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Original article (Orijinal araştırma)

Pest status, prevalence and molecular identification of *Hoplopteridius lutosus anatolicus* Osella & Lodos, 1979 (Coleoptera: Curculionidae) in saffron[1]

Safran alanlarında, *Hoplopteridius lutosus anatolicus* Osella & Lodos,1979 (Coleoptera: Curculionidae)'un zarar durumu, yaygınlığı ve moleküler teşhisi

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Abstract

Saffron (*Crocus sativus* L.) is an endangered plant species that has been cultivated in a small field in Safranbolu District of Karabük Province, Turkey. Although the existence of *Hoplopteridius lutosus anatolicus* Osella & Lodos, 1979 (Coleoptera: Curculionidae) was known for Turkey, the present study determined its pest status and, prevalence with respect to its endangered host plant saffron in the Safranbolu District of Karabük between 2015 and 2016. *Hoplopteridius lutosus anatolicus* larvae were found to harm saffron corms starting from the second half of May till the end of June with the harmful effects continuing even after harvest during storage. While the pest is not normally found in the newly established saffron fields, *H. lutosus anatolicus* establishes in these fields with a prevalence rate of 4-35% in subsequent years. In addition, CO1 sequences for the molecular identification were performed for quick and reliable identification in future studies and these novel sequences for the pest were uploaded to BOLD and GenBank databases.

Keywords: CO1, corm damage, *Crocus sativus*, molecular taxonomy, Molytinae, Safranbolu

Öz

Safranbolu ilçesinde küçük bir alanda kültürü yapılan safranın (*Crocus sativus* L.) nesli tehlike altındadır. Türkiye'de daha önce varlığı bilinen, ancak yaygınlığı ve konukçu bitkisi olan safrandaki durumu bilinmeyen Safran hortumlu böceği, *Hoplopteridius lutosus anatolicus* Osella & Lodos, 1979 (Coleoptera: Curculionidae)'nin Karabük ili Safranbolu ilçesindeki safran alanlarındaki yaygınlığı ve yoğunluğunun belirlenmesi amacıyla 2015-2016 yıllarında yapılmıştır. Çalışma sonucunda Safranbolu ilçesi safran alanlarında *H. lutosus anatolicus*'un safran soğanlarında larva zararının mayıs ayının ikinci yarısından haziran sonuna kadar olduğu, hasat sonrasında zararın depoda devam ettiği belirlenmiştir. Sürvey çalışmalarında yeni tesis edilen tarlalarda zararlı görülmezken ilerleyen yıllarda zararlının tarlaya yerleştiği ve bulaşıklık oranını artarak %4-35 olduğu saptanmıştır. Ayrıca ilerleyen dönemde yapılacak çalışmalarda zararlının hızlı ve güvenilir teşhisinin yapılabilmesi için CO1 gen dizilemesi yapılmıştır ve BOLD ve Genbank veri tabanına bu zararlı için yapılan ilk yükleme niteliğindedir.

Anahtar sözcükler: CO1, bitki soğanı zararı, *Crocus sativus*, moleküler taksonomi, Molytinae, Safranbolu

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Introduction

Of the 85 colchicums (*Crocus* spp.) found around the world, Turkey has 72 taxa consisting of 36 species and 36 subspecies, with 40 endemic taxa (19 species and 21 subspecies). Of these, only saffron is cultivated commercially (Kravkaz et al., 2006). Saffron (*Crocus sativus* L., Iridaceae) has been cultivated for a long time for cosmetic, culinary, dyeing and medicinal purposes. It is cultivated in very limited areas of Safranbolu District in Karabük Province and the Harran Plains in Şanlıurfa Province in Turkey. Saffron cultivation is a labor-intensive work based on patience with very delicate and attentive care for 3-4 years given to the saffron corms planted during August and September. Saffron is one of the most expensive spices in the world because about 100-140 thousand saffron flowers are needed to produce 1 kg of dry saffron and its cultivation is as difficult. China, India and Iran are the major saffron producers in the world.

There have been relatively few studies on saffron pests and diseases. It is known that *Rhizoglyphus robini* Claparede, 1869 and *Thrips tabaci* Lindeman, 1889 are economically damaging pests, as well as other mite, thrips and nematode species (Kafi et al., 2006; Rahimi et al., 2008). Satyagopal et al. (2014), reported that pest control is needed for *Anaphothrips obscurus* (Müller, 1776), *Thrips flavus* Schrank, 1776, *Microcephalothrips abdominalis* (Crawford, 1910), *Rhizoglyphus echinopus (*Fumouze & Robin, 1868) and *R. robini* since they damage the above ground parts of saffron. Also, Chandel et al. (1996) reported that *Mylabris macilenta* Marseul, 1873 feeds on saffron flowers and causes production losses in the saffron fields infected with the pest in India.

By decreasing the yield and sometimes totally destroying the whole crop, pests only add to the already overwhelming labor-intensive process of saffron cultivation, which threatens the viability of saffron farming. Therefore, in order to prevent economic losses, it is important to determine saffron pests and their relationship with the host plant for different periods during the cultivation process.

An accurate and fast taxonomic identification of pests is crucial for proper agricultural management. However, morphological identification methods are time-consuming, require high taxonomical expertise and usually only provides adult stage identification. To resolve this problem, molecular methods offer a reliable and easy DNA-based identification tool called DNA Barcoding. The DNA Barcoding identifies target species using short DNA sequences as barcodes (Hebert et al., 2003), in particular, 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, 2015).

The Molytinae (Curculionidae) genus *Hoplopteridius* Daniel, 1908 (Coleoptera: Curculionidae) has five species distributed only in the Palearctic Region and the species *Hoplopteridius lutosus* (Frivaldszky, 1835) represents four subspecies known from Bulgaria, Italy, Romania and Turkey (Alonso-Zarazaga et al., 2017). One of these subspecies, *Hoplopteridius lutosus anatolicus* Osella & Lodos, 1979 was described by Osella & Lodos (1979) from Safranbolu indicating it is a potential pest of saffron *Crocus vernus* (L.) Hill. In the present study, the status and prevalence of *H. lutosus anatolicus* was determined with data collected in 2015 and 2016 in Safranbolu District, Karabük Province, Turkey.

Material and Methods

Survey and sampling

Studies were conducted in the saffron fields of Safranbolu District, Karabük Province during 2015 and 2016 in order to determine the pest status and prevalence of *H. lutosus anatolicus* (Table 1). Sampling 0.1 ha, a total of 100 saffron corms were collected randomly in 10 different spots along 10 different rows in each field. The presence of the pest and health state of the saffron corms were then visually determined. The number of subsamples was increased according to the field size (Jarvis & Guthrie, 1987).

Table 1. Duration of the surveys and location of fields surveyed

Village	Field 1	Field 2
Merkez	0.1 ha, 5-6 years 41°14'34" N, 32°40'36" E, 425 m	-
Yazıköy	0.2 ha, 2-3 years 41°14'26" N, 32°44'23" E, 478 m	0.3 ha, 0-1 year 41°14'20" N, 32°44'43" E, 490 m
Çercen	0.1 ha, 2-3 years 41°11'26" N, 32°48'18" E, 609 m	0.2 ha, 3-4 years 41°11'43" N, 32°48'31" E, 645 m

Laboratory studies

Fifty damaged corms were randomly collected from each field and kept in a climate chamber at 24±1ºC, 65±5% RH in the dark to allow any insect larvae to mature. Species identification was confirmed based on adult insects from saffron plants obtained from these corms. The collected specimens were preserved in absolute ethanol. The specimens were identified morphologically and from genitalia preparations.

Molecular studies

DNA was extracted from two different populations. Abdomen and three pairs legs were removed, and DNA extracted 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 et al., 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. Sequences were aligned at codon level using MUSCLE (Edgar, 2004) in MEGA (Tamura et al., 2013). Finally, consensus sequences were uploaded to the BOLD and GenBank (accession number ADM7695) databases.

Results and Discussion

The study was conducted in five different fields in Safranbolu District, which is probably the complete range of this endangered host in saffron fields of Turkey. In order to determine the prevalence of the pest, two different surveys were conducted on 15 October 2015 after the plantation of corms and on 1 June 2016 just before harvesting. According to the survey, all saffron fields, except the newly planted ones, were found to be infested with the pest. The corms were damaged due to the feeding of the larvae on corms (Figure 1A). It was found that the pest matured into adults and went into hibernation state under the soil. Damage to corms was detected in all fields except the newly established field in Yazıköy. It was determined that corm damage was only caused by the larvae.

Prevalence of the pest and damage to corms were examined in the fields again in June 2016 just before saffron harvest. Pest damage to the corms had increased in four of the five fields, and the already damaged corms had putrefied. No damage to corms was observed in the newly established saffron field located in Yazıköy (Figure 2).

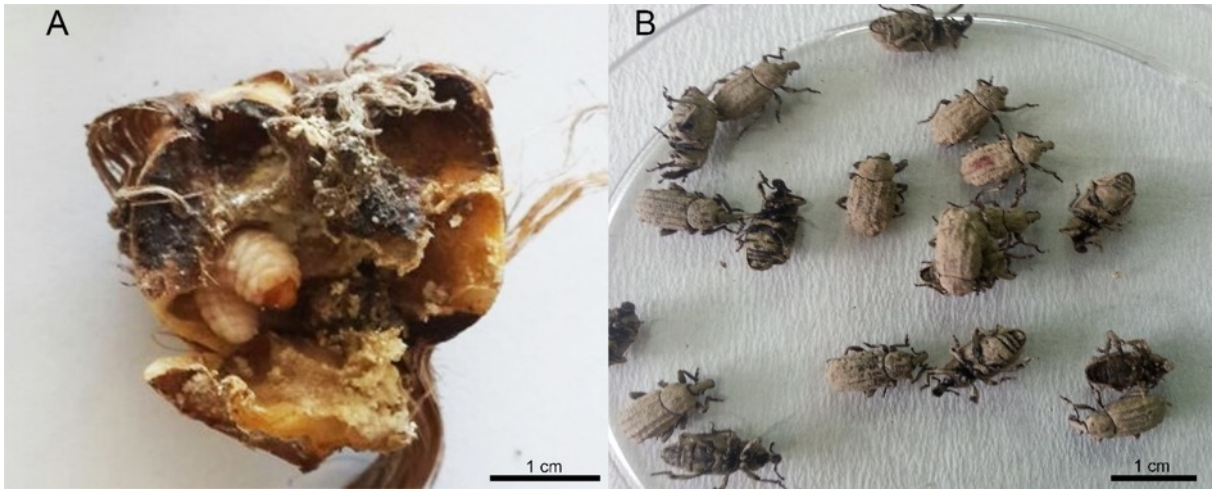

Figure 1. A) Corm damage by larvae of *Hoplopteridius lutosus anatolicus*; B) adults reared from larval stage in laboratory.

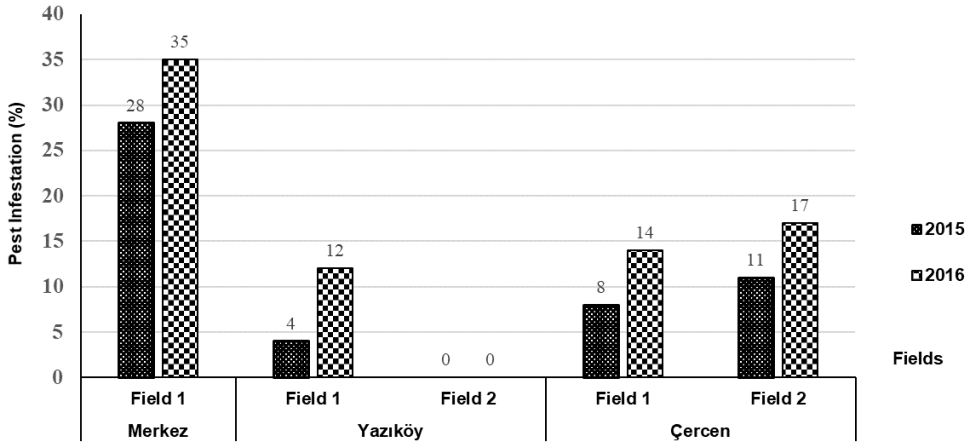

Figure 2. Prevalence of *Hoplopteridius lutosus anatolicus* on saffron corms in 2015 and 2016.

Prevalence rates of 4-28% and 11-35% were found in corms during the first and second samplings, respectively. It was also found that the prevalence rate rises with the age of the plantation (1-4 years). While the pest was not found in the newly planted field, damage to saffron corms was evident in the second year, and it increased in the third and fourth years. All previous studies (except Yücel et al., 2017) were for identification purposes only without any investigation of damage to saffron corms, economic loss, and host-pest interactions. Yücel et al. (2017) reported that the pest damaged saffron corms, which is consistent with our findings.

It is quite difficult to collect the pest since it goes into hibernation after maturing into an adult under the soil away from the corms. For this reason, the infected corms were collected before harvest in June in order to allow the larvae to mature to adults in the laboratory (Figure 1).

The larvae in culture matured into adults within an 87-d period between 3 June 2016 and 29 August 2016. Only 4-26% of larvae matured into adults because of the difficulties maintaining the infested corms in the laboratory (Figure 1B, Table 2). Although the adults were transferred onto sprouting saffron corms, no feeding or mating behavior was observed.

Table 2. Rate (%) of maturation of larvae to adults for 50 infested corms under laboratory conditions

Village	Field 1	Field 2
Merkez	26	-
Yazıköy	8	0
Çercen	4	14

From the present study, species *H. lutosus anatolicus* is reported for the second time in Turkey and for the first time in an agricultural production area. Habitus and genitalia images of the pest are given in Figure 3. Antennal and genitalia features are the morphological characteristics that are used for the identifications (Osella & Lodos, 1979). Osella & Lodos (1979) detected the pest in *Crocus vernus* (L.) (spring crocus) in Safranbolu (Karabük), Ballıdağ (Kastamonu) and Abant (Bolu). Also, Lodos et al. (2003) reported *H. lutosus anatolicus* in *Crocus* sp. in Safranbolu and *Hoplopteridius chaudoirii* (Hochhuth, 1847) in Erciyes Mountain (Kayseri).

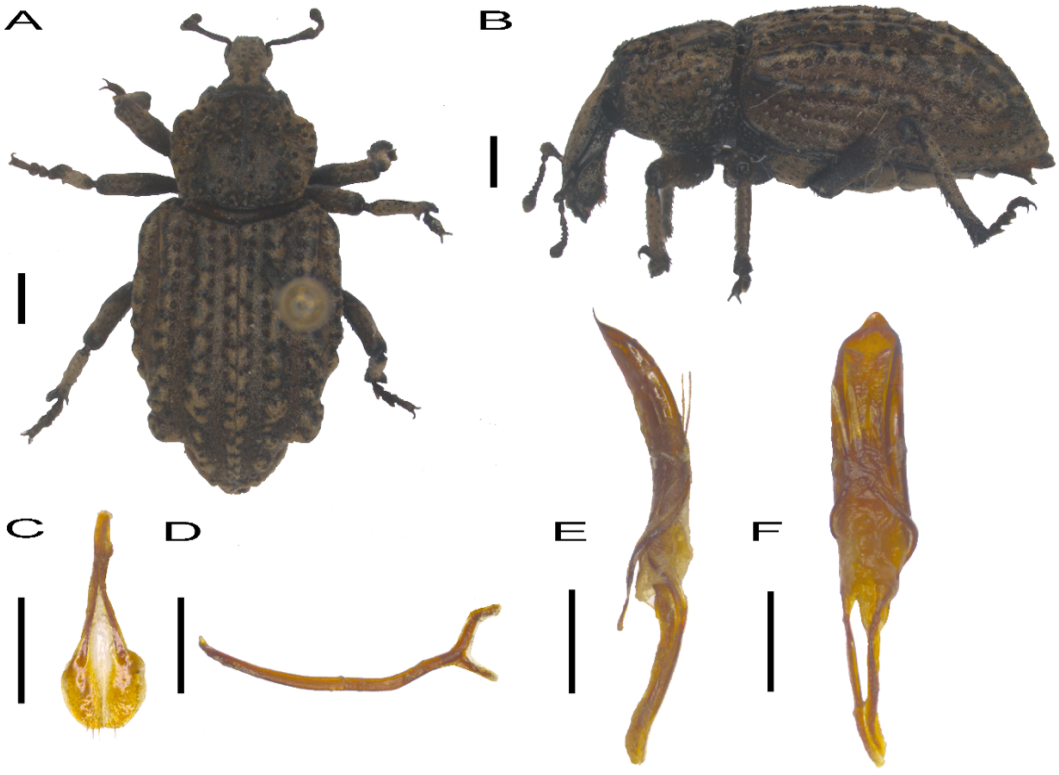

Figure 3. Images of *Hoplopteridius lutosus anatolicus*: A and B) habitus; C) spiculum ventrale (female genitalia); D) male genitalia as spiculum gastrale; E and F) aedeagus (Scale bars = 1 mm).

In Spain, *Ceutorhynchus pulvinatus* Gyllenhal, 1837 (Coleoptera: Curculionidae) was observed in high numbers and other Coleoptera species have been found in saffron fields (Cirujeda et al., 2016). However, no information is given about the level of damage to the saffron. *Mylabris macilenta* has also been found in saffron, but the report provided no information on damage (Chandel et al., 1996).

Only a limited number of studies has been conducted on *Hoplopteridius* and these studies were on detection and distribution (Osella & Lodos, 1979; Lodos et al., 2003). There are no reports about damage to saffron and prevalence of the pest in production areas. In the present study, considerable loss in saffron was confirmed by field observations and producer feedback. It was determined that the pest reaches a high density especially in aging saffron fields in which the producers have not applied adequate pest management. Saffron cropping provides two types of produce. The first is the production of saffron spices from the flowers, and the second is saffron corms sold as planting material. The insect damage is directly to the saffron corms. As evident in Figure 1, the corms are subject to severe damage. In addition, fungal infections occur in the damaged corms. As a result, these corms are no longer able to produce shoots and consequently no flowers. As a result, the usual 15 t/ha corm yield is reduced to 1-1.5 t/ha. Due to the high economic losses, most of the saffron producers have shifted to producing other crops.

In molecular studies, two CO1 sequences (639 and 658 bp), with a base composition of A = 29.4%; C = 19.6%; G = 16.1%; T = 34.9% were successfully obtained after performing quality control analysis. This study provided CO1 sequences of *H. lutosus anatolicus* for BOLD and GenBank databases for the first time. This is a new record for the genus *Hoplopteridius* as well. Due to the absence of sequences in these databases, it was not possible to confirm the morphological identification using molecular analysis. The Blast analysis showed at least 17% nucleotide distance from *Plinthus* Germar, 1817 which has been proposed as one of the closest genera to *Hoplopteridius* (Davidian, 2008). In BIN analysis, it was also determined that the distance to nearest neighbor species (*Plinthus pseudostarcki* Meregalli, 1985) is 17.4%. In addition, certain morphological identification specialists support the accuracy of the sequences. Further studies could be undertaken to increase the number of sequences of the genus in databases.

In conclusion, the present study determined *H. lutosus anatolicus* as a damaging pest for saffron and reported the species for the second time in Turkey after 36 years. The primary damage is caused by pest larvae feeding inside the saffron corms and consequently decreasing the chance of shooting. This, therefore, decreases the flower yield. Although the pest was not detected in a newly established fields, it is established by the second year with moderate prevalence, with damage to corms increasing in the third and fourth years. Saffron corms are stored after harvesting and sold in August as foundation stock. Feeding of the pest on corms permit fungal infections, that cause secondary damage and result in total rotting of the corms.

The present study has determined the status and prevalence of *H. lutosus anatolicus*, which is an important step in preserving and increasing the saffron production of Turkey, which is important both economically and culturally. Moreover, CO1 sequences were submitted to BOLD and GenBank databases for the first time for further DNA Barcoding studies and molecular identification, and are the first sequences submitted for this genus.

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References

Alonso-Zarazaga, M. A., H. Barrios, R. Borovec, P. Bouchard, R. Caldara, E. Colonnelli, L. Gültekin, P. Hlaváč, B. Korotyaev, C. H. C. Lyal, A. Machado, M. Meregalli, H. Pierotti, L. Ren, M. Sánchez-Ruiz, A. Sforzi, H. Silfverberg, J. Skuhrovec, M. Trýzna, A. J. Velázquez de Castro & N. N. Yunakov, 2017. Cooperative Catalogue of Palaearctic Coleoptera Curculionoidea. Monografías Electrónicas S.E.A., Vol. 8, Sociedad Entomológica Aragonesa S.E.A., Zaragoza, Spain, 729 pp.

Chandel, R. S., J. Kumar & P. K. Mehta, 1996. Saffron, *Crocus sativus* Linn. - A new host of blister beetle, *Mylabris macilenta* (Marshal) (Meloidae: Coleoptera). Journal of Insect Science, 79-91.

Cirujeda, A., M. M. Coca-Abia, F. Escriu, A. Palacio-Bielsa, A. I. Marí, P. Zuriaga, J. Aibar, M. Luis-Arteaga & C. Zaragoza, 2016. Estado fitosanitario del azafrán en Aragón (Espana): insectos, ácaros, nematodos, virus, bacterias y malas hierbas. Informacion Tecnica Economica Agraria, 112 (1): 3-19.

Davidian, G. E., 2008. Weevils of the genus *Plinthus* (Coleoptera, Curculionidae) from the Caucasus. (Unpublished) PhD Thesis, St. Petersburg, Russia, 193 pp.

Edgar, R. C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research, 32 (5): 1792-1797.

Folmer, O., M. Black, W. Hoeh, R. Lutz & 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.

Hebert, P. D. N., A. Cywinska, S. L. Ball & J. R. deWaard, 2003. Biological identifications through DNA barcodes. Royal Society, 270: 313-321.

Jalali, S. K., R. Ojha & T. Venkatesan, 2015. "DNA Barcoding for Identification of Agriculturally Important Insects, 13-23". In: New Horizons in Insect Science: Towards Sustainable Pest Management (Ed. A. K. Chakravarthy). Springer India, 431 pp.

Jarvis, J. L. & W. D. Guthrie, 1987. Ecological studies of the European corn borer (Lepidoptera: Pyralidae) in Boone County, Iowa. Environmental Entomology, 16: 50-58.

Kafi, M., A. Koocheki, M. H. Rashed & M. Nassiri, 2006. Saffron (*Crocus sativus*) Production and Processing. Science Publishers, Enfield, England, 244 pp.

Kravkaz, İ. S., H. Vurdu & E. Türkyılmaz, 2006. Colchicums (*Crocus* spp.) as a potential ornamental plant. Journal of Forestry Faculty, Gazi University, 6 (1): 135-140.

Lodos, N., F. Önder, E. Pehlivan, R. Atalay, E. Erkin, Y. Karsavuran, S. Tezcan & S. Aksoy, 2003. Faunistic Studies on Curculionidae (Coleoptera) of Western Black Sea, Central Anatolia and Mediterranean Regions of Turkey. Meta Basım Matbaacılık Hizmetleri, İzmir, Turkey, 83 pp.

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

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

Osella, G. & N. Lodos, 1979. *Hoplopteridius lutosus* (Frivaldsky): Brevi note sisytematiche (Coleoptera: Curculionidae-Hylobiinae). Türkiye Bitki Koruma Dergisi, 3 (3): 161-170.

Rahimi, H., A. Mokhtarian, M. Bazoobandi, H. Rahimi, M. Kiani & M. Behdad, 2008. Effects of sowing depth and summer irrigation on *Rhizoglyphus robini* population in Gonabad. Applied Entomology and Phytopathology, 76 (1): 1-14.

Satyagopal, K., S. N. Sushil, P. Jeyakumar, G. Shankar, O. P. Sharma, S. K. Sain, D. R. Boina, N. Lavanya, N. S. Rao, B. S. Sunanda, R. Asre, R. Murali, S. Arya, S. Kumar, V. Gupta, D. Kumar, V. K. Razdan, G. Mahendiran, H. P. Patnik, K.C. Sahu, S. N. Mohapatra, B. R. Patel, S. Khalko, N. Laskar, A. Roy, T. K. Hath & H. S. Yadava, 2014. AESA Based IPM Package for Saffron. National Institute of Plant Health Management, Rajendranagar, Hyderabad, India, 31 pp.

Tamura, Y., Y. Harada, S. Nishikawa, K. Yamano, M. Kamiya, T. Shiota, T. Kuroda, O. Kuge, H. Sesaki, K. Imai, K. Tomii & T. Endo, 2013. Tam41 is a CDP-diacylglycerol synthase required for cardiolipin biosynthesis in mitochondria. Cell Metabolism, 17: 709-718.

Yücel, C., A. Barış & D. Coral-Şahin, 2017. "Safranda zararlı *Hoplopteridius lutosus anatolicus* Osella & Lodos (Col.: Curculionidae) üzerine bir araştırma, 86". Uluslararası Katılımlı XIII. Ekoloji ve Çevre Kongresi (12-15 September 2017, Edirne, Turkey), 789 s.