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MORPHOMETRIC AND MOLECULAR IDENTIFICATION OF CAMPYLOMMA VERBASCI (HEMIPTERA: MIRIDAE) ON INFESTED SESAME IN ERBIL- KURDISTAN REGION- IRAQ

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Abstract

Sesame is an oil seed crop and is cultivated in many areas of the world, in Iraq, it is being grown especially in the Kurdistan region, including Erbil province. Campylomma verbasci is a piercing and sucking insect found on sesame plants in Erbil city during the summer season cultivation– in 2022. An investigation was carried out in the Grdarasha location within the sesame crop field to collect the above mentioned insect by using an aerial net and aspirator aimed to describe some morphological characters and identify the insect based on molecular techniques to determine genus and species. A set of specified primers were utilized to amplify the mitochondrial gene (COI) barcoding sequence. PCR products successfully resulted in 435bp of target DNA, and the sequence was sent to be sequenced, then the BLAST was performed in NCBI. The homogeneity of the query and subject sequence was very high. The BLAST showed that the sample was C. verbasci and it was the first time (in Iraq) to register these insect sequences in NCBI data reference.

Keywords: Bug, Miridae, Molecular Technique, PCR, Pest, Sesame.

الوصف المظهري والتشخيص الجزيئي لحشرة CAMPYLOMMA VERBASCI (HEMIPTERA:MIRIDAE) على السمسم في أربيل- إقليم كردستان– العراق

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الخلاصة

يعتبر السمسم من محاصيل البذور الزيتية ويزرع في العديد من مناطق العالم ومن ضمنها العراق، ويزرع بشكل خاص في إقليم كردستان بما في ذلك محافظة أربيل. تعد الحشرة *Campylomma verbasci* من الحشرات الثاقبة ماصة والموجودة على نباتات السمسم في مدينة أربيل خلال موسم الزراعة الصيفية لعام 2022، أجريت الدراسة للتحري عن الحشرة المذكورة اعلاه في منطقة گردرشه داخل حقل محصول السمسم، وجمعت باستخدام شبكة هوائية وشافطة ونقلت الى المختبر لغرض استخدامها لوصف بعض صفات المظهرية وتشخيص الحشرة وفق التقنيات الجزيئية لتحديد الجنس والنوع. استخدمت مجموعة من البادئات الخاصة لتضخيم تسلسل الترميز الشريطي لجين الميتوكوندريا (COI). أسفرت نواتج تفاعل البلمرة المتسلسل PCR عن 435 زوج قاعدي من الحمض النووي المستهدف وتم إرسال تسلسل القواعد النيتروجينية للتعرف عليها، ثم بعده تم إجراء BLAST في المركز الوطني لمعلومات التكنولوجيا الحيوية NCBI. حيث كان درجة التجانس مرتفعًا جداً، حيث أظهرت BLAST أن العينة *C. verbasci* تشخص للمرة الأولى على مستوى العراق وسجلت تسلسل هذه الحشرة في قاعدة بيانات NCBI.

كلمات مفتاحية: بق، Miridae، التقنية الجزيئية، PCR، آفة، السمسم.

Introduction

Sesame is regarded as the earliest oilseed crop known to humans, with the domestic variety first being farmed in India (25). The defatted Sesame meal is composed of approximately 50% protein, and large quantities of oxalic acid and fiber are contained in the seed hull (1). The oil contains Sesamin and Sesamolin lignans which is vital in oxidative stability and antioxidative activity (30). Chlorosesamone, obtained from the roots of sesame, has antifungal activity (9). Heteropteran insects commonly named true bugs comprises the largest group of hemimetabolous insects globally, having more than 42,000 described species in 5,800 genera and 140 families (8), moreover, the family of Miridae represents the most economically important heteropteran groups associated with plants therefore they are called plant bugs.

This family comprises many insects including pest insects, and others are predators that can be utilized for biological control programs (e.g., *Nesidiocoris tenuis* and *Cyrtorhinus lividipennis*) (24). In Canada, *Campylomma verbasci* (Meyer) has been recognized as a pest exerting damage to apple fruits (5, 20 and 23). In Columbia, it has been considered a significant pest of apples since the year 1991 (19). On susceptible cultivars, most of the injured fruits are aborting, while on other varieties, the fruit will recover and grow out of the damage (28), however, some malformed fruits remain on the tree.

This insect causes damage to apple trees in many countries around the world, including southern New England and New York (12 and 16) and in the Netherlands (27), while it had been traditionally considered a useful insect for biological control. Some authors have documented the *C. verbasci* as a predator of mites and small insects like aphids infesting orchard trees (4). However, in Turkey, *C. verbasci* is reported to be a pest insect on the sesame crop and causing damage to the crop (3).

To date, two main methods have been utilized for the identification of insects of both pests and natural enemies, which includes conventional morphological based method (29) and Molecular based technique (22). DNA barcoding utilizing partial DNA sequences, such as mitochondrial cytochrome C oxidase subunit I (COI), is a significant tool for identifying and distinguishing species in various animal taxa, including insects (10 and 11).

In Iraq, to our knowledge, no Authors have recorded the target bug on the sesame crop, and no one performed molecular techniques for the identification of *C. verbasci*, especially on sesame. Therefore, the main aims of this paper are to record the *C. verbasci* on sesame and identify it using mitochondrial genes, as well as register the mirid bug in Gene Reference (NCBI) in Erbil City, in addition to describe some morphological characteristics and body measurements.

Materials and Methods

Sample collecting and morphological identification: Samples of adult mirid bugs were collected fom infested sesame plants in the Grdarasha field- Erbil belonging to the College of Agricultural Engineering Sciences, Salahaddin University-Erbil. Samples were collected using aerial nets and aspirators during summer sesame season from July to September-2022. After then, the samples were brought to the laboratory and split randomly into two groups, each group contaning 100 individuals; the first group was for taking measurements and describing the body parts under dissecting a microscope and using a calibration micrometer ruler. The othe groups of *C. verbasci* was utilized to isolate DNA prior to molecular identification.

DNA extraction: The genomic DNA was extracted from certain body parts (e.g., head, thorax, legs + wings, and abdomen) of ten adult bugs by using the ZYMO Quick DNA Tissue- Insect Micro-prep Kit manufactured in the United States of America No. D6015 (2 and 17).

Quality and Quantity of Genomic DNA: Spectrophotometer (NANODROP1000 U.K.) was used to check the quantity and quality of isolated genomic DNA, and the spectrophotometer concentration for the samples were (40.34 ng/ µl).

PCR Amplification of mitochondrial Cytochrome Oxidase c subunit I (COI) and DNA sequencing: Polymerase Chain Reaction amplification for the COI partial gene was performed in 50µl of the reaction mixture, which contained 2x Taq DNA Polymerase Master Mix (AMPLIQON A/S Stenhuggervej 22), forward primer: C1-J-1718 (5' GGAGGATTTGGAAATTGATTAGTTCC 3') reverse primer C1-J-1718 (5' ACTGTAAATATATGATGAGCTCA 3') (12), DNA template and DNase free water as displayed in (table 1) by Bioresearch PTC-200 Gradient thermo-cycler. The used temperatures were as follows: denaturation at 95°C for 5 minutes, followed by 35 cycles, and primer annealing at 60°C for 40 seconds, finally, a one-minute extension at 72°C, followed by a ten-minute extension at 72°C (26). The COI partial gene was sequenced as a target gene using ABI Prism Terminator Sequencing Kit (Applied Biosystem).

Table 1 COI PCR Amplification Reagents.

No.	PCR components	Concentration	Volume (µl)
1	Master Mix	2x	25
2	Forward Primer	20 Pmol	3
3	Reverse Primer	20 Pmol	3
4	DNase free Water	-	15
5	Template DNA	50ng/µl	4
	Total		50

Sequence alignment and submission: The sequenced COI gene was run through the (BLAST) which can be found on the website: (https://blast.ncbi.nlm.nih.gov/Blast.cgi) where comparisons and alignments of query sequences with other organism sequences were performed to gain more homogeneity with other targets.

Statistical analysis: The statistical data processing was conducted for calculating the measurement means using the statistical software program – IBM SPSS version 26.0 (6).

Results and Discussion

Classification of the Mirid Bug.
The taxonomic hierarchy of *C. verbasci* (21) is as follows:
Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Hemiptera
Suborder: Heteroptera
Superfamily: Miroidea
Family: Miridae
Genus: *Campylomma* Reuter, 1878
Species: *Campylomma verbasci* (Meyer-Dur, 1843).

Description of Adults: The adult insect was first distinguished by possessing a green-gray color, and then it became green to brown. The Filiform antennae comprised four-segments and red compound eyes (Figure 1).

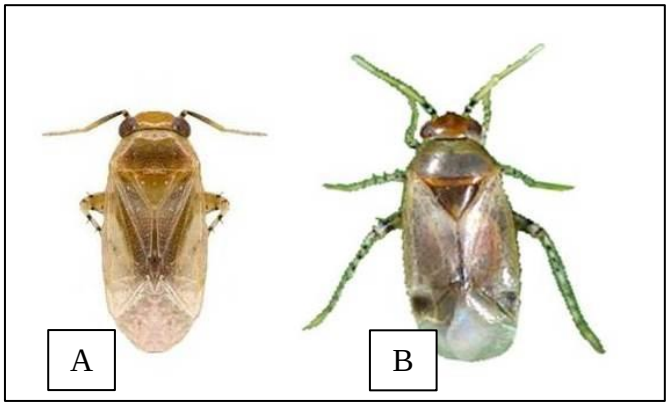

Figure 1 Adults of *C. verbasci* (A) Male and (B) Female.

Table 2 shows the various morphological characteristics of the adult stage, which include (the general coloration of the body, the color of the head, thorax, and abdomen, the number of antennae segments, the color of compound eyes, the rudiments of wings and covering of abdominal rings, color of the legs and presence of spines, and the general shape of the body). The morphometric results showed all most similar to other research studies (18).

Table 2 Important characters of the adult stage of *C. verbasci*.

No.	Adult character	Description
1	Color of Body	It appears grey-green at first, then becomes brownish-gray
2	Head	Yellow
3	Thorax	Pale yellow smooth
4	Abdomen	Pale green oval
5	Antennae	Four segments, the second segment is equal to the last two segments with a black base, and the first segment has a black-top
6	Eyes	Red compound
7	Pads of Wings	The forewings hemelytron and the hind wings are membranous, and they cover all abdomen and extend outward slightly beyond it
8	Legs	It has strong black spots and spines
9	Shape of Body	Oval

The observations were made on measurements of *C. verbasci* adults. Further, it was found that the length of the male bugs were 1.65 - 1.75 mm and averaged 1.70 ± 0.02 mm, whereas the range of male width was 0.76 - 0.98 mm and averaged 0.84 ± 0.06 mm, respectively. Moreover, the length of the female bugs was ranged between 2.11 - 2.65 mm and averaged 2.42 ± 0.16 mm, while the width ranged from 0.98 - 1.10 mm and averaged 1.02 ± 0.03 mm. Furthermore, the antennae are of filiform type, the length of the antennae of the male bugs was also measured, and they were 0.55 - 0.60 mm with an average of 0.57 ± 0.01 mm, while the length of fore, middle, and hind legs were ranged from 1.20 - 1.33 mm, 1.40 - 1.48 mm and 1.50 - 1.60 mm with an average of 1.28 ± 0.04 mm, 1.44 ± 0.02 mm and 1.55 ± 0.02 mm, respectively. In contrast, the length of the antennae of female bugs was also measured, which were found to be 0.62 - 0.70 mm with an average of 0.66 ± 0.02 mm, while the length of fore, middle, and hind legs ranged from 1.30 - 1.42 mm, 1.45 - 1.55 mm and 1.63 - 1.70 mm with an average of 1.36 ± 0.03 mm, 1.50 ± 0.02 mm and 1.66 ± 0.02 mm,

respectively. In addition, forewings are hemelytron and the hind wings are membranous, the length of fore wings and hind wings of male bugs ranged from 1.60 - 1.80 mm and 0.90 - 0.97 mm with an average of 1.71 ± 0.06 mm and 0.92 ± 0.02 mm, respectively, whereas the length of fore and hind wings of female bugs ranged from 1.92 - 2.25 mm and 0.92 - 1.25 mm with an average of 2.05 ± 0.10 mm and 1.05 ± 0.10 mm, respectively (Table 3).

The results of this study on antennae and compound eyes of *C. verbasci* are in agreement with those of (18), who showed that the antennae of adults consist of four segmented and compound eyes are reddish.

Table 3 Measurements of adult body parts of *C. verbasci*.

Life Stage	Particulars	Measurement (mm)		
		Min.	Max.	Mean ± SD
Adult				
Body				
Male	Length	1.65	1.75	1.70 ± 0.02
	Width	0.76	0.98	0.84 ± 0.06
Female	Length	2.11	2.65	2.42 ± 0.16
	Width	0.98	1.10	1.02 ± 0.03
Antennae				
Male	Length	0.55	0.60	0.57 ± 0.01
Female	Length	0.62	0.70	0.66 ± 0.02
Legs				
Male				
Fore-legs	Length	1.20	1.33	1.28 ± 0.04
Middle-legs	Length	1.40	1.48	1.44 ± 0.02
Hind-legs	Length	1.50	1.60	1.55 ± 0.02
Female				
Fore-legs	Length	1.30	1.42	1.36 ± 0.03
Middle-legs	Length	1.45	1.55	1.50 ± 0.02
Hind-legs	Length	1.63	1.70	1.66 ± 0.02
Wings				
Male				
Fore-wing	Length	1.60	1.80	1.71 ± 0.06
Hind-wing	Length	0.90	0.97	0.92 ± 0.02
Female				
Fore-wing	Length	1.92	2.25	2.05 ± 0.10
Hind-wing	Length	0.92	1.25	1.05 ± 0.10

Isolation of DNA Genomics: Genomic DNA was isolated from adult individuals; sample was extracted using the ZYMO Quick DNA Tissue-Insect Micro-prep Kit, model number D6015, made in the United States of America (Figure 2). The isolated DNA was electrophorized in 1.5% of Agarose gel.

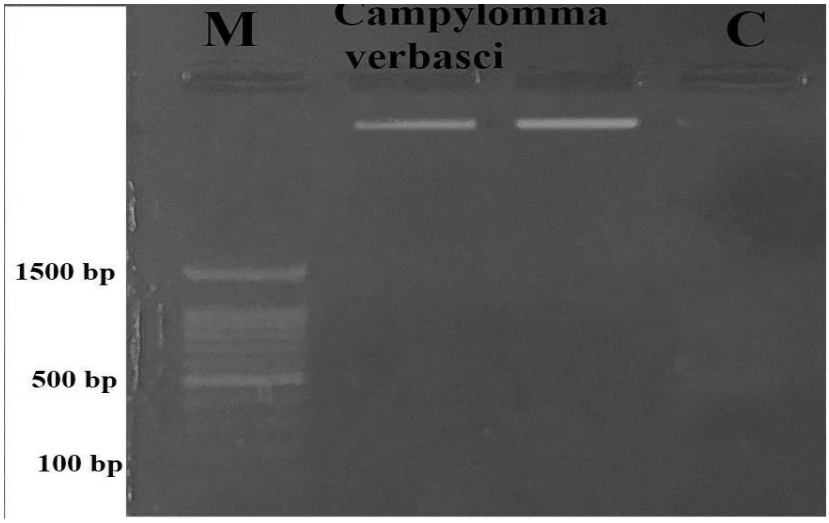

Figure 2 Genomic DNA isolated from samples of Mirid bugs.

Partially amplification of the COI gene by using PCR: Specific primers were designed from the mitochondrion gene, for using the sequences of cytochrome c oxidase subunit I synthesized by Micro-gene Company (South Korea). The primers could yield a band of ~500bp. The PCR product was electrophoresed and visualized by 1.5% of Agarose gel (Figure 3).

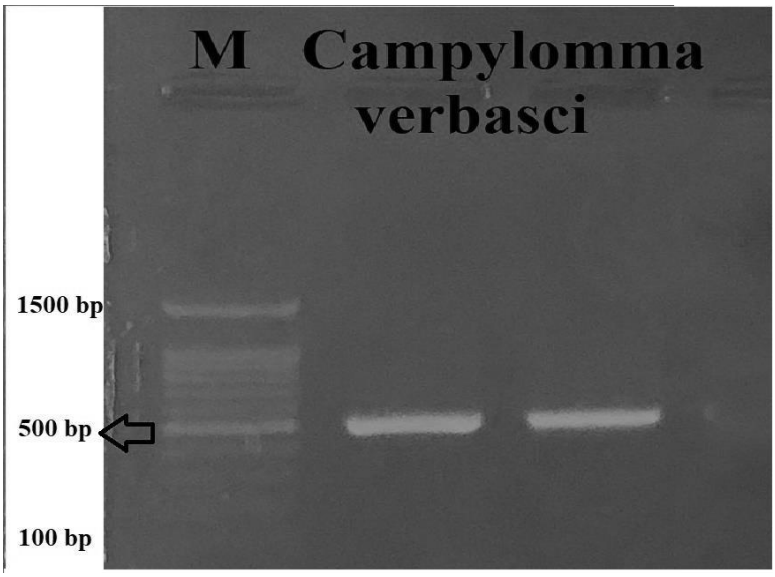

Figure 3 PCR amplification of partial Cytochrome C Oxidase I gene from Mirid bugs.

Partial cytochrome c oxidase I sequenced gene: DNA sequencing was performed separately using only forward primer C1-J-1718 by ABI 3130X genetic analyzer (Applied Biosystem). The sample products of PCR were used as an origin of DNA template for sequence specific PCR amplification (Figure 4).

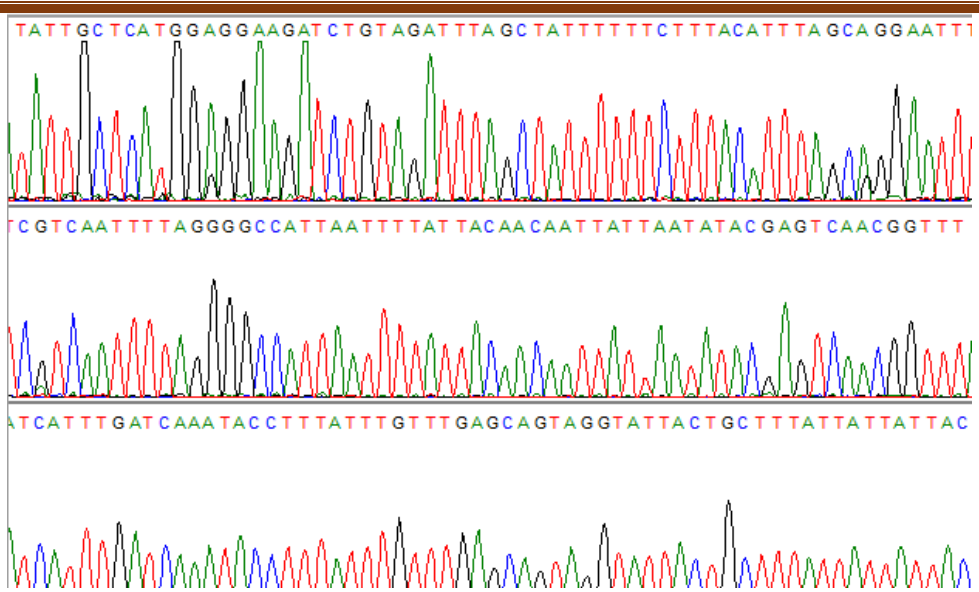

Figure 4 The COI sequence's results on the chromatogram.

Molecular identification of genus and species of Mirid bug: BLAST program (*http://blast.ncbi.nlm.nih.govl.*) was used to align COI sequence samples with sizes 400-450 from Gen bank for comparison of amplified sequences of this study with other saved species of the same bug sequences. The BLAST results showed that the highest and lowest matching of the number query sequence was 100%, which was *C. verbasci*. These results indicate that we submitted our query sequences to the NCBI Gen bank and gained the accession number MW548956. As it's known, there are many approaches for the identification of insects in general, including morphological and molecular methods. This study has focused on both methods to meet the goal of identification of insect samples accurately, the study started with the morphometric description of insect body parts, for this purpose, different parts of insect samples were measured, including the whole body of adults (starting from head to the end of the abdomen), antennae, legs, wings, all the measures are listed in the table 1, which were almost compatible with results of (18), despite slight differences in morphometric measurements between studied samples collected in Erbil and those reported in the literature. This could be due to the geographical distribution of the Miridae family all over the world. This helps researchers to recognize this species easily (15).

Regarding the molecular identification method, mitochondrial gen is the most commonly constant region in insects that have contributed to the field of DNA barcoding of the animal's identification (14). In this study, insect samples were collected, DNA was extracted, and as can be seen in Figure 1, the extracted DNA from the samples is active and noticed in gel wells with a size band of 1500bp. To amplify 500bp regions, specific primers were designed and used then PCR was carried out. The results of PCR listed in Figure 2 and the size band of 500bp were obtained, and this size could be used as a standard for *C. verbasci*, because the genus has not been identified molecularly by previous researchers. This shows that primers have successfully amplified the COI gene regions, and it could be said that because the samples have been collected from the same area, meaning no divergence across

their genomes. After the DNA sequences were sent for sequencing and blasting in NCBI, sequences were sent for Gen Bank, and accession numbers for each sample were obtained and then first recorded in NCBI. In contrast to our results, (7) stated that the complete mitochondrial genome (mitogenome) of other genera within the Miridae family can be amplified using long PCR and a primer walking as a sequencing strategy that results in 17544bp in length.

Conclusion: Piercing and sucking insects are a diversified group of insects including pestiferous and predators, the mirid bugs are considered pest insects of many plants around the world, in this paper, the Mirid bug, *C. verbasci*, was identified using molecular techniques depending on the mitochondrial gene. The target sequence was submitted to the gene reference (NCBI), and the accession number was received. It is interesting that registered species were put into the database for the first time in Iraq.

Reference

1- Abou-Gharbia, H. A., Shehata, A. A. Y., and Shahidi, F. (2000). Effect of processing on oxidative stability and lipid classes of sesame oil. Food research international, 33(5): 331-340.

2- Ahmed, H. S. (2022). Biology and ecology of oak leaf moth, *Euproctis melania* Staud. in Erbil Governorate, Iraq. PhD Dissertation, Salahaddin University-Erbil.

3- Atakan, E., Pehlivan, S., and Cevdet, K. A. Y. A. (2017). Adana ili susam (Sesamum indicum L.) üretim alanlarında bulunan zararlı ve faydalı türlerin araştırılması. Türkiye Entomoloji Bülteni, 7(2): 157-166.

4- Bartlett, D. (1996). Feeding and egg laying behaviour in *Campylomma verbasci* Meyer (Hemiptera: Miridae). Theses (Dept. of Biological Sciences)/ Simon Fraser University.

5- Boivin, G. and Stewart, R. K. (1982). Identification and evaluation of damage to McIntosh apples by phytophagous Mirids (Hemiptera: Miridae) in Southwestern Quebec. The Canadian Entomologist, 114(11): 1037-1045.

6- Corp, I. (2013). IBM SPSS statistics for windows, version 22.0. Armonk, NY: IBM Corp.

7- Dai, X., Xun, H., Chang, J., Zhang, J., Hu, B., Li, H., ... and Cai, W. (2012). The complete mitochondrial genome of the plant bug Nesidiocoris tenuis (Reuter)(Hemiptera: Miridae: Bryocorinae: Dicyphini). Zootaxa, 3554(1): 30-44.

8- Foottit, R. G., and Adler, P. H. (2009). Insect biodiversity: science and society, John Wiley and Sons.

9- Hasan, A. F., Begum, S., Furumoto, T., and Fukui, H. (2000). A new chlorinated red naphthoquinone from roots of Sesamum indicum. Bioscience, biotechnology, and biochemistry, 64(4): 873-874.

10- Hebert, P. D. N., Stoeckle, M. Y., Zemlak, T. S., and Francis, C. M. (2004). Identification of birds through DNA barcodes. PLoS biology, 2(10): 312.

11- Jung, S., Duwal, R. K., and Lee, S. (2011). COI barcoding of true bugs (Insecta, Heteroptera). Molecular Ecology Resources, 11(2): 266-270.

12- Kain, D. P., and Agnello, A. M. (2013). Relationship between plant phenology and Campylomma verbasci (Hemiptera: Miridae) damage to apple fruit. Environmental entomology, 42(2): 307-313.
13- Kambhampati, S., and Smith, P. T. (1995). PCR primers for the amplification of four insect mitochondrial gene fragments. Insect molecular biology, 4(4): 233-236.
14- Kim, J., and Jung, S. (2018). COI barcoding of plant bugs (Insecta: Hemiptera: Miridae). PeerJ, 6: 6070.
15- Knight, H. H. (1941). The plant bugs, or Miridae, of Illinois. Illinois Natural History Survey Bulletin, 22(1).
16- Leonard, D. E. (1965). Atractotomus mali and Campylomma verbasci (Heteroptera: Miridae) on apples in Connecticut. Journal of Economic Entomology, 58(5): 1031-1031.
17- Luo, B., Ma, F., Liu, H., Hu, J., Rao, L., Liu, C., ... and Liu, X. (2022). Cell-free DNA methylation markers for differential diagnosis of hepatocellular carcinoma. BMC medicine, 20: 1-12.
18- Mahmalji, M. Z., Almatni, W., Alimad, N., and El-nagar, M. E. (2010). Morphological Study on *Campylomma verbasci* Meyer-Dure (Hemiptera: Miridae) and Insuranceitt's Classification as Genera and Species. Egyptian Journal of Agricultural Research, 88(3): 667-682.
19- McBrien, H. L., Judd, G. J. R., and Borden, J. H. (1994). Campylomma verbasci (Heteroptera: Miridae): Pheromone-based seasonal flight patterns and prediction of nymphal densities in apple orchards. Journal of Economic Entomology, 87(5): 1224-1229.
20- McMullen, R. D., and Jong, C. (1970). The biology and influence of pesticides on Campylomma verbasci (Heteroptera: Miridae). The Canadian Entomologist, 102(11): 1390-1394.
21- Myers, P., Espinosa, R., Parr, C. S., Jones, T., Hammond, G. S., and Dewey, T. A. (2023). The Animal Diversity Web [online]. Accessed at https://animaldiversity.org/accounts/Campylomma_verbasci/classification/.
22- Rajesh, H. S., Udikeri, S. S., and Bhat, R. S. (2020). Molecular characterization of mirid bugs infesting cotton. Journal of Farm Sciences, 33(4): 441-447.
23- Reding, M. E., Beers, E. H., Brunner, J. F., and Dunley, J. E. (2001). Influence of timing and prey availability on fruit damage to apple by Campylomma verbasci (Hemiptera: Miridae). Journal of economic entomology, 94(1): 33-38.
24- Schaefer, C. W., and Panizzi, A. R. (2000). Economic importance of Heteroptera: a general view. In Heteroptera of economic importance, 25-30.
25- Shurtleff, W., and Aoyagi, A. (2022). History of Sesame (100 CE to 2022): Extensively Annotated Bibliography and Sourcebook, Soyinfo Center.
26- Šigut, M., Pyszko, P., Šigutová, H., Višňovská, D., Kostovčík, M., Kotásková, N., dorňák, O., Kolařík, M., and Drozd, P. (2022). Caterpillar Gut Microbiome: Fungi Are More Transient Than Bacteria.

27- Stigter, H. E. N. K. (1995). Campylomma verbasci, a new pest on apple in the Netherlands. In International Conference on Integrated Fruit Production 422: 140-144.

28- Thistlewood, H. M. A., Borden, J. H., Smith, R. F., Pierce, H. D., and McMullen, R. D. (1989). Evidence for a sex pheromone in the mullein bug, Campylomma verbasci (Meyer)(Heteroptera: Miridae). The Canadian Entomologist, 121(9): 737-744.

29- Waterhouse, C. O. (1890). Aid to the Identification of Insects, EW Janson, 2.

30- Wu, W. H. (2007). The contents of lignans in commercial sesame oils of Taiwan and their changes during heating. Food chemistry, 104(1): 341-344.