *Sündiken Dağlarinin (Eskişehir) Aphodiinae ve Rutelinae Alt Familyalarinin Sistematiği ve Faunasi (Coleoptera: Scarabaeidae)* (Publication No. 12345678) [Doctoral dissertation - Master's thesis, Gazi University]. Council of Higher Education Thesis center. https://tez.yok.gov.tr/UlusalTezMerkezi/

Nomenclature should be in absolute agreement with the current ICZN rules. The only acceptable type concepts are: holotype, paratype, etc. The following abbreviations should be adopted: gen. n., sp. n., stat. n. and comb. n. Journal of the Entomological Research Society uses the Open Journal Systems (OJS) platform, which will enable the journal to accept submissions online. For submitting a manuscript please go to web page http://www.entomol.org and register as author and submit your manuscript online.

Copyright form: You can download JERS copyrigth form in our web site, then sign it with all authors and send us.

URL: http://www.entomol.org

e-mails: jers@entomol.org

Address: Journal of the Entomological Research Society, P.box.110 Bahcelievler P.Isl.Mud. 06502, Ankara/TURKEY

CONTENTS